Genetic testing for hereditary mutations in the RET gene

August 2013

MSAC application no. 1152

Assessment report

Contracted Assessment Report for Application 1152 - Genetic testing for hereditary mutations in the RET gene

Online ISBN: 978-1-74186-111-2
Publications approval number: 10698

Internet site http://www.msac.gov.au/

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This report is a contracted technical report for use by the Medical Services Advisory Committee (MSAC) to inform its deliberations. MSAC is an independent committee which has been established to provide advice to the Minister for Health and Ageing on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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This report should be referenced as follows:

Newton S, Schubert C, Morona J, Fitzgerald P, Merlin T. (2013). *Genetic testing for hereditary mutations in the RET gene.* MSAC Application 1152, Assessment Report. Commonwealth of Australia, Canberra, ACT.

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Executive summary

Mutation testing of the *RET* gene

Purpose of application

In October 2010 the Department of Health and Ageing received an application from the Pathology Services Table Committee (PSTC) requesting a Medicare Benefits Schedule (MBS) listing to enable RET (rearranged during transfection) mutation testing for (i) patients with symptoms of multiple endocrine neoplasia type 2 (MEN2) and (ii) unaffected relatives of a patient with a documented RET mutation to determine the risk of disease. It was proposed that two new MBS items cover the use of diagnostic and predictive testing for mutations in the *RET* gene.

A team from Adelaide Health Technology Assessment (AHTA), University of Adelaide, was contracted to conduct a systematic review of the literature and an economic evaluation of RET mutation testing. A decision analytic protocol (DAP) was developed before commencement of the assessment and was approved by the Protocol Advisory Sub-Committee (PASC) of the Medical Services Advisory Committee (MSAC).

The *RET* proto-oncogene encodes a receptor tyrosine kinase, which is involved in processes such as neural crest differentiation, cell migration and proliferation (Burzynski et al. 2005). Hereditary mutations in this gene cause MEN2 and hereditary Hirschsprung's disease (colonic aganglionosis). Genetic testing may be indicated in a patient with one or more features of the syndrome (diagnostic testing) to make a diagnosis; or it can be used in unaffected relatives of a patient with a documented RET mutation (presymptomatic testing) in order to determine their risk of disease and reduce morbidity and mortality through early intervention.

Multiple endocrine neoplasia type 2 (MEN2)

MEN2 is a group of disorders associated with tumours of the endocrine system (generally the thyroid, parathyroid and adrenals). Nearly all patients develop a medullary thyroid carcinoma (MTC), and half of patients with MEN2A or MEN2B develop phaeochromocytomas (Margraf et al. 2009). Of those patients with MEN2A, 15–30% may also develop hyperparathyroidism, whereas patients with MEN2B are not at risk of parathyroid disease but will show other abnormalities such as ganglioneuromas, medullated corneal nerves and marfanoid body habitus (Eng 1999). Familial medullary thyroid cancer (FMTC) comprises families who only have MTC. However, some RET mutations are associated with both MEN2A and FMTC, so a clinical history is required to distinguish between the two conditions (Margraf et al. 2009).

RET mutation testing is currently performed in order to triage further investigations. If patients are found to have pathological RET mutations, they are investigated for further MEN2 features before receiving a total thyroidectomy. However, if patients are found to have no pathological RET mutations, they are either assumed to have a sporadic MTC or hyperparathyroidism, or are investigated for other hereditary disorders associated with phaeochromocytoma.

Proposal for public funding

The proposed MBS items are summarised in Table 1. The suggested fees are based on updated information on the current pricing of RET mutation testing in Australia and differ from the fees proposed in the final DAP.

It is a requirement that all individuals undergoing predictive testing should first receive genetic counselling and give informed consent (or assent in the case of children). It is also recommended that patients undergoing diagnostic RET mutation testing should undergo genetic counselling. As a consequence, it is suggested that the ordering of the genetic test for RET mutations should be limited to specialised genetic services that can provide accredited genetic counselling to patients and their family members.

Table 1 Proposed MBS item descriptors for RET mutation testing

Category 6 – Pathology services
MBS [item number] Detection of germline mutations in the *RET* gene in patients with: (a) medullary thyroid carcinoma (b) adrenal phaeochromocytoma under the age of 50 years (c) hyperparathyroidism plus a diagnosis of medullary thyroid cancer or phaeochromocytoma in a close relativea 1 or more tests Fee: $400 Prior to ordering these tests the ordering practitioner should ensure that the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.
MBS [item number] Detection of a known mutation in the *RET* gene in: (a) asymptomatic first- or second-degree relatives, at genetic risk, of a patient with a documented pathogenic RET mutation 1 or more tests Fee: $200 Prior to ordering these tests the ordering practitioner should ensure that the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

Current arrangements for public reimbursement

Currently, RET mutation testing is standard practice, although there is no MBS listing for any test that detects mutations of the *RET* gene. Patients are therefore encouraged to have their blood sample collected through a public hospital so that genetic testing is conducted and billed under state and territory public hospital arrangements. When patients are referred by a private facility, they are billed directly. Private health insurance generally provides a subsidy for testing only if the MBS also provides a rebate for the test (ALRC 2003; PaLMS 2011).

Three accredited pathology laboratories in Australia offer RET mutation testing (RCPA 2012). All offer polymerase chain reaction (PCR) amplification and DNA sequencing of the RET gene, with a 4-week to 3-month turnaround for results. The costs of RET mutation testing in Australia are summarised in Table 6 (page xxxiv).

There have been no previous MSAC considerations of RET mutation testing.

Prerequisites to implementation of any funding advice

RET mutation testing is currently classified as a Class 3 *in-vitro* diagnostic (IVD) by the Therapeutic Goods Administration (TGA). Laboratories offering the test in house must have National Association of Testing Authorities (NATA) accreditation, with demonstrated compliance with the suite of standards on the validation of in-house IVDs, as published by the National Pathology Accreditation Advisory Council, for each test manufactured.

Consumer impact statement

The public was invited to provide feedback on the draft protocol for undertaking this evaluation of RET mutation testing during October 2011. No public consultation responses were received from any relevant craft groups or consumer groups.

Clinical need

It is estimated that the prevalence of MEN2 is 2.5 per 100,000 in the general population (Raue & Frank-Raue 2010). In a population of 22.6 million people (ABS 2011), it is therefore estimated that approximately 500–600 Australians have this rare disorder. The best estimate of the population *suspected* of having MEN2 comprises those who are diagnosed with MTC. In 2007 there were a total of 456 males and 1,331 females newly diagnosed with thyroid carcinomas (AIHW 2010). Approximately 5–10% of thyroid carcinomas are medullary (Keatts & Itano 2006), so it is estimated that, of the 1,787 thyroid carcinomas diagnosed, 89–179 of them would be medullary.

In 2007 there were 150 diagnostic tests performed on the *RET* gene in Australia (Suthers 2008b). This is within the range of what would be expected given the estimated rate of

MTCs diagnosed. It is expected that having item numbers on the MBS to allow reimbursement for RET mutation testing would not significantly impact on the number of genetic tests being performed on patients, given that it is already considered standard practice in Australia[1] and 'best practice' world-wide (Brandi et al. 2001).

Only 25–30% of MTCs are hereditary (Raue & Frank-Raue 2010), so the use of the genetic test in the proband would rule out the need for further familial genetic testing or MTC surveillance in 65–70% of cases. It is therefore expected that only 22–54 Australian patients per year would have MTC caused by MEN2, resulting in their first-degree relatives requiring genetic screening. Based on data from the Familial Cancer Unit in South Australia, there are approximately 11.5 unaffected first- or second-degree relatives per proband (Suthers et al. 2006). In a study assessing uptake of genetic screening, when family members were contacted both by the proband and directly by letter from the Familial Cancer Unit, 40% of relatives undertook genetic screening within 2 years (Suthers et al. 2006). It is therefore estimated that, on average, 4.6 family members per proband would agree to predictive genetic testing. In 2007 there were 49 presymptomatic tests performed on the *RET* gene in Australia (Suthers 2008b), which is below the rate of what would be expected, assuming that more than one relative per proband would be tested. It is therefore estimated that having an item number on the MBS for detection of a known mutation in the *RET* gene in first- or second-degree relatives at genetic risk would increase the number of presymptomatic tests to approximately 101–248 per year.

Clinical place for proposed intervention

For the diagnosis of MEN2, RET mutation testing is used to triage (or replace, in the case of pentagastrin-stimulated calcitonin) biochemical screening and imaging in those patients with clinical features suggestive of MEN2.

Two clinical management algorithms have been provided for RET mutation testing in index cases *with* an MTC (Figure 1) and *without* an MTC (Figure 2), and for their close family members. The clinical scenario in Figure 1 is more common than in Figure 2, as an MTC is the first symptom in most MEN2 families due to its earlier and higher penetrance (Brandi et al. 2001). The left side of each management algorithm outlines the approach to the diagnosis and prediction of MEN2 in a setting without genetic testing (as the historical comparator), while the right side shows current clinical practice, which includes the use of genetic testing. The white text boxes and solid arrows relate to the diagnosis and treatment of people with clinical features suggestive of MEN2, while the black boxes and dashed arrows correspond to the management of their close family members.

[1] Health Expert Standing Panel (HESP) member and endocrinologist, R Clifton-Bligh, email received on 20 June 2011

Special emphasis should be given to material differences between the algorithms outlining the 'historical' and 'current' clinical management strategies for MEN2 in the type of healthcare resources and the frequencies of their use. Figure 1 shows that, in the absence of RET mutation testing (the historical setting), all patients with an MTC at presentation or detected through initial investigations would be monitored for further clinical features of MEN2, despite there being a 75% chance of the MTC being sporadic. It is also assumed that, in the absence of genetic testing, their first-degree family members would receive annual surveillance for MEN2 features. Family members would undergo a total thyroidectomy once early signs of MTC are detected by elevated calcitonin levels. In comparison, the main differences between this historical setting and the current setting (with RET mutation testing available) are: i) the *targeted* use of lifelong surveillance in patients and family members who have a definitive diagnosis of MEN2 or RET mutation, or the *avoidance* of this requirement in those patients and family members without a RET mutation; and ii) the use of prophylactic total thyroidectomy in family members with a confirmed RET mutation.

In Figure 2 it is shown that, in the absence of RET mutation testing, all those who present with an early onset adrenal phaeochromocytoma or hyperparathyroidism (plus a diagnosis of MTC or phaeochromocytoma in a close relative) who are found *not* to have an MTC would be assumed not to have MEN2. Therefore, the index case and their family members would not be screened or undergo surveillance. However, in the current setting where genetic testing is available, patients with this clinical profile who have a RET mutation would be diagnosed with MEN2 and therefore undergo prophylactic total thyroidectomy and lifelong surveillance. Their family members would also undergo cascade screening and those who also carry the RET mutation would undergo prophylactic thyroidectomy and lifelong surveillance.

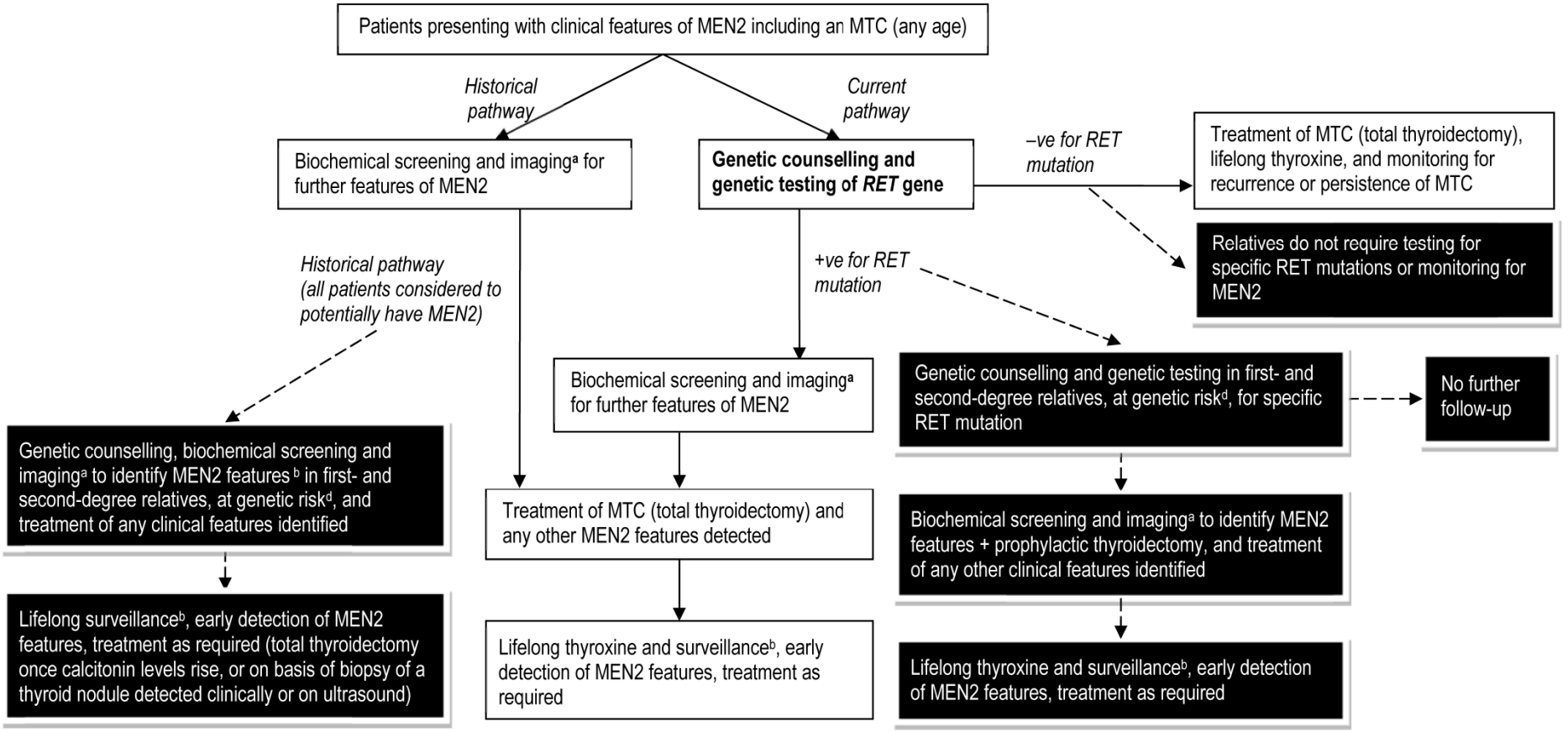

Figure 1 Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (MTC identified in index case prior to genetic testing)

[a] Biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma, serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism.

[b] Surveillance in those who have had a total thyroidectomy: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}) and calcitonin and carcinoembryonic antigen to detect persistence or recurrence of MTC.

[c] Historical surveillance in those at risk of MEN2 who have not had a total thyroidectomy: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}); pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC.

[d] Second-degree relatives would only be considered to be at genetic risk if first-degree relatives have a RET mutation, clinical features of MEN2, or if information regarding first-degree relatives is unavailable.

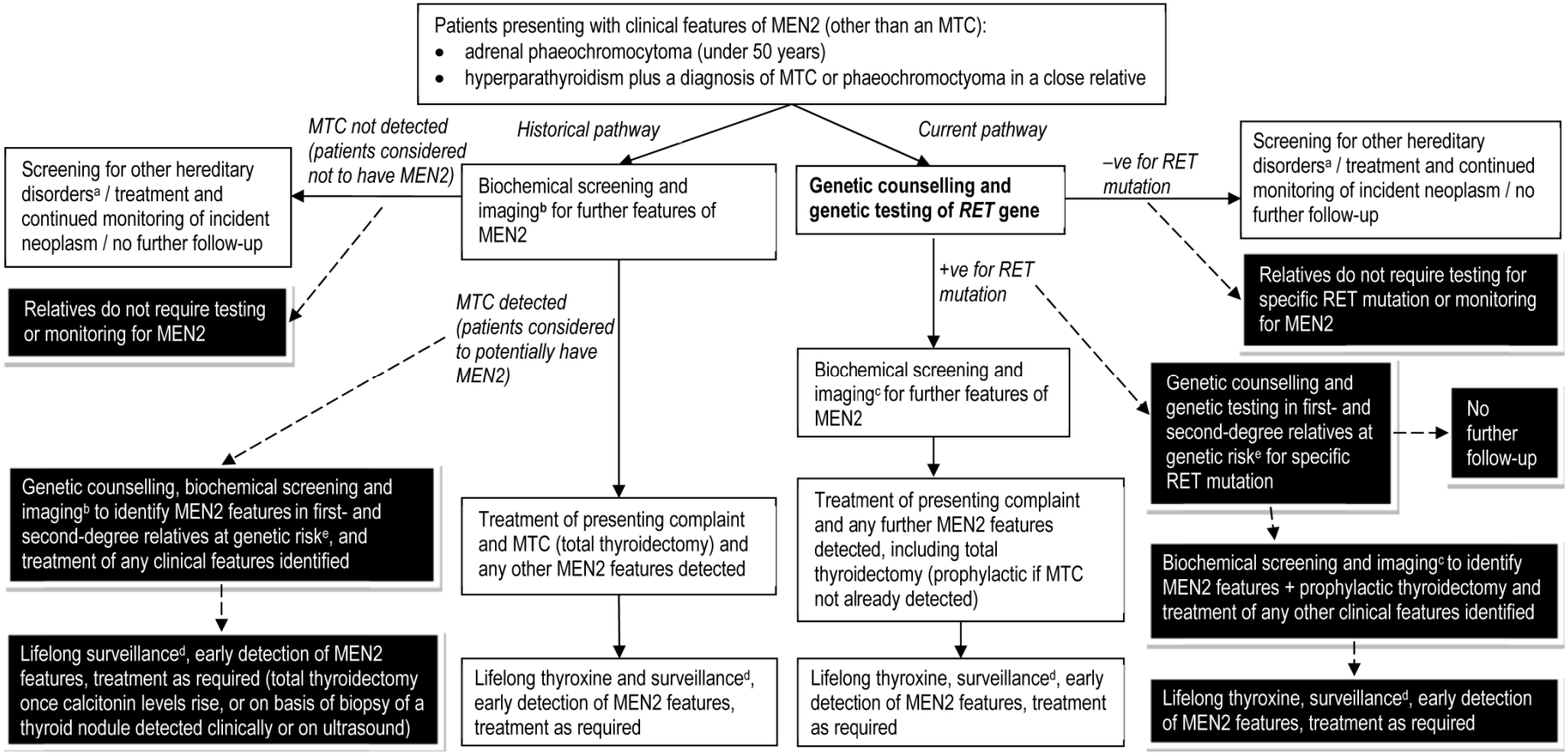

Figure 2 Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (no MTC in index case prior to genetic testing)

[a] Screening for other hereditary disorders: genetic testing of the VHL gene for von Hippel Lindau disease, genetic testing for SDHB, SDHC and SDHD mutations; if serum calcium and parathyroid hormone are elevated then additional testing for features of MEN1 (serum gastrin, serum insulin, serum glucagon, serum pancreatic polypeptide, serum vasoactive intestinal peptide, serum prolactin, growth hormone and adrenocorticotrophic hormone).

[b] Historical biochemical screening and imaging for further features of MEN2: pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC; plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical feature, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting clinical features.

[c] Current biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical features, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting feature.

[d] Historical surveillance: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}); plus pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC or calcitonin and carcinoembryonic antigen after surgery for MTC.
Current surveillance: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}) ± calcitonin and carcinoembryonic antigen after surgery for MTC.

[e] Second-degree relatives would only be considered to be at genetic risk if first-degree relatives have a RET mutation, or if information regarding first-degree relatives is unavailable.

Comparator

Comparators are usually selected by determining the technology (including testing strategy) most likely to be replaced, or added to, by the technology submitted for a new MBS item number. However, in the situation of RET mutation testing of patients suspected of having MEN2 or of their close family members, genetic testing is already standard practice. As a consequence, when determining the financial implications of RET mutation testing in this report, the comparator is considered to be genetic testing paid for either by the patient or by the states and territories through the public hospital system.

The comparator for financial implications therefore differs from the comparator used to benchmark the safety, effectiveness and cost-effectiveness of RET mutation testing. As RET mutation testing is a means of triaging biochemical screening and imaging (and a replacement for pentagastrin-stimulated calcitonin measurements) in patients suspected of having MEN2 and their close relatives, the comparator selected was biochemical screening and imaging *alone* for the diagnosis of MEN2. The screening and imaging investigations that patients receive depend on their presenting feature. For index patients, the comparator is outlined in the second column of Table 7 (page xxxvi) and Table 87, in Appendix I outlines the MBS items that correspond to these investigations.

There is no specific alternative test to determine individual *susceptibility* to MEN2. Without genetic testing the diagnosis of MEN2 would rely on tumour type and location, which is not possible to assess prospectively. However, close family members of someone with MEN2 would have lifelong surveillance to ensure early detection of disease. The surveillance regimen for those with MEN2 or at risk of MEN2 is outlined in Table 2 and the MBS items corresponding to these surveillance measures are given in Table 88 n Appendix I. The comparison for first-degree relatives (and second-degree relatives in a cascade fashion) is therefore between genetic counselling and RET mutation testing in addition to a prophylactic thyroidectomy, lifelong thyroxine and lifelong surveillance in those who carry a RET mutation, versus genetic counselling and lifelong surveillance (with a total thyroidectomy and lifelong thyroxine after a rise in calcitonin levels) for all at-risk relatives.

Table 2 Lifelong surveillance regimen for MEN2

Age	Surveillance
From 1–5 years	*Annual* General clinical examination Examination of thyroid (or thyroid bed if post-thyroidectomy) by neck ultrasound (and biopsy of any suspicious masses) Biochemical screen for phaeochromocytoma Screen for hyperparathyroidism (total and ionised serum calcium) ± calcitonin and carcinoembryonic antigen after surgery for MTC

Source: (Genetics Sub-committee of PSTC 2010)

Scientific basis of comparison

There were no studies identified, that met the inclusion criteria, on which to judge the safety of genetic testing for RET mutations. One historical controlled study (level III-3 interventional evidence) and 8 uncontrolled case series (level IV interventional evidence) reported on the safety of prophylactic thyroidectomies.

The diagnostic effectiveness of RET mutation testing was primarily determined by 9 historical controlled studies with a moderate to high risk of bias. These studies were supported by 171 uncontrolled case series (level IV interventional evidence and/or level IV diagnostic evidence) that provided uncontrolled data on the impact of RET mutation testing on health outcomes and/or diagnostic yield data.

Test performance could not be determined as no studies were identified that provided long-term clinical data that could be used as the reference standard for a MEN2 diagnosis.

One cohort study (level III-2 interventional evidence) was identified that assessed whether the expected change in management from RET mutation testing was associated with better health outcomes for individuals who underwent a prophylactic thyroidectomy.

Comparative safety

No studies were available that specifically reported on the safety of RET mutation testing or surveillance for MEN2. However, given the availability of RET mutation testing results in asymptomatic gene carriers being recommended to undergo prophylactic total thyroidectomy, rather than waiting for clinical signs of an MTC, the safety of prophylactic total thyroidectomy was assessed.

One historical controlled study (level III-3 interventional evidence) showed similar rates of mortality due to surgical complications in those who underwent surgery prior to knowledge of the link between RET mutation status and MEN2, versus those who underwent surgery knowing their RET mutation status (one death in each cohort). Twelve case series (level IV interventional evidence) reported on the rate of adverse events following total thyroidectomy. Transient hypoparathyroidism was reported in five patients (36.4%) in 4 of the 12 case series. Permanent hypoparathyroidism occurred in between 7.7% and 13.6% of patients from 4 of the 12 studies that reported adverse events after total thyroidectomy. Transient laryngeal nerve palsy was reported in between 4.5% and 5.9% of patients in 4 studies, and one case of permanent laryngeal nerve palsy was reported. Other complications included one case of arterial bleeding, one case of fluctuating thyroid hormone (at 1 year post-surgery) despite adequate compliance with thyroxine replacement, and one case of

permanent unilateral Horner's syndrome[2]. It is expected that the rate of surgical complications would be higher in those patients who undergo surgery at a later stage of disease, due to the more invasive surgery required to remove an MTC once the tumour has extended beyond the thyroid, although direct evidence was not available comparing the safety of prophylactic thyroid surgery against curative surgery.

Key results

There were no safety concerns (either physical or psychological) raised in any of the articles identified regarding RET mutation testing.

Overall conclusion with respect to comparative safety

RET mutation testing is a safe procedure for patients, involving a simple blood test. In those who are found to be RET mutation carriers, the treatment recommended is a prophylactic thyroidectomy to avoid the risk of developing an MTC. This procedure is associated with a risk of hypoparathyroidism and laryngeal nerve palsy, which is usually transient. The risk of adverse events with prophylactic surgery is likely to be lower than when patients are treated at a later disease stage.

Comparative effectiveness

Nine historical controlled studies (level III-3 interventional evidence) provided evidence showing that health outcomes are likely to be better for patients diagnosed with the addition of RET mutation testing.

Seven historical controlled studies reported on the incidence and severity of MTC in patients who underwent total thyroidectomy in the era prior to RET mutation testing compared with the era subsequent to the introduction of RET mutation testing. Those diagnosed and treated since RET mutation testing became available had almost half the risk of having an MTC at the time of surgery, compared with those whose treatment decisions were based on biochemical screening in the pre-RET mutation testing era (RR=0.53, 95% CI 0.32, 0.90). It is unknown whether any clinical benefit has occurred in index patients, or whether all the benefits found have been due to more effective management of family members.

One historical controlled study reported that age at diagnosis reduced for patients with MEN2A and FMTC between two surveys in Japan, one performed in 1996 (capturing data prior to the availability of RET mutation testing) and the other in 2002. Age at diagnosis in patients with MEN2B increased marginally, likely just through chance given the small

[2] Horner's syndrome is a combination of drooping of the eyelid and constriction of the pupil, with redness of the conjunctiva of the eye often present. It indicates a problem with the sympathetic nervous system.

sample; however, the MEN2B phenotype is more clearly diagnosed than the MEN2A, so genetic testing has probably had less impact on patients and their family members with or suspected of having MEN2B than MEN2A. Five additional historical controlled studies reported that the introduction of RET mutation testing allowed the age at time of total thyroidectomy to significantly reduce. One Australian study reported that the mean age decreased from 32 years to 16 years (Learoyd et al. 1997).

Both age at time of total thyroidectomy and severity of MTC are significant predictors of the risk of residual or recurrent disease (Schreinemakers et al. 2010). Six historical controlled studies reported a greatly reduced risk of persistence, recurrence or mortality in those who underwent total thyroidectomy with knowledge of their RET mutation status, compared with total thyroidectomy without this knowledge (RR=0.28, 95% CI 0.17, 0.45). However, this evidence is highly biased, as those in the historical cohort were followed up for longer time periods, allowing a greater chance of disease recurrence simply as a matter of time.

Assessment of individual components in an evidence linkage supported the conclusions based on direct evidence of the impact of testing on patient health outcomes. One historical controlled study and 3 case series reported instances of false positive results based on calcitonin levels, which led to patients either undergoing total thyroidectomy or being scheduled for surgery that was subsequently cancelled after a negative RET mutation status was identified. One single case of an individual free from RET mutations, in a family with known mutations, who had an MTC was noted (Halling et al. 1997). It is unknown whether this could be considered a false negative RET mutation test or a coincidental finding of a spontaneous MTC in a RET-mutation-negative family member of an FMTC kindred. Although a true comparison of accuracy was not able to be performed given the lack of long-term clinical follow-up data to use as a reference standard for MEN2 diagnosis, the limited evidence available would suggest that diagnoses made with the addition of RET mutation testing are likely to be more accurate than those made on the basis of biochemical screening. As the treatment option (thyroidectomy) is the same, irrespective of early or late identification of MEN2, and has proven effectiveness, it is unlikely that studies assessing the comparative effectiveness of thyroidectomy in an 'earlier (RET-mutation-tested)' versus 'later (non-RET-mutation-tested)' MEN2 diagnosed population are necessary or will be conducted.

Patients who are asymptomatic gene carriers are likely to undergo prophylactic total thyroidectomy on the basis of this knowledge. Prophylactic surgery is associated with having a lower stage of MTC disease at time of surgery, compared with surgery performed on the basis of calcitonin levels.

Overall, clinical management with the addition of RET mutation testing would appear to have superior effectiveness and at least non-inferior safety, compared with diagnosis and treatment of MEN2 without knowledge of RET mutation status.

Key results

There is evidence that RET mutation testing has allowed patients to undergo total thyroidectomy at an earlier age, and at an earlier stage of MTC disease, than before the introduction of RET mutation testing.

Key uncertainties

Both age and stage of disease at the time of surgery may be considered surrogate outcomes for survival. Longer term patient-relevant outcomes such as rates of mortality and disease recurrence were reported and were highly in favour of RET mutation testing; however, these results were confounded by different lengths of follow-up in the testing and non-testing study arms.

There is also a high risk of bias in the results due to the comparison against historical cohorts. This type of comparison means that it is unknown to what extent other factors might have influenced the results; for example, if significant advances in surgical methods or surveillance for features of MEN2 have occurred over the same time period as the introduction of RET mutation testing, it would be difficult to correctly attribute the clinical benefits.

Overall conclusion with respect to comparative clinical effectiveness

All the evidence regarding the comparative clinical effectiveness of RET mutation testing was at high risk of bias. This evidence suggests that the addition of RET mutation testing allows identification of patients at risk of MEN2 at a younger age, allowing prophylactic surgery to occur at a younger age and at a less advanced stage of MTC disease. As age and disease stage are predictors of MTC disease recurrence, it is probable that earlier identification will reduce the risk of disease recurrence in MEN2 patients. Assuming that the findings from the evidence base remain consistently in the same direction, even if the size of this effect is confounded by longer lengths of follow-up in the control arm and differences in patient care over time, the comparative clinical effectiveness of the addition of RET mutation testing would be superior to biochemical screening and imaging.

Economic evaluation

An economic evaluation was conducted for both (i) RET mutation testing in potential index cases—MTC or phaeochromocytoma under 50 years of age—and (ii) RET mutation testing in index cases and additional familial genetic testing in first- or second-degree relatives of identified RET-mutation-positive index cases.

As genetic testing is currently funded through state hospital budgets, an analysis of the comparison between existing clinical practice and funding arrangements against the proposed MBS listings would only identify a shift in the funding provider, but would not

identify whether or not the practice of RET mutation testing has economic merit. Therefore, the economic analysis undertaken for this assessment compares the proposed MBS listings for RET mutation testing against a hypothetical analysis of the historical scenario of medical surveillance before RET mutation testing was available.

In each case the model runs over 30 years and shows accumulated healthcare costs from a societal perspective, with discounting applied to both costs and outcomes (where applicable) at a rate of 5% per year.

With respect to the economic evaluation of genetic testing in potential index cases alone, a cost analysis (cost-minimisation) approach was used, as there is no evidence to suggest that health outcomes within the index case will be affected by genetic testing. The inputs into this model relate to the costs of genetic testing and monitoring (consultation, biochemical tests and imaging) for additional MEN2 symptoms. Resources used are based on the surveillance regimen described by the Genetics Subcommittee of the PSTC and current MBS fees (website, March 2013).

With respect to familial testing, a cost-utility analysis was undertaken, as the ability to identify RET-mutation-positive family members via testing allows for prophylactic thyroidectomy treatment and therefore both health costs and outcomes are affected. The inputs into this model relate to the costs of genetic testing, monitoring (biochemical/imaging etc.) and thyroidectomy (surgical, hospital and pharmaceutical). The health states, which are applicable to family members only, include: healthy (no surgery/surveillance); healthy (pre-surgery, with surveillance); healthy (no MTC) post-thyroidectomy (incorporating adverse effects of surgery); symptomatic MTC; and death. Health outcomes are measured as accumulated quality-adjusted life-years (QALYs). While a decrease in the rate of symptomatic MTC following thyroidectomy in patients receiving medical surveillance is associated with early identification of RET-mutation-positive patients through genetic testing, the quantification of this effect is uncertain and represents a major source of uncertainty in the model. Patient uptake rates with respect to both annual medical surveillance and genetic testing are also uncertain.

The cost-minimisation analysis of genetic testing in potential index cases demonstrates that cost savings occur within 5 years of testing. Over the course of 30 years, savings of approximately $535 per MTC patient tested, or $1,458 per phaeochromocytoma patient under 50 years of age tested, would be expected compared with a scenario where testing was not available.

With respect to the cost-utility analysis of genetic testing of potential index cases and family members of patients identified as RET-mutation-positive, the results indicate that availability of genetic testing 'dominates' (i.e. it results in both improved health outcomes and cost-savings), compared with the alternative scenario where testing is not available.

Sensitivity analyses suggest that the base-case economic conclusions are relatively robust.

With respect to diagnostic RET mutation testing in suspected index cases presenting with MTC, a net cost might be expected if i) high test costs ($1,150) are applied or ii) diagnostic yield increases substantially (i.e. testing only occurred in patients with suspected familial disease). With respect to diagnostic testing in suspected index cases presenting with phaeochromytoma, the costs of testing are most sensitive to test price.

The cost-utility model incorporating both diagnostic testing and familial screening is highly robust where the index cohort present with MTC. Adoption of RET mutation testing remained the dominant economic strategy (vs historical biochemical screening) across all analyses of alternative test price, diagnostic yield, uptake rates and relative risk (RR) below 0.97.

The cost-utility model incorporating both diagnostic testing of index cases presenting with phaeochromocytoma and predictive testing of their family members, was also relatively robust. Genetic testing remained the dominant economic strategy across alternative values of test price, and diagnostic yield. When uptake rates of testing or screening are reduced to 15% a relatively low ICER ($485/QALY) is obtained.

The base case estimate of RR is 0.25, however this is highly uncertain. In either model if the RR of MTC recurrence is increased to 1.0, then genetic testing has negative outcomes and is either dominated (resulting in neither health benefits nor savings) in the model where index patients present with MTC, or associated with a cost-saving of $4,721/QALY lost in the model where index patients present with phaeochromocytoma. However the assumption of zero clinical benefit may be considered unreasonable and not consistent with the available evidence. Where the index cohort present with MTC, any RR less than 0.97 results in genetic testing remaining dominant (gaining QALYs and saving money), and this applies to any RR less than 0.43 in the model where index cases present with phaeochromocytoma.

Key uncertainties

The lack of direct comparative evidence and the hypothetical nature of the economic comparisons mean that the actual quantification of both incremental costs and outcomes in the economic models are not expected to be particularly accurate. Furthermore, the model structure is simplistic and incorporates generalised assumptions that do not capture the distribution of patient age or risk profiles. For this reason the assumptions and inputs in the base case have been selected to be conservative with respect to the cost-effectiveness of RET. However, broad-ranging sensitivity analyses to nevertheless demonstrate that cost-effectiveness is maintained across a range of clinical scenarios.

Overall conclusion with respect to comparative cost-effectiveness

Despite the shortcomings of the model, the robust nature of the findings—that RET mutation testing results in cost savings and health outcome benefits when model inputs are varied over a wide range of possibilities—is reassuring. On this basis the conclusion—that RET mutation testing and subsequent targeted surveillance (in comparison with broader and increased reliance on imaging/biochemical surveillance) *is cost-effective*—is reasonably certain.

Financial / budgetary impacts

Diagnostic RET mutation testing is estimated to occur in 130–260 patients in 2013, increasing to 147–294 in 2015. The estimate of the population suspected of having MEN2 is based on those diagnosed with MTC (approximately 5–10% of all thyroid cancers) (Keatts & Itano 2006). An annual increase in thyroid cancers (and MTCs) of 6.3% has been projected based on the average annual increase in thyroid cancer in Australia 2005-09. One diagnostic RET test is required per patient.

The likely number of eligible family members who elect to have RET screening tests is estimated to be 150–359 in 2013, increasing to 169–406 in 2015. One predictive RET mutation test would be required per eligible family member. These estimations are based on the following assumptions:

- Between 25% and 30% of diagnostic RET mutation tests identify a patient with a positive hereditary mutation (Raue & Frank-Raue 2010).
- Each index patient has 11.5 first- or second-degree relatives eligible for predictive RET mutation testing (Suthers et al. 2006).
- Of eligible relatives, 40% accept familial testing (Suthers et al. 2006); i.e., uptake of the test occurs in 4.6 family members per index case.

The extent of uptake in eligible family members is uncertain, with lower uptake (of one or two relatives per index patient) previously reported in the Australian context (Suthers 2008b). The effect of this uncertainty on the financial and budgetary impact is explored in sensitivity analyses.

The cost of diagnostic RET mutation testing used in the base-case estimates is $400, and $200 for familial RET screening. These costs are based on the median quote for RET mutation testing of the 6 exons most commonly examined (exons 10, 11 and 13–16) provided from the pathology laboratories currently providing this service, and are substantially lower than the price previously estimated in the DAP. The financial and budgetary impacts using the DAP-based costs are provided in Appendix K. It is assumed that all testing is provided in an outpatient setting and, as such, the MBS will cover 85% of the cost of the test. A patient contribution of 15% is applied.

The total estimated cost to the MBS, based on an estimated number of 130–260 diagnostic and 150–359 predictive RET mutation tests performed in 2013, is $109,654, increasing to $123,906 in 2015 based on 147–294 diagnostic and 169–406 screening RET mutation tests performed (Table 3). However, an unknown proportion of patients may qualify for the Medicare Safety Net, in which case 100% of the scheduled fee is paid by the MBS. Allowing for application of the Medicare Safety Net, the overall true costs to the Commonwealth health budget would lie between the total costs to the MBS and the total combined costs of RET mutation testing, i.e. up to $129,005 in 2013 and $145,772 in 2015.

A cost saving would be observed in the state and territory systems due to transfer of testing services to the MBS; however, the costs of genetic counselling services provided in hospitals would continue as per current arrangements.

Under the current arrangements some patients who are referred through the public system receive genetic counselling services and testing at no direct cost[3]. With the listing of RET mutation testing on the MBS, assuming that most patients would receive testing as outpatients, Medicare would pay 85% of the scheduled fee and a patient contribution of 15% would apply (in addition to any 'gap' charges or out-of-pocket expenses). Patients who may be eligible for the Medicare Safety Net, and those whose pathology service bulk-bills tests listed on the MBS, may not be required to contribute a co-payment.

Table 3 Total costs of RET mutation testing

Year	2013[a]	2014[a]	2015[a]
Diagnostic RET mutation testing			
Number of diagnostic RET mutation tests[b]	130–260	138–277	147–294
Estimated expenditure on diagnostic RET mutation testing[c]	$52,071–$104,141	$55,351–$110,702	$58,838–$117,676
Patient co-payment[d]	$7,811–$15,621	$8,303–$16,605	$8,826–$17,651
Estimated MBS expenditure[e]	$44,260–$88,520	$47,048–$94,097	$50,012–$100,025
Familial (predictive) RET mutation testing			
Relatives eligible for testing	374–898	398–955	423–1,015
Number of relatives tested	150–359	159–382	169–406
Estimated expenditure on predictive RET mutation testing[h]	$29,941–$71,857	$31,827–$76,384	$33,832–$81,197
Patient co-payment[d]	$4,491–$10,779	$4,774– $11,458	$5,075–$12,179
Estimated MBS expenditure[e]	$25,450–$61,079	$27,053–$64,927	$28,757–$69,017
Total combined cost of RET mutation testing[i]	$129,005	$137,132	$145,772
Lower limit	$82,011	$87,178	$92,670

[3] NSW Government, Centre for Genetics Education (2013). Family Cancer Services. http://www.genetics.edu.au/Genetics-Services/family-cancer-services#SAFCC (cited 28/02/13; last updated 07/02/13)

Year	2013[a]	2014[a]	2015[a]
Upper limit	$175,999	$187,087	$198,873
Total patient co-payment[d]	$19,351	$20,570	$21,866
Lower limit	$12,302	$13,077	$13,901
Upper limit	$26,400	$28,063	$29,831
Total cost to the MBS[e]	$109,654	$116,562	$123,906
Lower limit	$69,710	$74,101	$78,770
Upper limit	$149,599	$159,024	$169,042

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] estimated based on a 5–10% incidence of medullary thyroid cancer in all thyroid cancers

[c] assuming that the cost of the diagnostic RET mutation test is $400 (see Table 6 on page xxxiv)

[d] assuming that most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[e] assuming that all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees, with no allowance for additional MBS if some patients qualify for the Medicare Safety Net

[f] estimated based on the identification of a positive hereditary mutation in the *RET* gene in 25–30% of tests performed; each patient was assumed to have, on average, 11.5 first- or second-degree relatives eligible for familial screening

[g] assuming an uptake rate of 40% in eligible family members

[h] assuming that the cost of the predictive RET mutation test is $200 (see Table 6 on page xxxiv)

[l] assuming that all patients qualify for the Medicare Safety Net, then the total cost to the MBS would equate to the total combined cost of RET mutation testing

Sensitivity analyses assuming upper estimates around disease incidence and a 100% uptake rate of familial screening were undertaken to provide an extreme upper limit of the predictable financial costs. The estimated cost of RET mutation testing to the MBS under these limits increases to $272,568 in 2015.

The proposed MBS item descriptors require that appropriate genetic counselling be provided to the patient prior to diagnostic testing or familial screening; further counselling may be required upon receipt of the test results. Genetic counselling services have not been accounted for in the financial and budgetary estimates, as the current distribution of counselling services is unlikely to change, with little impact expected to the overall health budget, MBS, and state and territory systems.

Listing RET mutation testing on the MBS is not expected to have any impact on the costs of the overall Australian healthcare system considered in its entirety. The practice of genetic testing and counselling is routine in diagnostic and familial screening of patients in a manner unchanged by the proposed listing and at a similar cost, which is currently borne by state government hospital budgets.

Other relevant factors

Clinical trials comparing the health outcomes of patients diagnosed with the addition of RET mutation testing, versus diagnosis without RET mutation testing, would now be considered unethical, as RET mutation testing has become standard clinical practice for patients

suspected of having MEN2. Although the evidence identified is at risk of bias, studies controlling for confounding factors are highly unlikely to now be performed.

Glossary and abbreviations

AHTA	Adelaide Health Technology Assessment
DAP	decision analytic protocol
DTC	direct-to-consumer
FMTC	familial medullary thyroid cancer
HESP	Health Expert Standing Panel
IMVS	Institute of Medical and Veterinary Science
IVD	*in-vitro* diagnostic (medical device)
MBS	Medicare Benefits Schedule
MEN2	multiple endocrine neoplasia type 2
MSAC	Medical Services Advisory Committee
MTC	medullary thyroid carcinoma
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
PaLMS	Pacific Laboratory Medicine Services
PASC	Protocol Advisory Sub-Committee
PCR	polymerase chain reaction
PICO	population, intervention, comparator and outcomes
PSTC	Pathology Services Table Committee
QALYs	quality-adjusted life-years
RCPA	Royal College of Pathologists of Australasia
RET	rearranged during transfection
RET M+	RET-mutation-positive
RET M–	RET-mutation-negative
RR	relative risk
TGA	Therapeutic Goods Administration

Introduction

A rigorous assessment of evidence is the basis of decision-making when funding is sought under Medicare.

The Medical Services Advisory Committee (MSAC) evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule (MBS), in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. The MSAC adopts an evidence-based approach to its assessments, based on reviews of the scientific literature and other information sources, including clinical expertise.

A team from Adelaide Health Technology Assessment (AHTA), School of Population Health, University of Adelaide, as part of its contract with the Department of Health and Ageing, was engaged to conduct a systematic review of the literature on RET (rearranged during transfection) mutation testing and to conduct economic modelling in order to inform the MSAC's decision-making regarding public funding of the intervention. A decision analytic protocol (DAP) was developed before commencement of the assessment and was approved by the Protocol Advisory Sub-Committee (PASC) of MSAC. AHTA sought input and advice from members of a Health Expert Standing Panel (HESP; see Appendix A), who are clinicians with expertise in the field.

This report summarises and critically appraises current evidence on RET mutation testing in (i) patients with symptoms of multiple endocrine neoplasia type 2 (MEN2) and (ii) unaffected relatives of a patient with a documented RET mutation to determine the risk of disease, in order to draw conclusions on the likely safety, effectiveness and cost-effectiveness of this testing in the event it is funded by Medicare.

Rationale for assessment

In October 2010 an application was received from the Pathology Services Table Committee (PSTC) by the Department of Health and Ageing requesting an MBS listing of genetic testing for mutations in the *RET* gene for (i) patients with symptoms of MEN2 and (ii) unaffected relatives of a patient with a documented RET mutation to determine the risk of disease. It was proposed that two new MBS items are created to cover the use of diagnostic and predictive testing for mutations in the *RET* gene.

Background

Mutation testing of the *RET* gene

The *RET* proto-onco-gene encodes a transmembrane receptor tyrosine kinase involved in processes such as neural crest differentiation, and cell migration and proliferation (Burzynski et al. 2005). Mutations in the *RET* gene are associated with MEN2 (MEN2A and B), familial medullary thyroid cancer (FMTC) and the seemingly unrelated syndrome concerning the congenital absence of the enteric ganglia (Hirschsprung's disease[4]). RET mutations that are causative of MEN2 are called *gain in function* mutations as they cause ligand-independent RET activation and constitutive cell signalling (Margraf et al. 2009).

MEN2 comprises a group of disorders that are associated with tumours of the endocrine system (generally the thyroid, parathyroid and adrenals) (Table 4). It includes three distinct phenotypes, the features of which are also outlined in Table 4. Nearly all patients develop a medullary thyroid carcinoma (MTC) and half of patients with MEN2A or MEN2B develop phaeochromocytomas (Margraf et al. 2009). Of patients with MEN2A, 15–30% may also develop hyperparathyroidism, whereas patients with MEN2B are not at risk of parathyroid disease but will show other abnormalities such as ganglioneuromas, medullated corneal nerves and marfanoid body habitus (Eng 1999). FMTC comprises families who only have MTC. However, some RET mutations are associated with both MEN2A and FMTC, so a clinical history is required to distinguish between the two conditions (Margraf et al. 2009).

As can be seen in Table 4, a clinical diagnosis of MEN2 would only be given once a minimum of two features are identified in a family, as a means of distinguishing this inheritable disease from sporadic MTC. Seventy-five per cent of cases of MTC are sporadic and the remainder are hereditary (i.e. MEN2A, MEN2B or FMTC) (Wells Jr & Santoro 2009). In the absence of genetic testing, patients with an MTC would be considered to *potentially* have MEN2 and would consequently, along with their first-degree family members, be recommended to undergo annual surveillance for additional clinical features of MEN2[5].

[4] The initial application proposed the use of genetic testing of the *RET* gene for patients suspected of having MEN2 or in those diagnosed with Hirschsprung's disease. However, no clinical benefit of using RET testing in Hirschsprung's disease could be determined through scoping searches of the literature or consultation with clinical experts (HESP members or through public consultation), as there was no ambiguity in the clinical presentation of Hirschsprung's patients, and so RET testing would be redundant. PASC decided that the assessment of RET testing should not include Hirschsprung's disease as an indication.

[5] HESP member and endocrinologist, R Clifton-Bligh, email received on 12 July 2011

Table 4 MEN2 phenotype definitions

Gene	Phenotype	Codon	Clinical characteristics	Risk	Timing of thyroidectomy
RET	MEN2A (55% of all cases)	634 611 618 620 630 631	Family (or individual) with MTC, and at least one individual developing hyperparathyroidism, phaeochromocytoma, or both.	2 (higher aggressiveness)	5 years of age
	MEN2B (5–10% of all cases)	918 883	MTC (with or without phaeochromocytoma) and characteristic clinical features: mucosal ganglioneuromas, gastrointestinal ganglioneuromas, eye abnormalities including corneal nerve thickening, and skeletal abnormalities including marfanoid body habitus.	3 (highest aggressiveness)	1st year of life
	FMTC (35–40% of all cases)	609 791 790 804 649 891 768	Four or more family members with MTC only. No clinical evidence of phaeochromocytoma, hyperparathyroidism, or any MEN2B-specific clinical features in affected or at-risk family members.	1 (high aggressiveness)	When calcitonin rises / 5–10 years of age

Source: (International *RET* Mutation Consortium 2006; Raue & Frank-Raue 2009, 2010); MTC = medullary thyroid carcinoma; FMTC = familial MTC

Most cases of MEN2 are caused by mutations on the *RET* proto-oncogene (over 98% of MEN2 families have known RET mutations) (Margraf et al. 2009). Furthermore, over 90% of people who have a RET mutation will develop MEN2 (Toledo et al. 2006). MEN2 is autosomal dominant, which means that offspring with one affected parent have a 50% chance of having MEN2 themselves. RET mutation testing is used as a means of *diagnosing* MEN2 in those with symptoms (distinguishing between those who have MEN2 and those who have the more common sporadic form of MTC), and as a way of *predicting* which family members will develop MEN2 based on whether they carry the pathogenic mutation of the *RET* gene. Given that specific genotype–phenotype relationships have become evident, the type of specific mutation found may also be used to determine the age at which a prophylactic thyroidectomy should be performed (Raue & Frank-Raue 2009).

Intended purpose

Proposed MBS listing

Based on the populations expected to benefit from RET mutation testing (those with clinical features of MEN2 or their family members), the proposed MBS items are suggested as:

1. A diagnostic test to detect germline mutations in the *RET* gene
2. A predictive test to detect mutations in the *RET* gene of family members of a proband.

In a diagnostic setting, the use of RET mutation testing would constitute Level 1 testing as defined by the National Pathology Accreditation Advisory Council, and would therefore not require formal pre-test genetic counselling or written consent (NPAAC 2007). However, expert opinion suggests that *all* patients undergoing RET mutation testing should participate in genetic counselling[6]. Predictive testing in unaffected relatives would constitute Level 2 testing, and therefore would need to be restricted to services which can provide accredited genetic counselling (NPAAC 2007).

Diagnosis of a pathogenic RET mutation in first-degree relatives of a proband allows for cascade testing of *their* first-degree relatives (i.e. second-degree relatives of the proband). Rather than restrict the proposed MBS item to first-degree relatives, the item has been worded to allow for situations where first-degree relatives are unavailable (e.g. have died), so second-degree relatives may be tested.

It is a requirement that all patients undergoing predictive testing should first receive genetic counselling and give informed consent (or assent in the case of children). It is also recommended that patients undergoing diagnostic genetic testing should undergo genetic counselling. It is therefore suggested that the ordering of the genetic test for RET mutations should be limited to specialised genetic services that can provide accredited genetic counselling to patients and their family members.

The proposed MBS items are summarised in Table 5. The suggested fees are only indicative and are substantially different from those originally proposed in the final DAP, which were $1150 for the diagnostic test and $480 for the predictive test. The propsed fees listed in Table 5 are based on updated information on the current pricing of RET mutation testing in Australia (Table 6).

[6] HESP member and endocrinologist, R Clifton-Bligh, email received on 22 June 2011

Table 5 Proposed MBS item descriptor for RET mutation testing

Category 6 – Pathology services
MBS [item number] Detection of germline mutations in the *RET* gene in patients with: (a) medullary thyroid carcinoma (b) adrenal phaeochromocytoma under the age of 50 years (c) hyperparathyroidism plus a diagnosis of medullary thyroid cancer or phaeochromocytoma in a close relative[a] 1 or more tests Fee: $400 Prior to ordering these tests the ordering practitioner should ensure that the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.
MBS [item number] Detection of a known mutation in the *RET* gene in: (a) asymptomatic first- or second-degree relatives, at genetic risk, of a patient with a documented pathogenic RET mutation 1 or more tests Fee: $200 Prior to ordering these tests the ordering practitioner should ensure that the patient (or their parent/guardian in the case of children) has given informed consent. Testing can only be performed after genetic counselling. Appropriate genetic counselling should be provided to the patient either by the treating practitioner, a genetic counselling service or a clinical geneticist on referral. Further counselling may be necessary upon receipt of the test results.

[a] It has been suggested by the Protocol Advisory Sub-Committee (PASC) that the definition of a 'close relative' be limited to first- or second-degree family members.

Screening of RET exons 10, 11 and 13–16 was initially considered as likely to identify all common known MEN2-associated mutations; however, increasing reports of mutations on exons 5 and 8 have led to a suggestion that these exons should be included as 'common' potential mutation sites and included in the routine screening of MTC patients (Elisei et al. 2012). When seeking test descriptions and costs from Australian pathology providers, it became apparent that broader screening is not common practice in Australia, although no data are available to confirm this. A full RET gene screen is only suggested where there is a strong suggestion of familial disease but no mutation is identified on any of the previously mentioned exons. Only one Australian laboratory indicated that they were readily able to undertake such testing.

MSAC may wish to consider whether the proposed listing of RET mutation testing for diagnostic purposes in potential index cases is intended to screen for the common mutations currently routinely tested for in Australia, or whether an increased—or even complete—gene screen is intended to be covered by the listing. Clearly, the number of exons tested will affect the proposed fee. While 6-exon (exons 10, 11 and 13–16) RET mutation testing is

assumed in the base-case analysis in the economic model, sensitivity analysis of a higher cost for more extensive testing is also included.

Table 6 Current fees for RET mutation testing in Australia (descriptions and prices from varying individual laboratories)

Test description (from source)	Price
Single mutation predictive testing	
RET gene predictive testing (one exon)	$200
MEN2A specific mutation (predictive)	$200
RET known mutation screen	$250
Diagnostic testing	
RET (common mutations; exons 10, 11 and 13–16)	$400
MEN2A full screen (exons 10, 11 and 13–16)	$400
RET (common mutations) URGENT (20% surcharge)	$480
RET gene: full screen (exons 10, 11 and 13–16)	$600
RET gene screen (complete)	$1,500.00

Source: Prices provided by the Institute of Medical and Veterinary Science (03/2013); Pacific Laboratory Medicine Services (published on internet and confirmed 02/2013); Peter MacCallum Cancer Centre (02/2013).

Clinical need

It is estimated that the prevalence of MEN2 is 2.5 per 100,000 in the general population (Raue & Frank-Raue 2010). In a population of 22.6 million people (ABS 2011), it is therefore estimated that 500–600 Australians would have this rare disorder.

The best estimate of the population suspected of having MEN2 are those who are diagnosed with MTC. In 2007 there were 456 males and 1,331 females newly diagnosed with thyroid carcinomas (AIHW 2010). Approximately 5–10% of thyroid carcinomas are medullary (Keatts & Itano 2006), so it is estimated that, of the 1,787 thyroid carcinomas diagnosed, 89–179 of them would be medullary. In 2007 there were 150 diagnostic tests performed on the *RET* gene in Australia (Suthers 2008b). This is within the range of what would be expected given the estimated rate of MTCs diagnosed. It is not expected that having item numbers on the MBS to allow reimbursement for RET mutation testing would significantly impact on the number of genetic tests being performed, given that testing is already considered standard practice in Australia[7] and 'best practice' internationally (Brandi et al. 2001).

Only 25–30% of MTCs are hereditary (Raue & Frank-Raue 2010), so the use of the genetic test in the proband would rule out the need for further familial genetic testing in 65–70% of cases. It is therefore expected that 22–54 Australian patients per year would have MTC caused by MEN2, resulting in their first-degree relatives requiring genetic screening. Based

[7] HESP member and endocrinologist, R Clifton-Bligh, email received on 20 June 2011

on data from the Familial Cancer Unit in South Australia, there are approximately 11.5 unaffected first- or second-degree relatives per proband (Suthers et al. 2006). In a study assessing uptake of genetic screening, when family members were contacted both by the proband and directly by letter from the Familial Cancer Unit, 40% undertook genetic screening within 2 years (Suthers et al. 2006). It is therefore estimated that, on average, 4.6 family members per proband would agree to predictive genetic testing. In 2007 there were 49 presymptomatic tests performed on the *RET* gene in Australia (Suthers 2008b), which is below the rate of what would be expected, assuming that more than one relative per proband would be tested. It is therefore estimated that having an item number on the MBS for detection of a known mutation in the *RET* gene in first- or second-degree relatives at genetic risk would likely increase the number of presymptomatic tests to approximately 101–248 per year.

Existing procedures for the diagnosis of MEN2 and screening for associated neoplasms

In the absence of genetic testing, patients with clinical features suggestive of MEN2 were investigated for other features of MEN2 as well as for hereditary disorders that may cause the features detected (Genetics Sub-committee of PSTC 2010). Those with early onset adrenal phaeochromocytoma or hyperparathyroidism (in combination with FMTC or phaeochromocytoma) would have been investigated for an MTC by pentagastrin-stimulated serum calcitonin and neck ultrasound.

In the current clinical setting with RET mutation testing as standard practice, it occurs as a triage to further investigations. If patients are found to have pathological RET mutations, they would be investigated for further MEN2 features before receiving a total thyroidectomy. However, if patients are found to have no pathological RET mutations, they would either be assumed to have a spontaneous MTC or hyperparathyroidism, or would be investigated for other hereditary disorders associated with phaeochromocytoma.

Table 7 summarises the investigations and management for MEN2 and other hereditary disorders that would be in use, for both patients presenting with disease and their close family members, in the settings with or without RET mutation testing.

Table 7 Co-administered and associated investigations and management

Clinical feature	Historical setting (RET mutation testing not available)[a]	Current setting (RET mutation testing available)
Medullary thyroid carcinoma	• Investigate for phaeochromocytoma: plasma or urine catecholamines (and adrenal imaging – e.g. adrenal CT scan or MRI and/or MIBG scan – if these are elevated) • Investigate for hyperparathyroidism: serum calcium (and parathyroid hormone if calcium is elevated) to assess for	• Investigate for MEN2 with RET mutation testing *Use RET mutation testing to triage further investigations.* Those with RET mutations: • Investigate for phaeochromocytoma: plasma or urine catecholamines (and adrenal

Clinical feature	Historical setting (RET mutation testing not available)[a]	Current setting (RET mutation testing available)
	hyperparathyroidism. • Total thyroidectomy, lifelong thyroxine and surveillance for all	imaging – e.g. adrenal CT scan or MRI and/or MIBG scan – if these are elevated) • Investigate for hyperparathyroidism: serum calcium (and parathyroid hormone if calcium is elevated) to assess for hyperparathyroidism. Those without RET mutations: • No further investigations required • Total thyroidectomy and lifelong thyroxine
Adrenal phaeochromocytoma (under 50 years of age)	• Investigate for MTC: pentagastrin-stimulated calcitonin and neck ultrasound • Investigate for hyperparathyroidism: serum calcium (and parathyroid hormone if elevated) Those with an MTC: • Total thyroidectomy, lifelong thyroxine and surveillance Those without an MTC: • Investigate for other hereditary disorders: genetic testing of the VHL gene for von Hippel Lindau disease; genetic testing for SDHB, SDHC and SDHD mutations; if serum calcium and parathyroid hormone are elevated, then assess for MEN (serum gastrin, insulin, glucagon, pancreatic polypeptide, vasoactive intestinal peptide, calcium, prolactin, adrenocorticotrophic hormone, growth hormone and somatomedin C)	• Investigate for MEN2 with RET mutation testing *Use RET mutation testing to triage further investigations* Those with RET mutations: • Investigate for hyperparathyroidism: serum calcium (and parathyroid hormone if elevated) • Prophylactic total thyroidectomy, lifelong thyroxine and surveillance Those without RET mutations: • Investigate for other hereditary disorders: genetic testing of the VHL gene for von Hippel Lindau disease; genetic testing for SDHB, SDHC and SDHD mutations; if serum calcium and parathyroid hormone are elevated, then assess for MEN (serum gastrin, insulin, glucagon, pancreatic polypeptide, vasoactive intestinal peptide, calcium, prolactin, adrenocorticotrophic hormone, growth hormone, and somatomedin C)
Hyperparathyroidism (plus a diagnosis of MTC or phaeochromocytoma in a close relative)	• Investigate for MTC: pentagastrin-stimulated calcitonin and neck ultrasound • Investigate for phaeochromocytoma: plasma or urine catecholamines (and adrenal imaging, e.g. adrenal CT scan or MRI and/or MIBG scan if elevated) Those with an MTC: • Total thyroidectomy, lifelong thyroxine and surveillance Those without an MTC: • No further investigations required	• Investigate for MEN2 with RET mutation testing *Use RET mutation testing to triage further investigations* Those with RET mutations: • Investigate for phaeochromocytoma: plasma or urine catecholamines (and adrenal imaging, e.g. adrenal CT scan or MRI and/or MIBG scan if elevated) • Prophylactic total thyroidectomy, lifelong thyroxine and surveillance Those without RET mutations: • No further investigations required
First-degree relatives of patients with a known pathogenic RET mutation	Genetic counselling plus lifelong surveillance Total thyroidectomy once calcitonin levels rise, or on the basis of biopsy of a thyroid nodule detected clinically or on ultrasound, followed by lifelong thyroxine	Genetic counselling plus genetic testing for known RET mutation In those with RET mutation: • Prophylactic total thyroidectomy, lifelong thyroxine and surveillance In those without RET mutation: • No further follow-up

[a] The comparative situation is termed 'historical' to illustrate a scenario that existed without the use of RET mutation testing. However, of interest is what tests *would be used currently in the absence of RET mutation testing*. Therefore, genetic testing for other diseases has been included in the comparative setting, despite not being available prior to the introduction of RET mutation testing.

CT = computed tomography; MIBG = meta-iodobenzylguanidine scintigraphy; MRI = magnetic resonance imaging; MTC = medullary thyroid carcinoma; SDHB = succinate dehydrogenase complex subunit B; SDHC = succinate dehydrogenase complex subunit C; SDHD = succinate dehydrogenase complex subunit D.

Patients with MEN2, those thought to have MEN2, and people at risk of developing MEN2 require lifelong surveillance. The surveillance tests offered are listed in Table 8.

In Australian practice the risk assessment of family members is already predominantly done by genetic testing of the *RET* gene (Fleming 2011). The listing of RET mutation testing on the MBS is therefore unlikely to have much impact on the use of current surveillance regimens. However, for the purposes of evaluating the cost-effectiveness of RET mutation testing, the historic clinical setting will be assessed—whereby genetic testing is not available and there is a reliance on annual surveillance for patients with an MTC and all close family members.

Prior to the introduction of RET mutation testing, decisions regarding the requirement for lifelong screening in asymptomatic family members were made based on family history. All first-degree relatives of a patient with an MTC would have been recommended to undergo lifelong surveillance. If a first-degree family member developed clinical features, their first-degree relatives (ie second-degree relatives of the proband) would then undergo annual surveillance using the principles of cascade testing.

With the introduction of RET mutation testing, the frequency of initial biochemical screening and imaging and surveillance within family members is likely to have changed for a variety of reasons:

(i) a better distinction between sporadic and hereditary cases of MTC (i.e. a more accurate diagnosis of the index case), such that fewer index cases require lifelong surveillance for MEN2

(ii) greater certainty regarding an individual's risk of developing MEN2, due to knowledge of the presence/absence of the RET mutation and understanding of the biomarker, facilitating better compliance with surveillance recommendations in those with a mutation

(iii) a better distinction between spontaneous and hereditary cases of MTC in the index case, and therefore that fewer families require screening and surveillance

(iv) the ability to distinguish between first-degree relatives who are, and who are not, at risk of developing MEN2 on the basis of RET mutations. Only those first-degree family members who have an RET mutation would undergo surveillance for clinical features of

MEN2, while those who are free from pathogenic RET mutations would avoid the need for lifelong surveillance. Thus, the genetic test would replace (and has replaced to date) routine lifelong screening of family members without RET mutations, resulting in fewer people undergoing annual surveillance

(v) earlier screening in second-degree relatives (and possibly broader) on the basis of genetic mutations in first-degree relatives, rather than waiting for the emergence of clinical features. Once a first-degree family member is found to have a RET mutation, their first-degree relatives may be genetically tested (using cascade testing). Although similar in principle to clinical practice before the introduction of genetic testing, identification of a RET mutation may occur years before the presentation of clinical features. It is therefore likely that a proportion of additional second-degree (and more distant) relatives are currently undergoing annual surveillance much earlier than they would have been before the introduction of RET mutation testing.

Points (i), (iii) and (iv) above are likely to have greatly reduced the number of index cases and family members recommended to undergo annual surveillance, while points (ii) and (v) are likely to have resulted in a slight increase in surveillance utilisation in specific populations.

Table 8 Lifelong surveillance regimen for MEN2

Age	Surveillance
From 1–5 years	*Annual* General clinical examination Examination of thyroid (or thyroid bed if post-thyroidectomy) by neck ultrasound (and biopsy of any suspicious masses) Biochemical screen for phaeochromocytoma Screen for hyperparathyroidism (total and ionised serum calcium) ± calcitonin and carcinoembryonic antigen after surgery for MTC

Source: (Genetics Sub-committee of PSTC 2010); MTC = medullary thyroid carcinoma

Treatment for MEN2 will depend on the presentation. Standard chemotherapy regimens and radiation therapy have been found to be ineffective methods of treating MTC, so treatment depends largely on the adequacy of surgical removal of the thyroid (Brandi et al. 2001). MTCs are treated with total thyroidectomy and central lymph node dissection at a minimum (Brandi et al. 2001; Lundgren et al. 2007). Following thyroidectomy, patients are required to take thyroxine over the remainder of their life. The primary treatment for phaeochromocytoma is resection, most often performed laparoscopically (Alderazi et al. 2005). The current treatment for hyperparathyroidism is parathyroidectomy, with the timing based on the degree of hypercalcaemia and/or associated clinical features (e.g. cognitive symptoms, renal stones, osteoporosis)[8]. Bisphosphonate treatment may be used in milder

[8] HESP member and endocrinologist, R Clifton-Bligh, email received on 20 June 2011

degrees of hypercalcaemia when patients show osteoporosis on bone densitometry testing[8]. It is not expected that treatment for those diagnosed with MEN2 who have an MTC at presentation will change with greater use of genetic testing. However, those who have an MTC who are found not to have a RET mutation (and therefore do not have MEN2) can avoid the need for lifelong surveillance for additional features of MEN2.

It is expected that those who are diagnosed with MEN2 prior to the development of an MTC (i.e. through initial presentation with early onset phaeochromocytoma) would receive a prophylactic thyroidectomy upon confirmation of having a RET mutation. As the penetrance of RET mutations in MEN2 patients is close to 100% (i.e. nearly all who have a mutation on the *RET* gene will eventually develop an MTC), the ideal treatment for MEN2 is a total thyroidectomy before clinical evidence of an MTC, to reduce disease-related morbidity and death (Margraf et al. 2009). MEN2A was the first disease in history where total removal of an organ was performed prior to development of a carcinoma solely on the basis of genetic testing (Lundgren et al. 2007). Since testing for RET mutations has been introduced, fewer patients have been diagnosed with metastatic MTC. RET mutation testing has resulted in fewer patients needing to undergo more extensive surgery such as a lymphadenectomy, and has replaced the need for palliation in some patients.

Treatment of family members who are found to have a RET mutation also involves a prophylactic total thyroidectomy (Brandi et al. 2001). Historically, pentagastrin-stimulated serum calcitonin measurements were used to identify those who had developed an MTC. In the absence of genetic testing, removal of the thyroid would only occur once calcitonin levels had risen, indicating the presence of a carcinoma. The present strategy of performing prophylactic thyroidectomy would therefore have resulted in an increased need for thyroxine in the years between when a prophylactic total thyroidectomy is performed and when that individual would have developed an MTC had prophylactic surgery not been performed.

Marketing status of technology

Regulatory status

In-vitro diagnostic medical devices (IVDs) are, in general, pathology tests and related instrumentation used to carry out testing on human samples, where the results are intended to assist in clinical diagnosis or in making decisions concerning clinical management (Therapeutic Goods Administration 2009).

The Therapeutic Goods Administration (TGA) regulatory framework for IVDs changed in July 2010, such that in-house laboratory tests now receive the same level of regulatory scrutiny as commercial kits. As RET mutation testing is currently only provided in-house, it would be classified as a Class 3 in-house IVD (Box 1). There is a transition period of 4 years to allow currently supplied goods to be included under the new regulation (IVD Australia 2010).

Laboratories that manufacture in-house Class 3 IVDs are required to notify the TGA of the types of IVDs manufactured in each laboratory for inclusion on a register. These laboratories must have National Association of Testing Authorities (NATA) accreditation, with demonstrated compliance with the suite of standards on the validation of in-house IVDs, as published by the National Pathology Accreditation Advisory Council, for each test manufactured. Manufacturers of Class 2, Class 3 and Class 4 IVDs must hold certification from a regulatory body to show compliance with a suitable conformity assessment procedure (Therapeutic Goods Administration 2009). NATA accreditation includes (but is not limited to) the use of direct sequencing on both DNA strands in all exons in which pathogenic mutations have been identified; and confirmation of the mutation on an independent polymerase chain reaction (PCR)[9].

As the national demand for RET mutation testing is likely to be low, it is probable that it would be undertaken by a small number of laboratories to ensure that they have sufficient throughput to maintain training and procedural quality. The staffing required will depend on the caseload, throughput and infrastructure of the laboratories that provide testing.

[9] MESP member and endocrinologist, R Clifton-Bligh, email received on 20 June 2011

Box 1 Classification of Class 3 *in-vitro* diagnostic medical devices

Therapeutic Goods (Medical Devices) Regulations 2002 –Schedule 2A

1.3 Detection of transmissible agents or biological characteristics posing a moderate public health risk or high personal risk

1. An IVD is classified as a Class 3 IVD medical device or a Class 3 in-house IVD if it is intended for any of the following uses:
    a. detecting the presence of, or exposure to, a sexually transmitted agent;
    b. detecting the presence in cerebrospinal fluid or blood of an infectious agent with a risk of limited propagation;
    c. detecting the presence of an infectious agent where there is a significant risk that an erroneous result would cause death or severe disability to the individual or foetus being tested;
    d. pre-natal screening of women in order to determine their immune status towards transmissible agents;
    e. determining infective disease status or immune status where there is a risk that an erroneous result will lead to a patient management decision resulting in an imminent life-threatening situation for the patient;
    f. the selection of patients for selective therapy and management, or for disease staging, or in the diagnosis of cancer;
    g. human genetic testing;
    h. to monitor levels of medicines, substances or biological components, when there is a risk that an erroneous result will lead to a patient management decision resulting in an immediate life-threatening situation for the patient;
    i. the management of patients suffering from a life-threatening infectious disease;
    j. screening for congenital disorders in the foetus.

    Note: For paragraph (f) An IVD medical device would fall into Class 2 under clause 1.5 if:
    k. therapy decisions would usually be made only after further investigation; or
    l. the device is used for monitoring.
2. Despite subsection (1) an IVD is classified as a Class 3 IVD medical device or a Class 3 in-house IVD if it is used to test for transmissible agents included in the Australian National Notifiable Diseases Surveillance System (NNDSS) list as published from time to time by the Australian Government.

Source: Commonwealth Consolidated Regulations [accessed March 2013]

Current reimbursement arrangements

Currently, there is no MBS listing for any test that detects mutations on the *RET* gene. No previous applications have been made to MSAC for public funding of RET mutation testing.

Patients are therefore encouraged to have their blood sample collected through a public hospital so that the state health system covers the cost of genetic testing. In those instances when patients are referred by a private facility, they will be billed directly, as private health insurance generally provides a subsidy for testing only if the MBS also provides a rebate for the test (ALRC 2003; PaLMS 2011).

The genetic testing website of the Royal College of Pathologists of Australasia (RCPA) lists three pathology laboratories that offer RET mutation testing (RCPA 2012). The Cancer Genetics Diagnostic Laboratory, Pacific Laboratory Medicine Services (PaLMS), Royal North Shore Hospital, Sydney, New South Wales, and the Peter MacCallum Cancer Centre in Victoria offer PCR amplification and DNA sequencing of RET exons 10, 11 and 13–16, with a

4-week to 3-month turnaround for receipt of results. The Molecular Pathology Division of the Institute of Medical and Veterinary Science (IMVS), Adelaide, South Australia, offers a gene screen for all exons and associated splice junctions by direct sequencing, with a turnaround of 3 months (RCPA 2012).

The costs of RET mutation testing in Australia are summarised in Table 6 (page xxxiv).

Consumer impact statement

No responses were made during the public consultation period for the DAP.

Approach to assessment

A DAP was developed prior to the commencement of the assessment, was informed by clinical experts (Appendix A) and public consultation, and was approved by the PASC of MSAC. The purpose of a DAP is to describe in detail a limited set of decision options associated with the possible public funding of a proposed medical service. The DAP also accurately captures current clinical practice, reflects the likely future practice with the proposed medical service, and describes all potentially affected healthcare resources. The guiding framework of the DAP was used throughout this assessment.

Objective

The objective of this assessment was to determine whether there is sufficient evidence of clinical need, safety, effectiveness and cost-effectiveness to recommend the public funding of genetic testing for hereditary mutations in the *RET* gene for (i) patients with symptoms of MEN2, and (ii) a family member of a patient with a known pathogenic RET mutation.

Clinical pathway

RET mutation testing is current standard practice, and is funded by either the states and territories or by patients directly. For the purposes of the financial analysis, the comparison is RET mutation testing funded by the states and territories versus testing funded through the MBS. However, for the purposes of assessing the safety, effectiveness and cost-effectiveness of RET mutation testing, the comparison is the historical setting where RET mutation testing was not available. RET mutation testing has been used as a replacement for pentagastrin-stimulated calcitonin testing and as a triage for biochemical screening and imaging.

Two management algorithms are presented, one for patients presenting with an MTC plus their first degree relatives (Figure 3) and the second for patients presenting with phaeochromocytoma or hyperparathyroidism, and their first degree relatives (Figure 4). On the left side of both algorithms is the historical setting, showing which investigations would be used in the absence of RET mutation testing (i.e. although it is classified historical, it is a hypothetical situation including the use of other genetic tests that would be used in the absence of RET mutation testing today, but that were not used before the introduction of RET mutation testing). On the right side of both algorithms is the current scenario, with RET mutation testing being standard clinical practice. Historically, without RET mutation testing, all patients with MTC were treated as if they had MEN2, and all patients and first-degree relatives would require ongoing surveillance for features of MEN2. Conversely, in the absence of RET mutation testing, all patients presenting with phaeochromocytoma or hyperparathyroidism would have been assumed *not* to have MEN2 unless an MTC was

present. Figure 3 is the more common algorithm, given the higher penetrance of MTC than phaeochromocytoma or hyperparathyroidism.

RET mutation testing is used to triage patients to further biochemical investigations and surveillance, and is a replacement for pentagastrin-stimulatedcalcitonin. Those who have pathological RET mutations associated with MEN2 would undergo a total thyroidectomy and receive lifelong thyroxine supplementation.

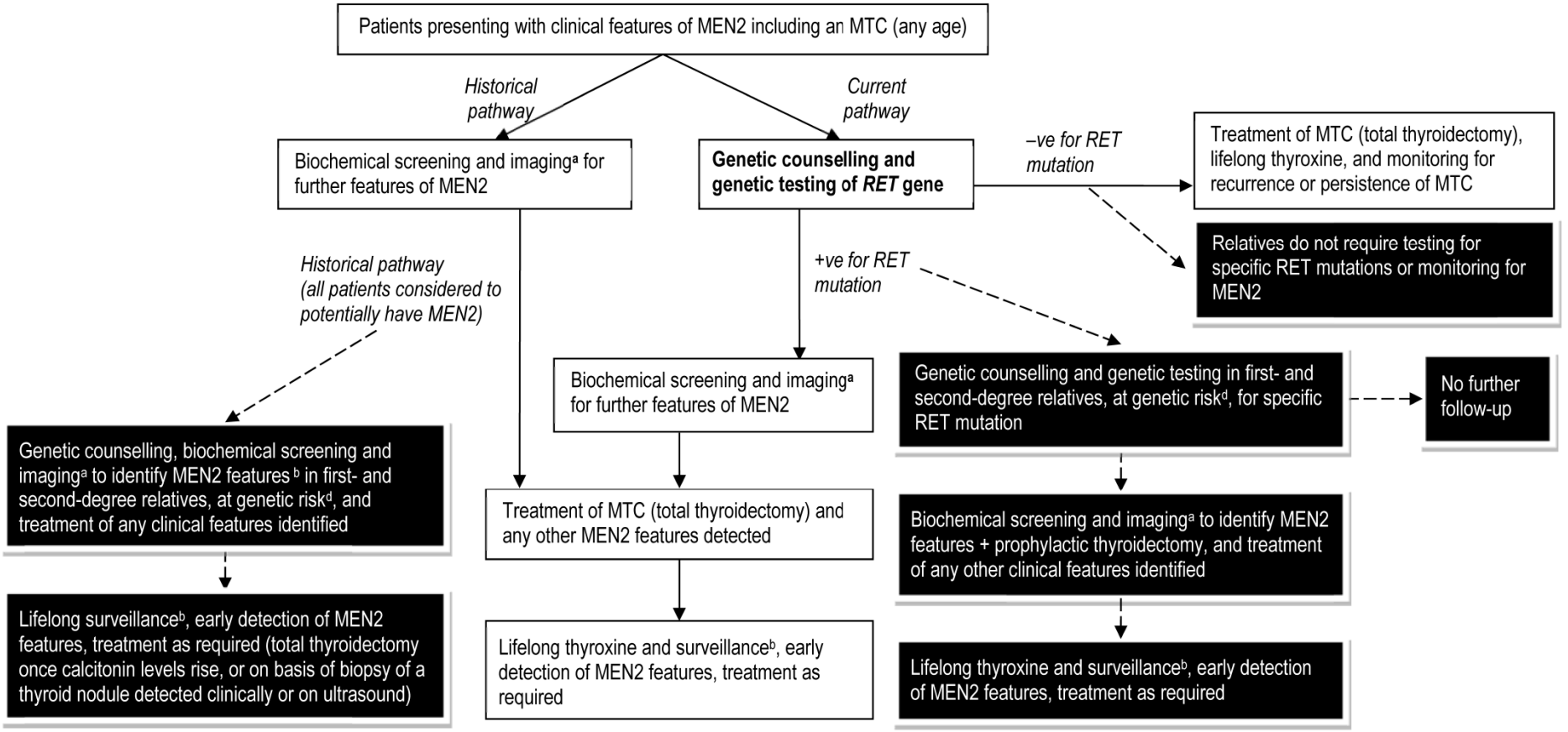

Figure 3 Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (MTC identified in index case prior to genetic testing)

[a] Biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma, serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism.

[b] Surveillance in those who have had a total thyroidectomy: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}) and calcitonin and carcinoembryonic antigen to detect persistence or recurrence of MTC.

[c] Historical surveillance in those at risk of MEN2 who have not had a total thyroidectomy: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}); pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC.

[d] Second-degree relatives would only be considered to be at genetic risk if first-degree relatives have a RET mutation, clinical features of MEN2, or if information regarding first-degree relatives is unavailable.

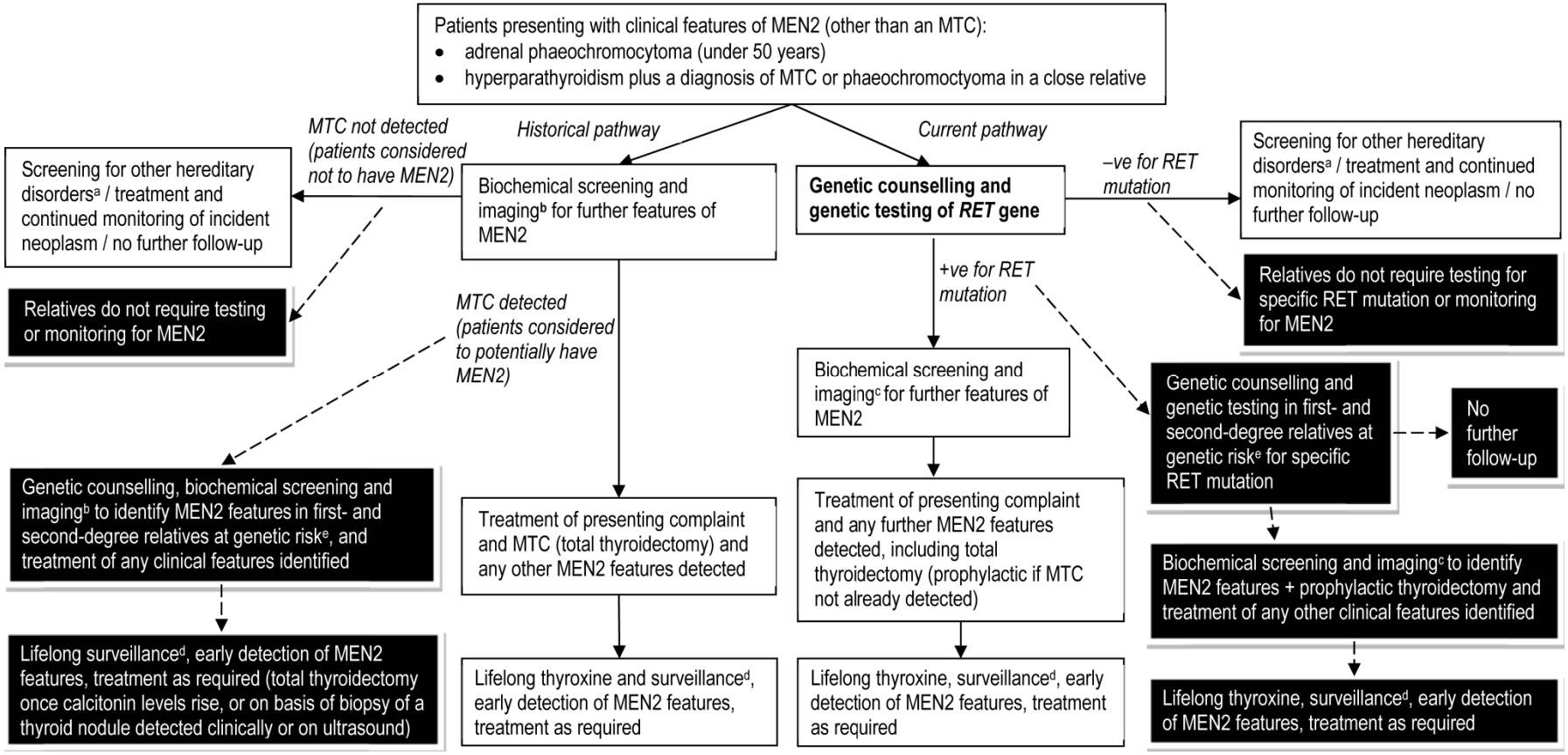

Figure 4 Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (no MTC in index case prior to genetic testing)

[a] Screening for other hereditary disorders: genetic testing of the VHL gene for von Hippel Lindau disease, genetic testing for SDHB, SDHC and SDHD mutations; if serum calcium and parathyroid hormone are elevated then additional testing for features of MEN1 (serum gastrin, serum insulin, serum glucagon, serum pancreatic polypeptide, serum vasoactive intestinal peptide, serum prolactin, growth hormone and adrenocorticotrophic hormone).

[b] Historical biochemical screening and imaging for further features of MEN2: pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC; plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical feature, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting clinical features.

[c] Current biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical features, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting feature.

[d] Historical surveillance: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}); plus pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for an MTC or calcitonin and carcinoembryonic antigen after surgery for MTC.
Current surveillance: annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca^{2+}) ± calcitonin and carcinoembryonic antigen after surgery for MTC.

[e] Second-degree relatives would only be considered to be at genetic risk if first-degree relatives have a RET mutation, or if information regarding first-degree relatives is unavailable.

Comparator

For the sake of determining the safety, effectiveness and cost-effectiveness of RET mutation testing and subsequent investigations and treatments, a comparison was made against investigations and timing of treatments that would occur without the use of RET mutation testing. The investigations would vary depending on the presenting feature leading to patients being suspected of having MEN2. Table 7 (page xxxvi) outlines the investigations and management strategies that are used with RET mutation testing and without RET mutation testing in index cases and asymptomatic close family members.

Questions for public funding

In order to clearly articulate the policy question to publicly fund RET mutation testing, details on the appropriate population, intervention, comparator and outcomes (PICO) of interest were sought from the PASC. The PICO approach outlines aspects of the following clinical questions that the assessment is intended to answer:

- Population – specification of the characteristics of the population or patients in whom the intervention is intended to be used;
- Intervention – specification of the proposed intervention;
- Comparator – specification of the therapy most likely to be replaced by, or added to, the proposed intervention; and
- Outcomes – specification of the health outcomes and the healthcare resources likely to be affected by the introduction of the proposed intervention.

Table 9 describes the PICO that were used to develop the clinical questions addressed by this evaluation. The questions were:

1. Is RET mutation testing safe, effective and cost-effective, in the diagnosis of patients suspected of having MEN2, when used to replace or triage the investigation of further features of MEN2 or other hereditary disorders, and to determine who should receive a prophylactic total thyroidectomy and/or lifelong surveillance?

2. Is RET mutation testing safe, effective and cost-effective, in first- and second-degree family members of patients diagnosed with MEN2, when used to determine who should receive prophylactic total thyroidectomy and/or lifelong surveillance?

Table 9 Summary of PICO to define clinical questions

Patients	Intervention	Comparator	Reference standard	Outcomes to be assessed
Patients presenting with clinical features of MEN2: • MTC (any age)	RET mutation testing *In those with RET mutation*: clinical investigations for phaeochromoctyoma and hyperparathyroidism, plus total thyroidectomy, lifelong thyroxine and surveillance *In those without RET mutation*: total thyroidectomy and lifelong thyroxine	Clinical investigations for phaeochromoctyoma and hyperparathyroidism plus total thyroidectomy, lifelong thyroxine and surveillance for all	Long-term clinical assessment	<u>Safety</u> Psychological and physical harms from testing <u>Effectiveness</u> *Direct evidence* Primary outcomes: Mortality Progression-free survival Quality of life Incidence and severity (TNM stage) of MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism Secondary outcomes: Incidence of symptoms arising from MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism Timing of thyroidectomy Age at diagnosis Rates and implications (physical and psychological) of surveillance <u>Cost-effectiveness</u> Cost, cost per event avoided, cost per life-year gained, cost per quality-adjusted life-year or disability-adjusted life-year, incremental cost-effectiveness ratio
Patients presenting with clinical features of MEN2: • adrenal phaeochromocytoma (under 50 years of age)	RET mutation testing *In those with RET mutation*: clinical investigations for hyperparathyroidism, plus prophylactic total thyroidectomy, lifelong thyroxine and surveillance *In those without RET mutation*: investigations for other hereditary disorders	Clinical investigations for MTC and hyperparathyroidism *In those with an MTC:* total thyroidectomy, lifelong thyroxine and surveillance *In those without an MTC:* investigations for other hereditary disorders		
Patients presenting with clinical features of MEN2: • hyperparathyroidism plus a diagnosis of MTC or phaeochromoctyoma in a close relative	RET mutation testing *In those with RET mutation*: clinical investigations for phaeochromoctyoma, plus prophylactic total thyroidectomy, lifelong thyroxine and surveillance *In those without RET mutation*: no further investigations	Clinical investigations for MTC and phaeochromoctyoma *In those with an MTC:* total thyroidectomy, lifelong thyroxine and surveillance *In those without an MTC:* no further investigations		
First-degree relatives of patients with a diagnosis of MEN2 or a known pathogenic RET mutation	Genetic counselling plus genetic testing for known RET mutation *In those with RET mutation*: prophylactic total thyroidectomy, lifelong thyroxine and surveillance *In those without RET mutation:* no further follow-up	Genetic counselling plus lifelong surveillance Total thyroidectomy once calcitonin levels rise, or on the basis of biopsy of a thyroid nodule detected clinically or on ultrasound, followed by lifelong thyroxine		

MTC = medullary thyroid cancer; TNM Classification of Malignant Tumours is a cancer staging system whereby T describes the size of the primary tumour, N the involvement of regional lymph nodes, and M the presence of distant metastasis.

Diagnostic assessment framework

This assessment of RET mutation testing is based on the framework outlined in the MSAC *Guidelines for the Assessment of Diagnostic Technologies* (MSAC 2005).

The effectiveness of a diagnostic or predictive test depends on whether it improves patient health outcomes. The clinical benefit can be assessed by studies that directly investigate the impact of the test on health outcomes or, alternatively, in some situations by linking evidence from different studies within the diagnostic or predictive pathway.

Direct evidence

In a very simplified manner, comparative **direct evidence** would present data on patients suspected (due to signs/symptoms) or at risk (due to family history) of having MEN2. These people would receive either clinical screening alone or RET mutation testing. Both study arms would have patients undergo further investigations and treatments on the basis of the test results (Figure 5). If one study arm was better at identifying MEN2 and appropriately targeting treatment than the other study arm, this would be reflected in a difference in the health outcomes between the patient groups.

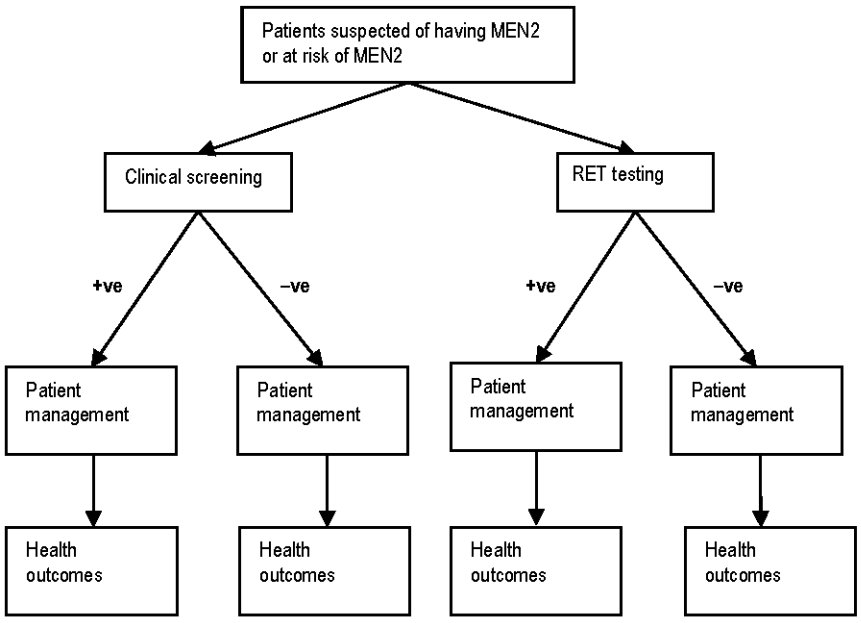

Figure 5 Ideal structure of comparative direct diagnostic evidence

Linked evidence

Scoping literature searches indicated that the available direct evidence was limited, so a **linked evidence** approach was used to supplement the evidence base. This included assessing evidence on test performance/diagnostic accuracy, identifying studies addressing

changes in clinical management as a consequence of RET mutation testing, and evaluating the likely treatment effectiveness in people found to have a *RET* gene mutation.

The questions addressing each linkage are given as follows:

Linkage 1 – Test accuracy

1. Is genetic testing for mutations in the *RET* gene, to triage further clinical investigations, as accurate as, or more accurate than, usual clinical diagnosis to identify patients with MEN2?

2. Is genetic testing for mutations in the *RET* gene, plus annual screening, as accurate as, or more accurate than, annual screening to diagnose relatives of patients with a known RET mutation?

The reference standard

The reference standard is long-term clinical assessment (ideally over the life-time of the patient). However, if a person is identified as having a pathological RET mutation associated with MEN2 prior to the development of an MTC, they would be strongly encouraged to have a prophylactic thyroidectomy to remove the possibility of developing an MTC. After removing the thyroid it is not possible to determine whether the individual would have developed an MTC or not (unless histopathological evidence shows evidence of microscopic disease), so the reference standard is imperfect and would potentially classify many people as receiving a false positive diagnosis by RET mutation testing.

Furthermore, the distinction between a spontaneous MTC and a familial MTC (as occurs in MEN2) is not always able to be made on the basis of clinical information when assessing the individual alone (Table 4, page xxxii). An individual with an MTC may have a *de novo* germline RET mutation but still not meet the classification of MEN2 or FMTC, as they will not have developed hyperparathyroidism or phaeochromocytoma, nor have sufficient family members with clinical signs of disease, to be classified as having MEN2.

Linkage 2 – Change in patient management

It was assumed that there is a change in patient management based on results of RET mutation testing, as those patients and relatives who have a RET mutation, but do not yet have an MTC, are likely to undergo a prophylactic thyroidectomy (and receive lifelong thyroxine). In the absence of RET mutation testing, this would only occur after clinical signs of disease are found (i.e. raised calcitonin levels). All patients who have an MTC would be assumed to potentially have MEN2 and receive lifelong screening accordingly. This would also occur when a RET mutation is confirmed. However, in patients where a pathological RET mutation is not found, screening for additional features of MEN2 would not be required.

In the absence of RET mutation testing, family members of index cases with an MTC would undergo annual screening for features of MEN2. With the addition of RET mutation testing, only those family members who are RET-mutation-positive would require screening.

Although these changes to clinical management are clear from expert opinion, relevant literature would also be collated to answer the following questions:

3. Does genetic testing for mutations in the *RET* gene, in addition to usual diagnostic assessment, change the management of patients suspected of MEN2 when compared with usual diagnostic assessment alone?

4. Does genetic testing for mutations in the *RET* gene, to triage annual screening and prophylactic thyroidectomy, change the management of relatives of patients with a known RET mutation when compared with usual clinical surveillance?

Linkage 3 – Likely impact of change in patient management from RET mutation testing on patient health outcomes

The key difference in clinical management that may impact on patient health outcomes related to RET mutation testing is the use of prophylactic surgery to remove the thyroid in those at risk of developing an MTC due to a RET mutation. Therefore, the key question regarding patient health outcomes concerns the benefit of prophylactic surgery versus surgery upon clinical confirmation of development of an MTC. The research question was therefore:

5. In patients or family members with a RET mutation, is a prophylactic thyroidectomy and replacement thyroxine as safe and effective as a thryoidectomy and replacement thyroxine performed after calcitonin levels rise, or on the basis of biopsy of a thyroid nodule detected either clinically or on ultrasound?

Review of literature

Literature sources and search strategies

The medical literature was searched to identify relevant studies and reviews for the period between 1993 and July–August 2012. Searches were conducted via the electronic databases listed in Table 10.

Table 10 Electronic databases searched

Database	Period covered
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	1993 – August 2012

Database	Period covered
Web of Science – Science Citation Index Expanded	1993 – July 2012
Current Contents	1993 – July 2012
Embase.com (including Embase and Medline)	1993 – July 2012
PubMed	1993 – July 2012
CINAHL	1993 – August 2012
EconLit	1993 – July 2012

The search strategy used on the Medline platform through PubMed was as follows:

(("Proto-Oncogene Proteins c-ret/analysis"[Mesh] OR "Proto-Oncogene Proteins c-ret/genetics"[Mesh] OR "Proto-Oncogene Proteins c-ret/physiology"[Mesh]) OR "ret"[All Fields]) AND (MEN2[All Fields] OR "Multiple Endocrine Neoplasia"[All Fields])

Other databases were searched with similar text words and medical subject headings that were relevant to the database.

Selection criteria

Direct evidence

Criteria were pre-specified to determine eligible studies to address the main research questions concerning the diagnostic safety and effectiveness of RET mutation testing. These are outlined in Table 11 and Table 12.

Table 11 Inclusion criteria for identification of studies relevant to assess the safety and effectiveness of RET mutation testing in those suspected of having MEN2

Characteristic	Criteria		
Study design	All study designs in the 'Intervention' column of Table 19 were included.		
Population	Patients suspected of having MEN2 due to:		
	An MTC	Adrenal phaeochromocytoma	Hyperparathyroidism plus a diagnosis of MTC or phaeochromocytoma in a close relative
Intervention/test	RET mutation testing plus clinical investigations for phaeochromocytoma and hyperparathyroidism in those RET M+	RET mutation testing plus clinical investigations for MTC and hyperparathyroidism in those RET M+, or investigations for other hereditary disorders in those RET M–	RET mutation testing plus clinical investigations for phaeochromocytoma

Characteristic	Criteria		
Comparator	Clinical investigations for phaeochromocytoma and hyperparathyroidism	Clinical investigations for MTC and hyperparathyroidism, and investigations for other hereditary disorders in those without an MTC	Clinical investigations for MTC and phaeochromocytoma
Outcome	Safety—psychological and physical harms from genetic testing and clinical screening Effectiveness— Primary outcomes: mortality, progression-free survival, quality of life, incidence and severity (TNM stage) of MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism Secondary outcomes: incidence of symptoms arising from MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism, timing of thyroidectomy, age at diagnosis, rates and implications (physical and psychological) of surveillance		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 12 Inclusion criteria for identification of studies relevant to assess the safety and effectiveness of RET mutation testing in close relatives

Characteristic	Criteria		
Study design	All study designs in the 'Intervention' column of Table 19 were included.		
Population	Close family members of:		
	Those with a pathological *RET* gene mutation	Those suspected of having MEN2 due to MTC	Those suspected of having MEN2 due to phaeochromocytoma or hyperparathyroidism
Intervention/test	RET mutation testing and lifelong surveillance for those who RET M+	RET mutation testing	RET mutation testing
Comparator	Clinical investigations and lifelong surveillance	Clinical investigations and lifelong surveillance	No surveillance for family members of index cases without MTC or RET mutation
Outcome	Safety—psychological and physical harms from genetic testing and clinical screening Effectiveness — Primary outcomes: mortality, progression-free survival, quality of life, incidence and severity (TNM stage) of MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism Secondary outcomes: incidence of symptoms arising from MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism, timing of thyroidectomy, age at diagnosis, rates and implications (physical and psychological) of surveillance		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Linked evidence

In the absence of strong direct evidence, a linked evidence approach was also attempted, where evidence of diagnostic accuracy and change in clinical management and treatment effectiveness are linked to provide an assessment of the effectiveness of RET mutation testing in those suspected of having, or at risk of having, MEN2 disease.

The inclusion criteria for selecting studies for such an assessment are outlined in Table 13 to Table 17.

Table 13 Inclusion criteria for identification of studies relevant to assess the diagnostic accuracy of RET mutation testing in those suspected of having MEN2

Characteristic	Criteria		
Study design	All study designs in the 'Diagnostic accuracy' column of Table 19 were included.		
Population	Patients suspected of having MEN2 due to:		
	An MTC	Adrenal phaeochromocytoma	Hyperparathyroidism plus a diagnosis of MTC or phaeochromocytoma in a close relative
Intervention/test	RET mutation testing plus clinical investigations for phaeochromocytoma and hyperparathyroidism in those RET M+	RET mutation testing plus clinical investigations for MTC and hyperparathyroidism in those RET M+, or investigations for other hereditary disorders in those RET M–	RET mutation testing plus clinical investigations for phaeochromocytoma
Comparator	Clinical investigations for phaeochromocytoma and hyperparathyroidism	Clinical investigations for MTC and hyperparathyroidism, and investigations for other hereditary disorders in those without an MTC	Clinical investigations for MTC and phaeochromocytoma
Reference standard	Lifelong clinical assessment		
Outcome	Diagnostic accuracy outcomes: Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values, diagnostic odds ratios, receiver–operator characteristic curves, area under the curve, accuracy		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified.		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 14 Inclusion criteria for identification of studies relevant to assess the diagnostic accuracy of RET mutation testing in family members of those with a RET mutation or suspected of having MEN2

Characteristic	Criteria		
Study design	All study designs in the 'Diagnostic accuracy' column of Table 19 were included.		
Population	Close family members of:		
	Those with a pathological *RET* gene mutation	Those suspected of having MEN2 due to MTC	Those suspected of having MEN2 due to phaeochromocytoma or hyperparathyroidism
Intervention/test	RET mutation testing and lifelong surveillance for those who are mutation +	RET mutation testing	RET mutation testing
Comparator	Clinical investigations and lifelong surveillance	Clinical investigations and lifelong surveillance	No surveillance for family members of index cases without MTC or RET mutation
Reference standard	Lifelong clinical assessment		
Outcome	Diagnostic accuracy outcomes: Sensitivity and specificity (and therefore rates of false positives and negatives), positive and negative likelihood ratios, positive and negative predictive values, diagnostic odds ratios, receiver–operator characteristic curves, area under the curve, accuracy		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified.		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Table 15 Inclusion criteria for identification of studies relevant to assess a change in patient management as a result of RET mutation testing in those suspected of having MEN2

Characteristic	Criteria		
Study design	All study designs in the 'Intervention' column of Table 19 were included. In the event that large numbers of pre-test/post-test case series were identified, all would be reviewed but only those that were large case series and/or with long-term follow-up would have data extracted.		
Population	Patients suspected of having MEN2 due to:		
	An MTC	Adrenal phaeochromocytoma	Hyperparathyroidism plus a diagnosis of MTC or phaeochromocytoma in a close relative
Intervention/test	RET mutation testing plus clinical investigations for phaeochromocytoma and hyperparathyroidism in those RET M+	RET mutation testing plus clinical investigations for MTC and hyperparathyroidism in those RET M+, or investigations for other hereditary disorders in those RET M–	RET mutation testing plus clinical investigations for phaeochromocytoma
Comparator	Clinical investigations for phaeochromocytoma and hyperparathyroidism	Clinical investigations for MTC and hyperparathyroidism, and investigations for other hereditary disorders in those without an MTC	Clinical investigations for MTC and phaeochromocytoma
Outcome	Rates of treatment, method of treatment, rates of referral, type of referral, hospital separations and re-admissions, hospital length of stay		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified.		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 16 Inclusion criteria for identification of studies relevant to assess a change in patient management as a result of RET mutation testing in close family members of those with a known RET mutation or suspected of having MEN2

Characteristic	Criteria		
Study design	All study designs in the 'Intervention' column of Table 19 were included. In the event that large numbers of pre-test/post-test case series were identified, all would be reviewed but only those that were large case series and/or with long-term follow-up would have data extracted.		
Population	Close family members of:		
	Those with a pathological *RET* gene mutation	Those suspected of MEN2 with MTC	Those suspected of MEN2 due to phaeochromocytoma or hyperparathyroidism (without MTC)
Intervention/test	RET mutation testing and lifelong surveillance for those who are RET M+	RET mutation testing	RET mutation testing
Comparator	Clinical investigations and lifelong surveillance	Clinical investigations and lifelong surveillance	Assuming not MEN2, therefore no surveillance for family members of index cases without MTC or RET mutation
Outcome	Rates of treatment, method of treatment, rates of referral, type of referral, hospital separations and re-admissions, hospital length of stay		
Search period	1993 – July 2012		
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified.		

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Although RET mutation testing impacts on the management of a large proportion of both the index cases and their relatives, many of the changes are in relation to ruling out the need for surveillance strategies, rather than concerning different treatment strategies, and so would not alter patient health outcomes. The only change in clinical management that could impact on the health of patients is the effect of prophylactic total thyroidectomy (either in the index case presenting with features of MEN2 without MTC) and ongoing surveillance for an MTC, compared with the previous management involving a total thyroidectomy in response to rising levels of calcitonin or on the basis of biopsy results from a thyroid nodule detected clinically or through ultrasound.

Table 17 Inclusion criteria for identification of studies relevant to assess treatment effectiveness following a change in patient management as a result of RET mutation testing

Characteristic	Criteria
Study design	Level I, II and III-1 evidence listed in the Invention column of Table 19 were included. Should there be sufficient good-quality evidence available from higher level evidence for each type of treatment intervention, then lower level evidence would be reviewed but data would not be extracted.
Population	Index case: Patients with a pathogenic RET mutation who present with adrenal phaeochromocytoma or hyperparathyroidism, plus a diagnosis of MTC or phaeochromocytoma in a close relative, who do not have an MTC upon presentation Relatives: Family members with a pathological *RET* gene mutation
Intervention/test	Index case: Treatment for presenting symptom plus a prophylactic total thyroidectomy, lifelong thyroxine and surveillance Relatives: A prophylactic total thyroidectomy, lifelong thyroxine and surveillance
Comparator	Index case: Treatment for presenting symptom plus lifelong surveillance, and a total thyroidectomy once calcitonin levels rise, or on basis of biopsy of a thyroid nodule detected clinically or through ultrasound, plus lifelong thyroxine and surveillance Relatives: A total thyroidectomy once calcitonin levels rise, or on basis of biopsy of a thyroid nodule detected clinically or through ultrasound, plus lifelong thyroxine and surveillance
Outcome	*Effectiveness:* Primary outcomes: mortality, progression free survival, quality of life, incidence and severity (TNM stage) of MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism Secondary outcomes: incidence of symptoms arising from MTC, phaeochromocytoma, parathyroid hyperplasia/neoplasia and hyperparathyroidism, timing of thyroidectomy, age at diagnosis, rates and implications (physical and psychological) of surveillance *Safety:* Psychological and physical harms from testing
Search period	1993 – July 2012
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified.

MTC = medullary thyroid carcinoma; TNM Classification of Malignant Tumours is a cancer staging system, whereby T describes the size of the primary tumour, N the involvement of regional lymph nodes, and M the presence of distant metastasis

Search results

Figure 6 details the steps undertaken to select studies eligible for this assessment, following screening against the inclusion criteria. Studies were selected by one assessor and reviewed by another assessor when inclusion was in doubt. Final selection was a consensus decision.

Prisma flowchart

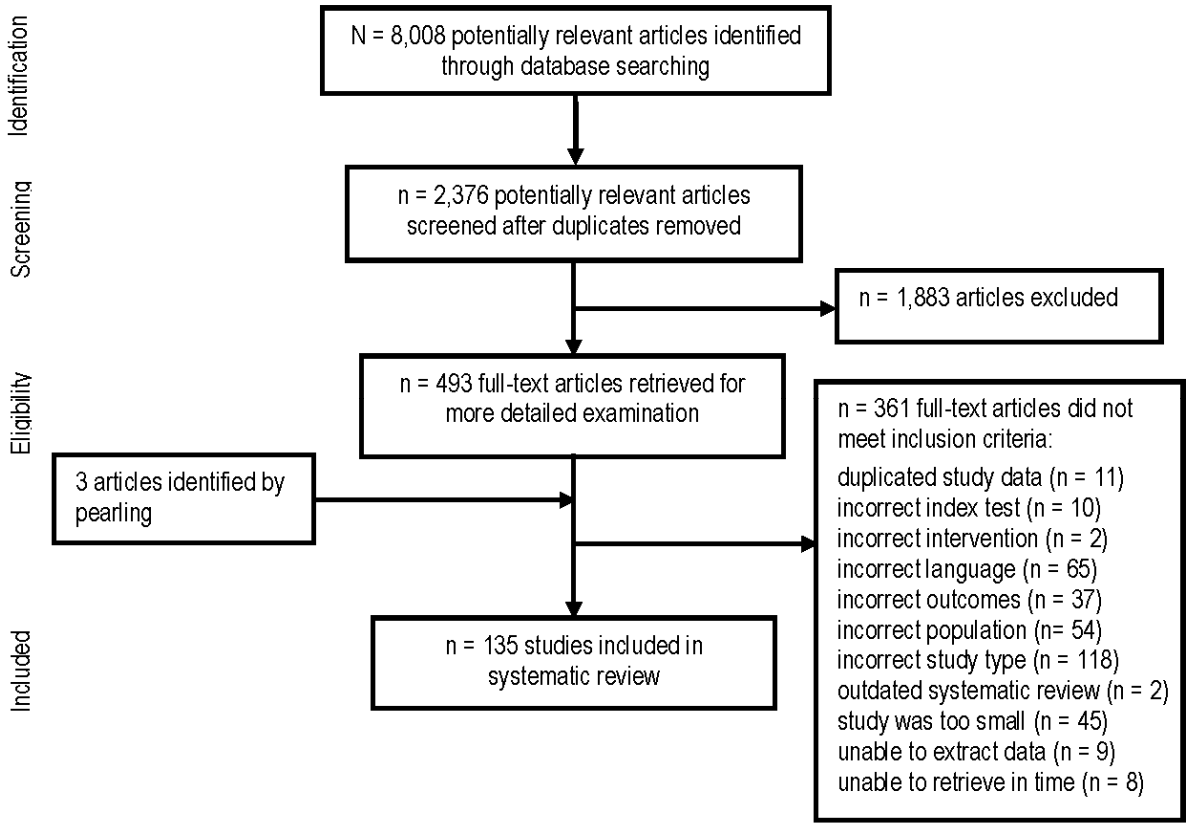

Figure 6 Summary of the process used to identify and select studies for the review
Source: adapted from (Liberati et al. 2009)

The study profiles of all included studies are shown in Table 97, to Table 100 in Appendix L. Full text articles that did not meet the inclusion criteria are listed in Appendix M according to the reason for exclusion.

Data extraction and analysis

Appraisal of the evidence

Studies included in the evidence base were appraised in three stages:

Stage 1: Appraisal of the applicability and quality (strength of the evidence) of individual studies included in the assessment.

Stage 2: Appraisal of the precision, size of effect and clinical importance of the results for primary outcomes in individual studies—used to determine the safety and effectiveness of RET mutation testing.

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of RET mutation testing in the context of Australian clinical practice.

Stage 1: Strength of the evidence

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC) (NHMRC, 2000).

These dimensions (Table 18) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention. The last two require expert clinical input as part of the determination.

Table 18 Evidence dimensions

Type of evidence	Definition
Strength of the evidence:	
Level	The study design used, as an indicator of the degree to which bias has been eliminated by design[a]
Quality	The methods used by investigators to minimise bias within a study design
Statistical precision	The *p*-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used

[a] See Table 19

The three sub-domains (level, quality and statistical precision) are collectively a measure of the strength of the evidence.

The 'level of evidence' reflects the effectiveness of a study design to answer a particular research question. Effectiveness is based on the probability that the design of the study has reduced or eliminated the impact of bias on the results. The NHMRC evidence hierarchy provides a ranking of various study designs ('levels of evidence') by the type of research question being addressed (Table 19).

Table 19 Designations of levels of evidence for interventions and studies of diagnostic accuracy (including table notes) (NHMRC 2008, 2009a)

Level	Intervention[a]	Diagnostic accuracy[b]
I[c]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d] among consecutive persons with a defined clinical presentation[6]
III-1	A pseudorandomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard[d] among non-consecutive persons with a defined clinical presentation[e]

Level	Intervention[a]	Diagnostic accuracy[b]
III-2	A comparative study with concurrent controls: ▪ non-randomised, experimental trial[f] ▪ cohort study ▪ case-control study ▪ interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for Level II and III-1 evidence
III-3	A comparative study without concurrent controls: ▪ historical controlled study ▪ two or more single arm study[g] ▪ interrupted time series without a parallel control group	A diagnostic case-control study[e]
IV	A case series with either post-test or pre-test/post-test outcomes	A study of diagnostic yield (no reference standard)[h]

Sources: (NHMRC 2008, 2009a)

Explanatory notes

[a] Definitions of these study designs are provided in (NHMRC 2000; pp. 7–8).

[b] These levels of evidence apply only to studies of assessing the accuracy of diagnostic or screening tests. To assess the overall effectiveness of a diagnostic test, there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett and Haynes 2002). The evidence hierarchy given in the 'Intervention' column should be used when assessing the impact of a diagnostic test on health outcomes relative to an existing method of diagnosis/comparator test(s). The evidence hierarchy given in the 'Screening' column should be used when assessing the impact of a screening test on health outcomes relative to no screening or alternative screening methods.

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity and thus are rated on the likelihood that the results have been affected by bias, rather than whether the systematic review itself is of good quality. Systematic review *quality* should be assessed separately. A systematic review should consist of at least 2 studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies (and study designs) might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al. 2003).

[e] Well-designed population-based case-control studies (e.g. population-based screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease is compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease, are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin and Miller 2002).

[f] This also includes controlled before-and-after (pre-test/post-test) studies, as well as adjusted indirect comparisons (i.e. using A vs. B and B vs. C to determine A vs. C with statistical adjustment for B).

[g] Comparing single arm studies, i.e. case series from 2 studies. This would also include unadjusted indirect comparisons (i.e. using A vs. B and B vs. C to determine A vs. C but where there is no statistical adjustment for B).

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternative when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed.

Some harms (and other outcomes) are rare and cannot feasibly be captured within randomised controlled trials, in which case lower levels of evidence may be the only type of evidence that is practically achievable; physical harms and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question, e.g. level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence.

Note C: Each individual study that is attributed a 'level of evidence' should be rigorously appraised using validated or commonly used checklists or appraisal tools to ensure that factors other than study design have not affected the validity of the results.

Source: Hierarchies adapted and modified from: (Bandolier 1999; Lijmer et al. 1999; NHMRC 2009a; Philips et al. 2001)

In terms of assessing the quality of the identified studies, it was planned that studies assessing test performance (diagnostic accuracy) would be graded according to pre-specified quality and applicability criteria using the QUADAS-2 tool (Whiting et al. 2011). However, no test performance studies were identified that compared RET mutation testing accuracy against the reference standard of lifelong clinical assessment. Many diagnostic yield studies were found, but these do not allow independent confirmation of a RET mutation that has been identified. Given the lack of comparator, these studies do not provide useful information on test performance and would universally rate poorly using the QUADAS-2 tool, and so their quality was not formally evaluated.

The appraisal of historical controlled studies and cohort studies was conducted using the modified checklist by Downs and Black (Downs & Black 1998). Studies were considered to have a high risk of bias if they scored ≤17, a moderate risk if they scored >18 and ≤21, and a low risk if they scored >22, out of 26.

The appraisal of uncontrolled before-and-after case series was assessed according to a checklist developed by the UK National Health Service (NHS) Centre for Reviews and Dissemination (Khan 2001). The six questions were scored 0–1 and summed to give an estimate of study quality: ≤2 = poor quality; > 2 & ≤ 4 = moderate quality; > 4 = high quality.

Stage 2: Precision, size of effect and clinical importance

Statistical precision was determined using statistical principles. Small confidence intervals and p-values give an indication as to the probability that the reported effect is real and not attributable to chance (NHMRC 2000). Studies needed to be appropriately powered to ensure that a real difference between groups would be detected in the statistical analysis. In the available direct evidence that compared RET mutation testing versus no testing, the size of the difference in effect (relative or absolute) and corresponding confidence intervals were inspected to determine whether the observed differences were clinically important.

Relevance of the evidence

The outcomes being measured in this report are clinically relevant, particularly for the direct evidence. However, the use of the supporting linked evidence approach means that clinically relevant outcomes could not be provided for some of the linkages (notably test performance). Inadequately validated (predictive) surrogate measures of a clinically relevant outcome should be avoided (NHMRC 2000).

Stage 3: Assessment of the body of evidence

Appraisal of the body of evidence (i.e. all the individual studies identified to address each clinical question) was conducted along the lines suggested by the NHMRC in their guidance on clinical practice guideline development (NHMRC 2008). Five components are considered essential by the NHMRC when judging the body of evidence:

1. the evidence base—which includes the number of studies sorted by their methodological quality
2. the consistency of the study results—whether the better quality studies had results of a similar magnitude and in the same direction, i.e. homogeneous or heterogeneous findings
3. the potential clinical impact—appraisal of the precision, size and clinical importance or relevance of the primary outcomes used to determine the safety and effectiveness of the test
4. the generalisability of the evidence to the target Medicare population
5. the applicability of the evidence—whether the studies delivered RET mutation testing in a similar manner to how it will be delivered in Australian clinical practice.

A matrix for assessing the body of evidence for each research question, according to the components above, was used for this assessment (Table 20) (NHMRC 2008).

Table 20 Body of evidence matrix

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]	One or more level I studies with a low risk of bias or several level II studies with a low risk of bias	One or two level II studies with a low risk of bias or an SR / several level III studies with a low risk of bias	One or two level III studies with a low risk of bias, or level I or II studies with a moderate risk of bias	Level IV studies, or level I to III studies / SRs with a high risk of bias
Consistency[b]	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population(s) studied in body of evidence are the same as the target population	Population(s) studied in the body of evidence are similar to the target population	Population(s) studied in the body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population[c]	Population(s) studied in the body of evidence differ to target population and it is hard to judge whether it is sensible to generalise to target population
Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

SR = systematic review; several = more than 2 studies

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer

Source: adapted from (NHMRC 2008)

After each of the five components in the matrix are rated, the overall assessment of the body of evidence is integrated and conclusions are drawn regarding the net clinical benefit of RET mutation testing in the context of Australian clinical practice.

Expert advice

The HESP has been established as a panel of MSAC and is a pool of experts collated from various medical fields who are nominated by their associated professional body or by applicants. HESP members are engaged to provide practical, professional advice to evaluators that directly relates to each application and the service being proposed for the MBS. HESP members are not members of either MSAC or its subcommittees—the Evaluation Sub-Committee (ESC) and the PASC. Their role is limited to providing input and guidance to the assessment groups to ensure that the pathway is clinically relevant and takes into account consumer interests. HESP members' advice is to inform the deliberations that MSAC presents to the Minister.

Results of assessment

Is it safe?

Summary of safety

No studies mentioned any safety concerns regarding RET mutation testing or surveillance for features of MEN2.

One historical controlled study (level III-3 interventional evidence) reported one death from surgical complications in the pre-RET mutation testing era, compared with one death from surgical complications after diagnosis by RET mutation testing, with similar numbers of patients treated in both scenarios (n=29 in pre-RET mutation testing era; n=31 in RET mutation testing era). No further details on the nature of these deaths or information on confounding factors were reported.

Twelve case series (level IV interventional evidence) reported on rates of adverse events due to total thyroidectomy, performed after RET mutation testing identified the patients as having (k=2), or being at risk of having (k=10), MEN2. Transient hypoparathyroidism was the most commonly reported adverse event, mentioned in 8 studies, with rates between 5.0% and 36.4%. Permanent hypoparathyroidism occurred in up to 13.6% of patients. Temporary laryngeal nerve palsy occurred in 4.5–5.9% of patients, and one case of permanent laryngeal nerve palsy was reported (1.3% of 1 study).

Other complications following total thyroidectomy were one case of arterial bleeding requiring re-operation, one case of permanent unilateral Horner's syndrome, and one paediatric case with fluctuating thyroid function test results despite good thyroxine replacement compliance at 1-year follow-up.

There were no studies identified that provided data on any psychological or physical harms from RET mutation testing plus clinical screening, as per the inclusion criteria outlined in Table 11 and Table 12. However, one of the consequences of the introduction of RET mutation testing was that more patients had prophylactic total thyroidectomies, prior to clinical evidence of disease, and this could result in harms. One historical controlled study (level III-3 interventional evidence) and 12 case series (level IV evidence) provided evidence on the rates of adverse events following total thyroidectomies. These studies are outlined in Table 21 and Table 25.

The most serious complication following total thyroidectomy was death, which was reported in two patients, one of whom was diagnosed with RET mutation testing, and the other was based on clinical testing (Table 21) (Diaz & Wohllk 2012). The specific surgical complications leading to these deaths were not reported. The surgical techniques employed with those who died from surgical complications, and the stage of disease of these patients, was not

mentioned. It is therefore unclear whether RET mutation testing affects the likelihood of surgical complications.

Table 21 Deaths following total thyroidectomy

Study and location	Level of evidence and quality assessment	Study population	Pre-RET mutation testing era	RET mutation testing era
(Diaz & Wohllk 2012) Chile	III-3 interventional evidence High risk of bias (9/26)	N=60 MEN2 patients who underwent total thyroidectomy	n=29	n=31
			13/29 (44.8%): 3 from hypertensive crisis 1 from surgical complications 9 from distant metastases	1/31 (3.2%): Died from surgical complications

Twelve case series reported on the rates of adverse events following RET mutation testing and subsequent total thyroidectomies (Table 22). The most common adverse event related to total thyroidectomy surgery was transient hypoparathyroidism or hypocalcaemia, requiring calcium substitution postoperatively. Eight studies mentioned this outcome, which occurred in 5.0–36.4% of patients (Table 22). Permanent hypoparathyroidism was identified in 4 studies, with between 5.9% and 13.6% of patients requiring ongoing calcium substitution (Dralle et al. 1998; Lau et al. 2009; Schellhaas et al. 2009; Spinelli et al. 2010).

Laryngeal nerve palsy was reported in 5 studies, with transient laryngeal nerve palsy reported in 4.5–5.9% of patients, and permanent laryngeal nerve palsy reported in one patient (1.3%) (Dralle et al. 1998; Gimm et al. 2002; Lau et al. 2009; Rodriguez Gonzalez et al. 2002; Schellhaas et al. 2009).

Three other complications were reported in individual patients. One patient had arterial bleeding that required re-operation the day after the thyroidectomy (Schellhaas et al. 2009), one patient developed permanent unilateral Horner's syndrome (Heizmann et al. 2006), and one paediatric patient had fluctuating thyroid function test results despite good thyroxine replacement compliance at 1-year follow-up (Lau et al. 2009).

It is unclear how these complication rates would compare with surgery performed after clinical signs of MTC are detected, and this cannot be determined given the non-comparative nature of the studies identified.

Table 22 Adverse events following total thyroidectomy

Study	Study design and quality appraisal	Population	Adverse events
In patients with MTC			
(Spinelli et al. 2010) Italy	IV interventional evidence Moderate quality	N=13 children with MEN2 who underwent surgery for MTC:	Permanent 1/13 (7.7%) developed postoperative permanent hypocalcaemia

Study	Study design and quality appraisal	Population	Adverse events
	(4/6)	7 (54%) MEN2A 4 (31%) FMTC 2 (15%) MEN2B	All other patients showed no sequelae related to surgery
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=5 members of an MTC family with a RET L790F mutation who had abnormal pentagastrin-stimulated calcitonin levels and a thyroidectomy (including index patient) 3 had clinical signs of disease	Transient 1/5 (20%) developed temporary hypoparathyroidism
In asymptomatic family members			
(Lau et al. 2009) Hong Kong	IV interventional evidence High quality (5/6)	N=22 asymptomatic relatives from 8 MEN2A families, who underwent prophylactic total thyroidectomy based on RET mutation status	Transient 8/22 (36.4%) temporary hypoparathyroidism requiring short-term calcium or vitamin D supplementation 1/17 (5.9%) transient recurrent nerve palsy (at follow-up, laryngeal nerve function described as normal) 1/17(5.9%) had arterial bleeding, requiring re-operation the day after thyroidectomy Permanent 3/22 (13.6%) had permanent hypoparathyroidism requiring oral calcium and vitamin D supplementation (median 49 months follow-up) 1/22 (4.5%) paediatric patient had fluctuating thyroid function test results despite good thyroxine replacement compliance (at 1-year follow-up)
(Wells Jr & Skinner 1998) USA	IV interventional evidence High quality (5/6)	N=18 RET M+ first-degree relatives from 7 MEN2A kindreds with no clinical symptoms of disease, who had a thyroidectomy	0/18 (0%) had surgical complications from total thyroidectomy
(Schellhaas et al. 2009) Germany	IV interventional evidence High quality (5/6)	N=17 patients with mutation in codon 634 operated on prophylactically (14 from MEN2A 3 with apparent familial MTC)	9/17 (52.9%) no perioperative complications Transient 5/17 (29.4%) temporary hypoparathyroidism (none required calcium or vitamin D supplementation at long-term follow-up) 1/17 (5.9%) transient recurrent nerve palsy (at follow-up, laryngeal nerve function described as normal) 1/17(5.9%) had arterial bleeding, requiring re-operation the day after thyroidectomy Permanent 1/17 (5.9%) had permanent hypoparathyroidism requiring oral calcium supplementation (15 years follow-up)
(Dralle et al. 1998) Germany	IV interventional evidence Moderate quality (4/6)	N=75 RET M+ patients <20 years of age who have undergone a prophylactic total thyroidectomy 57 underwent additional	Transient 20/75 (26.7%) transient hypoparathyroidism 4/75 (5.3%) laryngeal nerve palsy Permanent 5/75 (6.7%) hypoparathyroidism

Study	Study design and quality appraisal	Population	Adverse events
		lymph node dissections	3/75 (4%) persistent hypercalcitoninaemia 1/75 (1.3%) laryngeal nerve palsy
(Gimm et al. 2002) Germany, Austria	IV Interventional evidence Moderate quality (4/6)	N=40 patients with RET codon 790/791 mutations who underwent thyroid operations 22 thyroidectomy with cervicocentral lymph node dissection 8 thyroidectomy with lymph node dissection extending beyond cervicocentral compartment	Transient 2/40 (5%) transient hypoparathyroidism 2/40 (5%) laryngeal nerve palsy Permanent No permanent complications for any patients
(Heizmann et al. 2006) Switzerland	IV interventional evidence Moderate quality (4/6)	N=14 RET M+ patients who were presymptomatic, from 2 MEN2A kindreds	Transient All patients received oral calcium substitution for several days postoperatively 2/14 (14.3%) required intravenous calcium substitution for 2 days Permanent 1/14 (7.1%) had permanent unilateral Horner's syndrome (63-year-old) No recurrent nerve palsy or persistent hypocalcaemia
(Decker et al. 1996) USA	IV Interventional evidence Moderate quality (4/6)	N=11 RET M+ children who underwent prophylactic thyroidectomy	Transient 1/11(9.1%) children required transient calcium and vitamin D replacement postoperatively Permanent No wound or recurrent nerve complications
(Frank-Raue et al. 1996) Germany	IV Interventional evidence Moderate quality (4/6)	N=9 patients identified as RET M+ undergoing a prophylactic thyroidectomy	Permanent No recurrent laryngeal nerve damage or hypoparathyroidism
(Rodriguez Gonzalez et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=22 RET M+ asymptomatic MEN2A patients with normal basal and pentagastrin-stimulated calcitonin levels who received a prophylactic thyroidectomy	Transient 2/22 (9.1%) transitory hypoparathyroidism, treated with calcium for 1 and 3 months (both had central neck dissection) 1/22 (4.5%) with transitory unilateral recurrent nerve injury, which improved 1 month after operation with normal laryngoscopy
(Yoshida et al. 2009) Japan	IV interventional evidence Moderate quality (3/6)	N=12 adults who underwent total thyroidectomy for MTC and had MEN2	0/12 (0%) patients experienced morbidity after surgery

FMTC = familial medullary thyroid carcinoma; MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Is it effective?

Direct evidence

Summary of effectiveness

Nine historical controlled studies (level III-3 interventional studies), with a high risk of bias and confounding, reported on health outcomes in cohorts of patients who were diagnosed either prior to the introduction of RET mutation testing, or after the addition of RET mutation testing.

The studies consistently showed lower rates of disease persistence or recurrence after total thyroidectomy in RET-mutation-positive patients whose mutation status was known prior to surgery, compared with those whose mutation status was unknown when surgical decisions were being made. However, this outcome is confounded by the fact that the historical cohorts had longer follow-up periods than the recent cohorts, and the rates of persistence/recurrence were not adjusted for length of follow-up.

Those who were diagnosed with the addition of RET mutation testing had a lower risk of MTC at the time of undergoing total thyroidectomy than those who were treated before the availability of RET mutation testing. Lower severity of disease at the time of surgery allows less invasive surgical procedures to be performed. It is therefore expected that patient outcomes would be better since the introduction of RET mutation testing, although this could not be confirmed as unconfounded long-term data are not available.

Age at diagnosis was found to be lower in one historical controlled study (level III-3 interventional study) for patients with MEN2A and FMTC since the introduction of RET mutation testing.

Over 50 uncontrolled studies, many of small sample size, provided additional data on the outcomes mentioned above. Results were often heterogeneous because of the different populations and circumstances studied, but were largely consistent with the findings observed in patients receiving RET mutation testing in the historical controlled studies. The only exception was that mortality rates following RET mutation testing were higher in the uncontrolled studies than in the controlled study, but this was likely due to their longer lengths of follow-up. The mortality rates following RET mutation testing in the uncontrolled studies were still lower than the rates reported for the pre-RET mutation testing era in the historical controlled study.

The diagnostic effectiveness of RET mutation testing was assessed using studies meeting the inclusion criteria given in Table 11 and Table 12. Nine historical controlled studies were identified. The majority (8/9) were considered to be at high risk of bias, with 1 considered to have a moderate risk of bias. As those patients who were included in the comparator arms (pre-RET mutation testing) were from a different time period than those in the intervention arms (with RET mutation testing available), it is possible that the observed results were due to confounding factors, for example changes in surgical techniques or screening procedures over time. Another flaw in the included studies was that the health outcomes of those who were determined not to have MEN2 were often not reported. It is therefore possible that

cases of MEN2 were missed through the diagnostic and screening process, but no discussion of the clinically negative patients or those with wildtype RET were generally included.

Mortality

Seven studies reported on the rate of death following RET mutation testing and subsequent treatments, compared with a pre-RET mutation testing scenario (k=1 historical controlled study) or without a comparator arm (k=10 case series). The historical controlled study was in MEN2 patients who had undergone total thyroidectomy (Table 23). Of those patients diagnosed and treated without knowledge of their RET mutation status, 31% died from distant metastases, compared with no patients diagnosed since the use of RET mutation testing. This study had a high risk of bias, as the use of historical controls meant that length of follow-up was inevitably different between the two study arms. The length of follow-up was not stated but, as it was reported that RET mutation testing was initiated in 1997, the maximum follow-up in the study for those diagnosed with RET mutation testing would be 5 years. It is unknown at what stage of disease patients commonly had a total thyroidectomy in either scenario. While this study may be hypothesis-generating, the lack of details in the article and the high risk of bias inherent in the study design means that no strong conclusions can be made regarding the risk of mortality following RET mutation testing, compared with diagnosis without RET mutation testing.

Table 23 Comparative mortality in pre-RET mutation testing era and RET mutation testing era

Study and location	Level of evidence and quality of assessment	Study population	Pre-RET mutation testing era	RET mutation testing era
(Diaz & Wohllk 2012) Chile	III-3 interventional evidence High risk of bias (9/26)	N=60 MEN2 patients who underwent total thyroidectomy	n=29	n=31
			13/29 (44.8%): 3 from hypertensive crisis 1 from surgical complications 9 from distant metastases	1/31 (3.2%): Died from surgical complications

Ten case series reported on deaths after RET mutation testing (level IV interventional evidence) (Table 24). The studies are ordered by risk of bias, then by sample size. These studies were very heterogeneous in regards to their populations and description of outcomes. Most studies reported that deaths were related to MTCs, whereas 1 study reported that half of all deaths were due to phaeochromocytomas.

Table 24 Mortality following RET mutation testing and subsequent treatments

Study and location	Level of evidence	Study population	Intervention	Mortality
(Gonzalez et al. 2003) Mexico	IV Interventional evidence High quality (5/6)	N=17 RET M+ patients who had MTC or CCH 14 MEN2A 3 MEN2B (all 3 symptomatic) 11 had thyroidectomy	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15 Clinical screening Thyroidectomy	2/17 (9.5%) died (mean follow-up 6.7 years, range 1–24 years): 1 patient had increased calcitonin prior to death at age 20 years, suggesting tumour activity/metastases 1 patient died from skeletal metastases detected
(Milos et al. 2008) Worldwide (Romania, Germany, Chile, Brazil, Argentina, Hungary, Spain, The Netherlands, Czech Republic, Poland, USA)	IV interventional evidence Moderate quality (4/6)	N=92 carriers of RET C634W mutation from 20 unrelated MEN2A families 81 had thyroidectomy 68 had MTC 7 had CCH	RET mutation testing (method not stated) Thyroidectomy	18/92 (19.6%) died (mean follow-up 12 years, range 1–29 years) Mean age of death 41 years Cause of death unknown in 2 cases PCC dominant cause in 8/16 (mean age 42 years, range 18–67 years) Metastatic MTC cause of death in 4/16 (ages 21, 29, 69 years): 2 died from myocardial infarction 1 died from lung cancer 1 died from an accident
(Frohnauer et al. 2000) USA	IV Interventional evidence Moderate quality (4/6)	N=23 members from 5 MEN2A kindreds who had a RET codon 804 mutation 14 had a thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis, confirmed by direct DNA sequencing of exon 14 Thyroidectomy	1/23 (4.3%) died from widespread metastases at age 12 years, 6 years after diagnosis and thyroidectomy
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=50 index cases and family members with RET codon 634 mutation, who underwent surgery 43 had clinical disease 7 were clinically asymptomatic gene carriers	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15 Total thyroidectomy, with a central cervical lymph node dissection in those with increased calcitonin levels	7/50 (14%) died of MTC, all of whom had disseminated disease at diagnosis Length of follow-up not stated

Study and location	Level of evidence	Study population	Intervention	Mortality
(Patocs et al. 2006) Hungary	IV interventional evidence Moderate quality (3/6)	N=40 patients from 18 families who had had a thyroidectomy due to hereditary MTC or CCH: 33 MEN2A 1 MEN2B 6 MTC families without PCC or hyperparathyroidism	RET mutation testing by single-strand conformation polymorphism analysis, restriction enzyme digestion, and direct DNA sequencing of exons 10, 11, 13, 14 and, in MEN2B phenotype, exons 15 and 16	2/40 (5%) deaths from MTC C609S: 0/3 C609Y: 0/2 C634F: 1/9 C634Y: 0/9 C634S: 0/4 C634R: 0/2 C634W: 0/2 V804M: 1/4 V804L: 0/4 M918T: 0/1
(Quayle et al. 2004) USA	IV interventional evidence Moderate quality (3/6)	N=39 patients with MEN2 or FMTC diagnosed when over 50 years of age: 36 MEN2A 3 FMTC Mutations: 5 RET codon 608 15 RET codon 618 6 RET codon 620 12 RET codon 634 1 unknown	RET mutation testing (method not stated)	5-year MTC specific survival: 87% 10-year MTC specific survival: 83% Overall 5-year survival: 86% Overall 15-year survival: 74% When older patients were compared with younger patients, the differences in MTC-specific survival (p=0.04) and overall survival (p>0.001) were statistically significant Stage-specific survival was similar in both groups 3/39 (7.7%) patients had distant metastases occurring in the liver and bone (n=1), liver, lung and kidney (n=1), and liver and skin (n=1) All 3 died MTC-specific deaths
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	N=14 symptomatic RET M+ members from 4 large MEN2A families	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11	3/14 (21.4%) died from MTC 2/14 (14.3%) died from PCC 1/14 (7.1%) died from an unrelated myocardial infarction
(Yoshida et al. 2009) Japan	IV interventional evidence Moderate quality (3/6)	N=12 adults who underwent total thyroidectomy for MTC and had MEN2	RET mutation testing (method not stated) Total thyroidectomy (unclear whether treatment decisions influenced by RET mutation)	1/12 (8.3%) patients had surgery that was not considered curative, and died of advanced metastatic MTC at 1 year postoperatively
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	N=8 RET M+ members of a 3-generation FMTC family (including index case) underwent total thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of the index case	0/8 (0%) patients died during median 10-year follow-up period

Study and location	Level of evidence	Study population	Intervention	Mortality
			Analysis of exon 10 in family members Total thyroidectomy with either central neck dissection or modified radical neck dissection	
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Low quality (2/6)	N=10 index cases with a RET Y791F mutation: 3 with apparently sporadic MTC 3 with FMTC/MEN2A/MEN2B 1 with PCC 3 with HSCR N=21 RET M+ family members	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	3/31 (9.7%) deaths during 15-year follow-up period

CCH = C-cell hyperplasia; FMTC = familial MTC; HSCR = Hirschsprung's disease; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Progression-free survival

Six historical controlled studies (level III-3 interventional evidence) reported on the percentage of patients who were disease free or who had residual or recurrent disease following total thyroidectomy (Table 25). The majority of these studies were considered to have a high risk of bias, as confounding factors (such as surgical or screening techniques and lengths of follow-up varying between study arms) could not be ruled out. These studies are presented in Figure 7. The 6 studies were consistent in the direction of effect, indicating that fewer patients who had been diagnosed with RET mutation testing subsequently had residual disease, recurrent disease or died, compared with those who were diagnosed without knowledge of RET mutations. However, as all these studies were historical controlled studies, it is possible that a component of these findings is due to longer follow-up in those who were diagnosed in the earlier time period (before RET mutation testing). The more time that has passed since diagnosis, the greater the proportion of patients who cumulatively have further signs of disease. Skinner et al. (1996) reported that the four patients who had recurrence had it detected at a mean of 10.5 years (range 5–18 years) follow-up. Assuming that recurrence would occur at a similar time-point for those who had undergone diagnosis including RET mutation testing, the mean follow-up of 1.3 years in the RET mutation testing era was too short to conclude if patients were likely to develop recurrence or not. Likewise, Lallier et al. (1998) reported a case of recurrence at 5 years post-thyroidectomy.

Table 25 Persistence or recurrence after total thyroidectomy in the pre-RET mutation testing era and RET mutation testing era

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era	Follow-up
(Rohmer et al. 2011) France	III-3 interventional evidence Moderate risk of bias (18/26)	N=170 patients with a RET mutation who underwent a total thyroidectomy younger than 21 years of age: 109 MEN2A 24 MEN2B 37 FMTC	RET M+	n=38	n=132	
			Disease-free patients	23/37 (62.1%)	117/129 (90.6%)	Median 5.8 years (range 0.01–28.7 years)
(Schreinemakers et al. 2010) Sweden	III-3 interventional evidence High risk of bias (17/26)	N=93 patients with a RET mutation who underwent a total thyroidectomy younger than 20 years of age	RET M+	n=25	n=68	
			Residual or recurrent disease at follow-up	7/18 (38.8%)	9/42 (21.4%)	Median 7 years (IQR 3, 11 years)
(Lallier et al. 1998) Canada	III-3 interventional evidence High risk of bias (15/26)	N=13 MEN2 patients (children) who underwent total thyroidectomy between 1981 and 1997 with RET mutation testing: 5 had codon 620 mutation 1 had codon 643 mutation without RET mutation testing: (codons unknown)	Clinically positive / RET M+	n=7	n=6	
			Persistence or recurrence	1/7 recurrence 5 years postoperatively	0/6 (0%)	Pre-RET 2–14 years RET 1–2 years
			Time free of disease (time to disease, or time to follow-up, for individual patients)	3 years 8 years 5 years 4 years 5 years 2 years 14 years	2 years 1 year 1 year 1 year 1 year 1 year	
(Skinner et al. 1996) USA	III-3 interventional evidence High risk of bias (13/26)	N=38 children who underwent thyroidectomy prior to 16 years of age for MEN2A or presence of a RET mutation	RET M+	n=24	n=14	
			Persistence or recurrence	1/24 (4.2%) persistence 4/24 (16.7%) recurrence	0/14 (0%)	Mean: Pre-RET 9.3 years RET 1.3 years
(Sanchez Sobrino et al. 2011) Spain	III-3 interventional evidence High risk of bias (10/26)	N=8 members of a family with MEN2A due to RET C634Y mutation	RET M+	n=5	n=3	
			Free of disease	0/5 (0%)	3/3 (100%)	Total >20 years
			Time free of disease	17 years 9 years 6 years 16 years 11 years	9 years 7 years 7 years	

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era	Follow-up
(Diaz & Wohllk 2012) Chile	III-3 interventional evidence High risk of bias (9/26)	N=60 MEN2 patients who underwent total thyroidectomy		n=29	n=31	
			Biochemically cured	8/29 (27.6%)	28/31(90.3%)	Not stated
			Persistent	8/29 (27.6%)	2/31 (6.5%)	
			Dead	13/29 (44.8%): 3 hypertensive crisis 1 surgical complications 9 distant metastases	1/31 (3.2%): Died from surgical complications	

FMTC = familial MTC; IQR = interquartile range; RET M+ = RET-mutation-positive

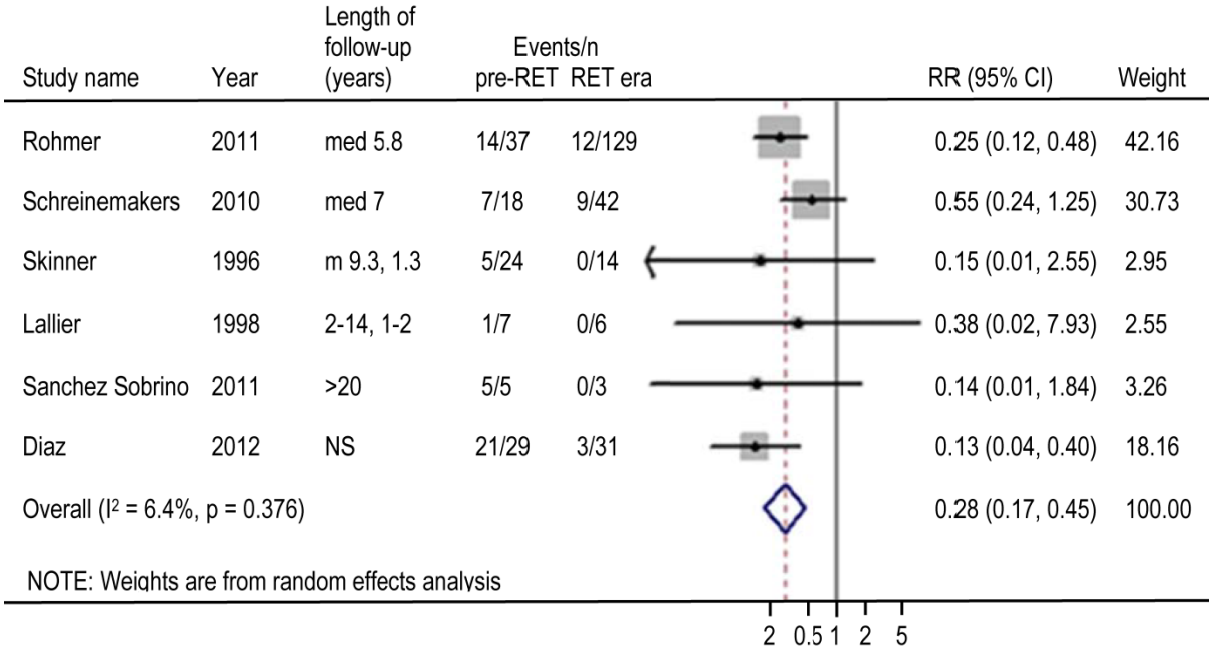

Figure 7 Risk of persistence of disease or mortality, RET mutation testing era versus pre-RET mutation testing era

m = mean; med = median; NS = not stated; RET era = RET mutation testing era.
References for studies: Rohmer (Rohmer et al. 2011); Schreinemakers (Schreinemakers et al. 2010); Skinner (Skinner et al. 1996); Lallier (Lallier et al. 1998); Sanchez Sobrino (Sanchez Sobrino et al. 2011); Diaz (Diaz & Wohllk 2012)

Twenty-three uncontrolled studies reported on the rates of persistence or recurrence of disease after total thyroidectomy in RET-mutation-positive patients with MTC (n = 8) and/or in asymptomatic relatives who had inherited a RET mutation (n =16) (see Table 73, Appendix B). The patients included adults and children with clinical signs of MEN2A, MEN2B and FMTC, with raised calcitonin levels or other early signs of disease at baseline, and those with no signs of disease receiving prophylactic treatment. Given the different phenotypes and disease stages included in each study, it is not surprising that results were highly

variable. It was not always explicit in the articles whether treatment was initiated before or after, identification of a RET mutation.

In those patients who developed clinical signs of disease prior to total thyroidectomy, the rate of persistence or recurrence was between 4.0% (Dralle et al. 1998), and 56.7% (Quayle et al. 2004). In patients where surgery was considered prophylactic, rates of persistence or recurrence varied between zero and 58.3%, with a median of 9%. Skinner et al. (2005) performed *post hoc* analyses comparing those patients who were operated on before 8 years of age with those operated on after they turned 8 years of age. In the 22 patients who received surgery before 8 years of age, there were no instances of metastasis to cervical lymph nodes and no instances of persistence or recurrence. However, in the 28 patients operated on after the age of 8 years (range 8–19 years), 6 patients (21.4%) suffered from persistence or recurrence.

Incidence and severity of MTC

Seven historical controlled studies (level III-3 interventional evidence) compared the incidence and severity of MTC in patients who had been diagnosed and treated prior to RET mutation testing becoming available, against those who were diagnosed and treated with the addition of RET mutation testing (Table 26). These studies were based in Australia, Canada, the United States of America, France, Sweden, the Netherlands and Spain. All the studies were at risk of bias and confounding (although 1 study was rated as moderate quality as the reporting was very good). Overall, data on the incidence and severity of MTC in these studies were more informative than for the other longer term outcomes, where the difference in length of follow-up between arms became a problem in interpretation of the data. Incidence and severity of MTC were determined by histopathology in those who underwent total thyroidectomy, and immediately after surgery in both the intervention and control arms.

The results of these 7 studies were heterogeneous in size of effect but consistent in their direction of effect, showing that the risk of having an MTC detected by histopathology was significantly greater in those patients who were diagnosed in the pre-RET mutation testing era (Table 26). On average, those diagnosed and treated since RET mutation testing became available had almost half the risk of having an MTC at time of treatment (RR=0.53, 95% CI 0.32, 0.90).

Patients are likely to be identified earlier through genetic screening than through clinical screening. Those undergoing surgery at a particular age are therefore more likely to have a lower risk profile if they have been identified by RET mutation testing.

The majority of the studies restricted the comparison of outcomes to those who were RET-mutation-positive. However, Learoyd et al. (1997) also discussed the management and outcomes of family members in MEN2 families who were found to be negative for RET

mutations. Four family members had undergone a total thyroidectomy based on an elevated calcitonin response to pentagastrin-stimulation testing. Two of these were performed without knowledge of mutation status and showed normal thyroid histological characteristics. In another family two members underwent a total thyroidectomy despite being found to not have a RET mutation. These two patients were found to have C-cell hyperplasia. Three members from one family had elevated calcitonin responses but refused thyroidectomy on religious grounds. These people were later found to be RET-mutation-negative. One additional person from a MEN2 family was indicated for surgery based on elevated calcitonin, but surgery was not performed as genetic screening became available and this family member was found not to have a RET mutation.

Table 26 Incidence and severity of MTC at time of total thyroidectomy

Study and location	Level of evidence and quality assessment	Study population	Histology results	Pre-RET mutation testing era	RET mutation testing era	Significance (p-value)
(Rohmer et al. 2011) France	III-3 interventional evidence Moderate risk of bias (18/26)	N=170 patients with a RET mutation who underwent a total thyroidectomy younger than 21 years of age: 109 MEN2A 24 MEN2B 37 FMTC	RET M+	n=38	n=132	
			Normal or CCH or microscopic MTC	26/37(70.3%)	120/129 (93%)	p=0.001
			MTC	11/37(29.7%)	9/129 (7.0%)	
			Lymph node metastases: N0 N1 Nx	 20/37 (54.1%) 6/37 (16.2%) 11/37 (29.7%)	 80/129 (62.0%) 9/129 (7.0%) 9/129 (7.0%)	NS
(Schreinemakers et al. 2010) Sweden	III-3 interventional evidence High risk of bias (17/26)	N=93 patients with a RET mutation who underwent a total thyroidectomy younger than 20 years of age	RET M+	n=25	n=68	
			Median age	10.6 years	7.8 years	p=0.02
			CCH	7/25 (28%)	22/67 (32.8%)	NS
			MTC	18/25 (72%)	43/67 (64.2%)	
			Normal histology	0/25 (0%)	2/67 (3.0%)	
			Lymph node metastases	5/25 (20%)	3/68 (4.4%)	p=0.02
(Learoyd et al. 1997) Australia	III-3 interventional evidence High risk of bias (16/27)	N=164 individuals from families with MEN2 and known RET mutations: 56 were RET M+ 108 were RET M–	RET M+	n=45	n=7	
			Mean age (range)	32 years (6–65 years)	16 years (7–28 years)	
			CCH	1/45 (2%)	4/7 (57%)	p<0.001
			MTC	44/45 (98%)	3/7 (43%)	
			RET M–	n=2	n=2	
			CCH	0/2 (0%)	2/2 (100%)	
			MTC	0/2 (0%)	0/2 (0%)	

Study and location	Level of evidence and quality assessment	Study population	Histology results	Pre-RET mutation testing era	RET mutation testing era	Significance (p-value)
(Lallier et al. 1998) Canada	III-3 interventional evidence High risk of bias (15/26)	N=13 MEN2 patients (children) who underwent total thyroidectomy between 1981 and 1997 with RET mutation testing: 5 codon 620 and 1 codon 643 mutations	Clinically positive / RET M+	n=7	n=6	
			Mean age (range)	11.8 years (1.5–16 years)	9.1 years (6–14 years)	
			CCH without MTC	0/7 (0%)	2/6 (33.3%)	
			MTC	7/7 (100%)	1/6 (16.7%)	
			Disease-free	0/7 (0%)	3/6 (50%)	
(Skinner et al. 1996) USA	III-3 interventional evidence High risk of bias (13/26)	N=38 children who underwent thyroidectomy prior to 16 years of age for MEN2A or presence of a RET mutation	RET M+	n=24	n=14	
			Mean age (range)	10.6 years (5–15 years)	10.5 years (5–15 years)	
			CCH without MTC	4/24 (16.7%)	3/14 (21.4%)	
			MTC without nodal metastases	12/24 (50%)	11/14 (78.6%)	
			MTC with nodal metastases	1/24 (4.2%) Spread to cervical lymph nodes	0/14 (0%)	
			MTC, unsampled lymph nodes	7/24 (29.2%)	0/14 (0%)	
(Sanchez Sobrino et al. 2011) Spain	III-3 interventional evidence High risk of bias (10/26)	N=8 individuals from a family with MEN2A due to RET C634Y mutation	RET M+	n=5	n=3	
			Age range	23–58 years	6–34 years	
			CCH	0/5 (0%)	2/3 (66.7%)	
			MTC	5/5 (100%)	1/3 (33.3%)	
(Lips et al. 1994) The Netherlands	III-3 interventional evidence High risk of bias (7/26)	N=14 members of 4 large MEN2A families who had a thyroidectomy: 8 were based on RET mutation carrier status 6 were based on raised pentagastrin-stimulated calcitonin levels who were later found to be RET M–	RET M+	Not stated	n=8	
			C-cell clusters or nodules and small irregular foci of MTC		8/8 (100%)	
			RET M–	n=6	Not stated	
			C-cell clusters or nodules	2/6 (33.3%)		

CCH = C-cell hyperplasia; FMTC = familial medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

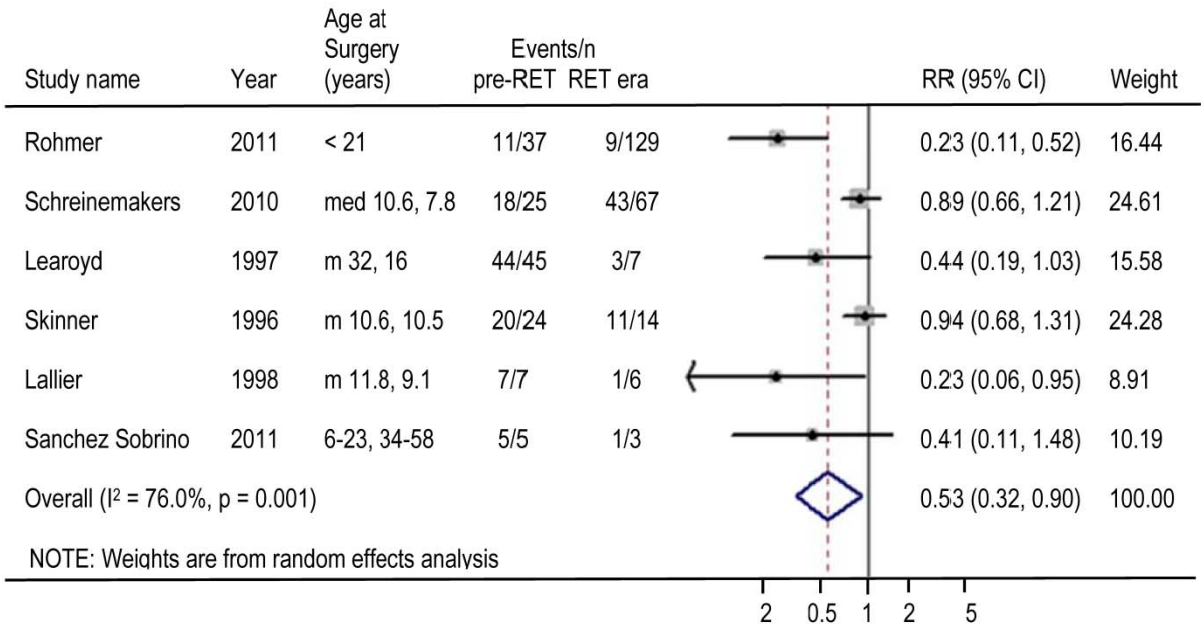

Figure 8 Risk of having an MTC at time of total thyroidectomy, RET mutation testing era versus pre-RET mutation testing era

m = mean; med = median; RET era = RET mutation testing era
References for studies: Rohmer (Rohmer et al. 2011); Schreinemakers (Schreinemakers et al. 2010); Learoyd (Learoyd et al. 1997); Skinner (Skinner et al. 1996); Lallier (Lallier et al. 1998); Sanchez Sobrino (Sanchez Sobrino et al. 2011).

Twelve uncontrolled studies (level IV interventional evidence) reported on the incidence and severity of MTC in patients who developed clinical signs of disease prior to total thyroidectomy (Table 74, Appendix C). The populations included a mix of index patients (probands) and family members who also showed clinical signs or symptoms. Given the starting population, it is not surprising that the vast majority of these patients had MTC at the time of total thyroidectomy (median 100%, range 64.3–100%), rather than C-cell hyperplasia or no disease.

Fifty-one uncontrolled studies (level IV interventional evidence) provided information on the rate of MTCs in RET-mutation-positive family members who had undergone total thyroidectomy (Table 75, Appendix C). In family members a total thyroidectomy is often considered 'prophylactic' if the surgery is based on a genetic diagnosis, in the absence of clinical manifestations (i.e. the patient is asymptomatic and there is no evidence of palpable cervical masses) (Rodriguez Gonzalez et al. 2002). However, MTCs or C-cell hyperplasia are often evident on histopathological examination of the thyroid specimen, with MTCs evident in 0–100% of RET-mutation-positive family members. The median rate of MTCs was 67.6% (interquartile range (IQR) = 50%, 85.7%). Given that, in many of the studies, the populations received prophylactic surgery, this is still very high and is indicative of the high penetrance of MTCs in those with a RET mutation.

Machens et al. (2005) stratified patients who underwent a thyroidectomy for C-cell hyperplasia or MTC into three risk categories according to which RET codon was mutated. They found that patients with RET codon 918 mutations (responsible for MEN2B) had the highest risk (100%) of MTC at the time of surgery, while patients with RET codon 609–634 mutations had a high risk (72.6%) of MTC at the time of surgery, and those with RET codon 786–891 mutations had the lowest risk (45.1%).

Incidence of phaeochromocytoma and hyperparathyroidism

One very small historical controlled study (level III-3 interventional evidence) provided the incidence of phaeochromocytoma and hyperparathyroidism in patients who were diagnosed clinically or with the addition of RET mutation testing (Table 27). This study was too small to draw any conclusions, but it is not expected that there would be any difference in the incidence of identified phaeochromocytoma and hyperparathyroidism between the two diagnostic modalities. In both scenarios (diagnosed prior to RET mutation testing availability or diagnosed after) treatment for phaeochromocytoma would only occur after clinical signs of disease, and patients in both arms would continue to undergo surveillance. The article describing the Australian historical controlled study (level III-3 interventional evidence) supported this, reporting that knowledge of having a RET mutation did not impact on the frequency of post-thyroidectomy pentagastrin testing or screening for additional features of phaeochromocytoma or hyperparathyroidism (Learoyd et al. 1997).

The only exception to this would be that availability of RET mutation testing could allow earlier distinction between patients with MEN2A and those from FMTC families. If, based on the genotype, patients are classified as having FMTC rather than MEN2A, screening for other features of MEN2A (phaeochromocytoma or hyperparathyroidism) would not be required. However, no differences in health outcomes are expected.

Table 27 Incidence of phaeochromocytoma and hyperparathyroidism

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era
(Sanchez Sobrino et al. 2011) Spain	III-3 interventional evidence High risk of bias (10/26)	N=8 individuals from a family with MEN2A due to C634Y mutation	RET M+	n=5	n=3
			Phaeochromocytoma	2/5 (40%) 2 x bilateral	1/3 (33.3%) Bilateral
			Hyperparathyroidism	0/5	0/3

RET M+ = RET-mutation-positive

Fifteen uncontrolled studies (level IV interventional evidence) reported on the penetrance of phaeochromocytoma and hyperparathyroidism over varying lengths of time (Table 76, **Error! Reference source not found.**). Phaeochromocytoma was observed in 0–76.4% of patients, with a median of 25%. These studies were often limited by short-term follow-up,

so the figures presented are not representative of lifetime penetrance of phaeochromocytoma.

It is well known that the rate of phaeochromocytoma and hyperparathyroidism differ by phenotype of MEN2 and by age. The greatest detail on the rate of phaeochromocytoma in those with RET mutations was provided by Machens et al. (2006). This case series was rated as poor quality, as few details were provided on the inclusion criteria for the study or the method of RET mutation testing. However, they reported that, in those with the highest risk phenotype (mutation in codon 918, corresponding to MEN2B), the penetrance of phaeochromocytoma was 43% by age 30 years and 100% by age 35 years. In those with a high-risk phenotype (mutation in codon 609–634, corresponding to some types of FMTC and MEN2A), the penetrance by age 30 years was 8%, which increased to 18% by age 35 years and 54% by age 50 years. In those with the lowest risk (mutation in codon 768–891, corresponding to FMTC), the penetrance of phaeochromocytoma was zero until age 50 years, when it was 4%. In the largest case series (Frank-Raue et al. 2011) over half of the phaeochromocytoma cases were identified after clinical symptoms were evident rather than being identified through screening.

Twelve uncontrolled studies reported on the rate of hyperparathyroidism in RET-mutation-positive index cases (Table 76, **Error! Reference source not found.**). Up to 15.4% of patients with MEN2A, MEN2B and FMTC combined were diagnosed with hyperparathyroidism. In 1 study 38.5% of patients showed evidence of hypercellular parathyroid pathology on exploration, but this was not correlated to signs of clinical disease (Etit et al. 2008). The largest study reported that the median age at which hyperparathyroidism was diagnosed was 46 years but the range (28–82 years) indicated wide variability (Frank-Raue et al. 2011). The vast majority (87.5%) of cases of hyperparathyroidism were identified through screening (Frank-Raue et al. 2011).

Thirty uncontrolled studies (level IV interventional evidence) provided rates of phaeochromocytoma and hyperparathyroidism in RET-mutation-positive family members (Appendix D). The studies included a mix of patients with MEN2A, MEN2B and FMTC. The overall penetrance of phaeochromocytoma varied between zero and 50%.

The 23 case series reporting on the rate of hyperparathyroidism in patients with MEN2 in RET-mutation-positive family members showed rates of up to 27.3%. Schuffenecker et al. (1998) reported that the mean age of hyperparathyroidism diagnosis was 33.7 years (range 12–70 years), with a penetrance of 14% by age 30 years, 26% by age 40 years and 48% by age 60 years. These rates are unlikely to have been altered at all by RET mutation testing.

Secondary effectiveness outcomes

Age at diagnosis

One article was identified that compared the age at time of diagnosis in patients with an MTC between a survey performed in 1996, and a survey performed in 2002 (Kameyama & Takami 2004). Age at diagnosis in 2002 was lower for patients with MEN2A, FMTC and sporadic MTC, but not with MEN2B. The reduction in age in the survey in 2002 was attributed to RET mutation testing allowing earlier diagnosis of symptomatic patients (rather than requiring more than one disease feature in the family), earlier distinction between MEN2A and FMTC, and pre-clinical diagnosis within family members. Given the smaller numbers of people with MEN2B, the lack of reduction in age may either be due to chance or as a consequence of MEN2B having a clearer phenotype. There is also a higher rate of *de novo* mutations in MEN2B than in MEN2A, such that the proportion of familial cases detected through screening will be much lower than with MEN2A.

Table 28 Mean age (years) at diagnosis

Study and location	Level of evidence and quality assessment	Study population	Population	Pre-RET mutation testing era	RET mutation testing era
(Kameyama & Takami 2004) Japan	III-3 interventional evidence High risk of bias (10/26)	N=905 MTC patients: 634 patients in 1996: 175 MEN2A 49 FMTC 20 MEN2B 390 sporadic MTC 271 patients in 2002: 83 MEN2A 14 FMTC 11 MEN2B 163 sporadic MTC	MEN2A FMTC MEN2B Sporadic MTC	40.3±15.3 43.6±15.6 26.5 ±8.8 48.5±13.9	35.6±14.4 34.6±12.3 30.5±10.1 47.6±14.0

FMTC = familial medullary thyroid carcinoma

Twelve additional uncontrolled studies reported the age at diagnosis for index cases (Table 78, Appendix E). Given that these patients were detected due to symptoms, the age at diagnosis varies largely on genotype and phenotype. The age ranged from a mean of 13.5±2.1 years for patients with MEN2B to a median of 62 years for patients with a RET codon 804 mutation.

Table 79 (Appendix E) outlines the age at diagnosis for family members of someone with a confirmed RET mutation in 12 uncontrolled studies. Punales et al. (2003) reported that those with clinical disease were, on average, 8 years older than those without clinical disease. Historically, asymptomatic gene carriers would not have been able to be diagnosed until they showed clinical or biochemical signs of disease.

Machens et al. (2005) reported that the time to diagnosis of MTC in patients with the highest risk (mutation in RET codon 918, corresponding to MEN2B) was 14.3 years (95% CI 10.3, 18.4). In those with a high risk (mutation in RET codons 609–634, corresponding to some types of FMTC and MEN2A), the time to diagnosis of MTC was 30.1 years (95% CI 26.6, 33.5). In those with the least high risk (mutation in RET codons 768–891, corresponding to FMTC), the time to diagnosis of MTC was 51.6 years (95% CI 46.5, 56.6). However, the authors did not report if diagnosis was based on clinical and/or biochemical signs or on RET mutation status.

Mean age at time of thyroidectomy

Five historical controlled studies (level III-3 interventional evidence) reported on the age at time of total thyroidectomy in a cohort of patients treated in the era prior to RET mutation testing, and a cohort of patients treated after RET mutation testing was available (Table 29). Since the introduction of RET mutation testing, patients have undergone total thyroidectomy at a much younger age. Schreinemakers et al. (2010) reported that the mean age at surgery was significantly different between those who were disease free after surgery (mean 8.6 years) compared with those who had residual or recurrent disease (mean 12.1 years, p=0.002).

Table 29 Age at time of total thyroidectomy

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era	Significance (p-value)
(Rohmer et al. 2011) France	III-3 interventional evidence Moderate risk of bias (18/26)	N=170 RET M+ patients who underwent a total thyroidectomy younger than 21 years of age: 109 MEN2A 24 MEN2B 37 FMTC	RET M+	n=38	n=132	
			Mean age at thyroidectomy	10.7± 6.6 years	8.3± 4.4 years	p=0.003
(Schreinemakers et al. 2010) Sweden	III-3 interventional evidence High risk of bias (17/26)	N=93 RET M+ patients who had undergone total thyroidectomy younger than 20 years of age	RET M+	n=25	n=68	
			Median age at thyroidectomy (IQR)	10.6 years (7.4–13.2)	7.8 years (5.3–12.2)	p=0.022
(Learoyd et al. 1997) Australia	III-3 interventional evidence High risk of bias (16/27)	N=164 people from 26 families with MEN2 RET mutations: 56 were RET M+ 108 were RET M–	RET M+	n=45	n=7	
			Mean age at surgery (range)	32 years (6–65)	16 years (7–28)	Not stated
(Lallier et al. 1998) Canada	III-3 interventional evidence	N=13 patients with MEN2 who underwent total	Clinically positive / RET M+	n=7	n=6	

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era	Significance (p-value)
			Mean age at surgery (range)	11.8± 4.9 years (1.5–15)	9.0± 3.3 years (5–14)	Not stated
(Sanchez Sobrino et al. 2011) Spain	III-3 interventional evidence High risk of bias (10/26)	N=8 family members with MEN2A due to a RET C634Y mutation	RET M+	n=5	n=3	
			Mean age at surgery (range)	37.6± 14.8 years (23–58)	16.7± 15.1 years (6–34)	Not stated

N/A = Not applicable; RET M+=RET mutation positive; RET M–=RET mutation negative.

Two of the historical controlled studies further compared whether patients with RET mutations were treated appropriately according to the 1999 consensus statement from the Seventh International Workshop on Multiple Endocrine Neoplasia and the 2009 French guidelines (Rohmer et al. 2011; Schreinemakers et al. 2010). For the highest risk group (mutations in codon 918, 883 and 922) surgery was recommended before 6 months of age, for the higher risk group (mutations in codon 630, 634, 609, 611, 618 and 620) before age 5 years, and for the least high risk group (mutations in codons 768, 790, 791, 804 and 891) prior to age 10 years (Rohmer et al. 2011; Schreinemakers et al. 2010). Across both these studies, only 3 out of 62 (4.7%) patients were treated according to the age-appropriate guidelines in the historical cohort, compared with 42 out of 197 (21.3%) in the cohorts diagnosed since the introduction of RET mutation testing (Table 30). The difference in the rates between cohorts was significant for both studies. Schreinemakers et al. (2010) reported that age at time of thyroidectomy was an independent prognostic variable for persistent or recurrent disease in multivariate analyses.

Both the Rohmer et al. (2011) and Schreinemakers et al. (2010) studies noted that, of those with residual or recurrent disease, none had been operated on at an age recommended by the guidelines. This finding should not be surprising, given that the guidelines were developed with reference to the age at which treatment may be considered curative.

Table 30 Age-appropriate surgery

Study and location	Level of evidence and quality assessment	Study population	Outcome measure	Pre-RET mutation testing era	RET mutation testing era	Significance (p-value)
(Rohmer et al. 2011) France	III-3 interventional evidence Moderate risk of bias (18/26)	N=170 RET M+ patients who underwent a total thyroidectomy younger than 21 years of age: 109 MEN2A 24 MEN2B 37 FMTC	RET M+	n=38	n=132	
			Age-appropriate surgery	2/37 (5.4%)	21/129 (16.3%)	p=0.091
(Schreinemakers et al. 2010) Sweden	III-3 interventional evidence High risk of bias (17/26)	N=93 RET M+ patients who had undergone total thyroidectomy younger than 20 years of age	RET M+	n=25	n=68	
			Age-appropriate surgery	1/25 (4%)	21/68 (30.9%)	p=0.004

FMTC = familial medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Two uncontrolled studies reported a mean age at time of total thyroidectomy in index cases of 24.6±12.2 years and 43.2±22.2 years (Table 31). Due to the lack of a comparator, it is unknown based on this data whether age at time of thyroidectomy has changed for index cases; however, it is hypothesised that little change would be found in those whose MTC is identified before RET mutation testing, as the treatment for MTC would be the same regardless of mutation.

Table 31 Mean age at total thyroidectomy (index cases)

Study and location	Level of evidence	Study population	Intervention	Mean age at thyroidectomy
(Chiefari et al. 1998) Italy	IV Interventional evidence Moderate quality (4/6)	N=16 RET M+ patients from 8 families with hereditary MTC, who underwent thyroidectomy	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14 Clinical screening Total thyroidectomy	Mean = 24.6±12.2 years (range = 10–45 years)
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=6 index cases with a RET Y791F mutation who underwent total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	Mean = 43.2±22.2 years (range = 14–69 years)

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Table 80 (Appendix F) outlines the mean age at total thyroidectomy in family members who were screened and found to have RET mutations as reported in 19 uncontrolled studies. Family members often had a total thyroidectomy at a younger age than index patients, prior

to any clinical signs of disease. Mean age at time of thyroidectomy in the studies varied between 7.5 years and 52.8 years, with a median of 25.1 years.

Rates of surveillance

Nine direct studies of the impact of RET mutation testing on health outcomes mentioned rates of surveillance in those who were RET-mutation-positive or -negative (Table 32). Prior to RET mutation testing being available, all family members would have been recommended to undergo surveillance for features of MEN2. However, after the introduction of RET mutation testing, those who had no pathological RET mutations were able to cease surveillance and were told that their descendants would not require evaluation for MEN2 either (see also 'Change in Management' in linked evidence section).

Those who were RET-mutation-positive were recommended to continue surveillance for features of MEN2 (Learoyd et al. 1997); however, a small proportion of RET-mutation-positive patients refuse further clinical and/or biochemical examinations (Romei et al. 2011).

Table 32 Rates of surveillance

Study and location	Level of evidence and quality of assessment	Study population	RET M+	RET M–
(Learoyd et al. 1997) Australia	III-3 interventional evidence High risk of bias (16/27)	N=164 patients from 26 MEN2 families: 56 were RET M+ 108 were RET M–	Unchanged from pre-RET mutation testing era	All discontinued screening
(Alvares Da Silva et al. 2003) Brazil	IV Interventional evidence Moderate quality (4/6)	N=229 members spanning 6 generations of a large extended FMTC family with a RET G533C mutation 76 members were RET M+	10/76 (13.2%) RET M+ family members presented with low pentagastrin-stimulated calcitonin levels and surgery was delayed 3/76 (3.9%) refused further clinical investigation	153/153 (100%) RET M– family members had normal pentagastrin-stimulated calcitonin levels and were excluded from further clinical investigation
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	N=60 RET M+ family members of patients with MTC re-classified from sporadic MTC to FMTC or MEN2A due to a RET mutation: 35 showed clinical and/or biochemical signs of disease on screening 30 (29 FMTC, 1 MEN2A) underwent total thyroidectomy 5 refused treatment	5/60 (8.3%) RET M+ refused clinical and/or biochemical examinations 20/20 (100%) RET M+ clinically unaffected underwent yearly clinical and biochemical assessment	Not stated

Study and location	Level of evidence and quality of assessment	Study population	RET M+	RET M–
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=33 RET M– family members of a MEN2A family with a RET codon 618 mutation	Not stated	33 RET M– family members who had previously undergone biochemical surveillance, ceased surveillance and were told that their descendants would not need to be evaluated for disease
(Shimotake et al. 1996) Japan	IV interventional evidence Moderate quality (3/6)	N=6 children who had an affected parent from a MEN2 family and were without clinical signs of disease underwent RET mutation testing 3 were RET M+	Not stated	3/6 were found not to have RET mutation These family members were released from endocrine screening
(Karga et al. 1998) Greece	IV interventional evidence Poor quality (2/6)	N=25 asymptomatic first-degree relatives from 12 unrelated Greek families 9 MEN2A 1 FMTC 3 probable FMTC (only 3 members diagnosed with MTC)	Not stated	20 RET M– family members excluded from further screening
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=15 RET M+ family members from an MTC index patient with a RET L790F mutation 8 had abnormal pentagastrin-stimulated calcitonin levels	7/15 (46.7%) had normal calcitonin levels and were advised to have annual follow-ups to check calcitonin levels 3/7 (42.9%) refused follow-up	Not stated
(Kinlaw et al. 2005) USA	IV interventional evidence Poor quality (2/6)	N=15 RET M+ family members (including index case) of a MEN2A family with a RET C609S mutation	11/15 (73.3%) underwent further biochemical and clinical screening 4/15 (26.7%) refused further evaluation	Not stated
(Uchino et al. 1999) Japan	IV interventional evidence Low quality (2/6)	N=6 clinically unaffected members from MEN2A families with mutations on RET codon 634 All had raised calcitonin levels.	2 adult patients refused treatment 1 patient, aged 7 years, is being monitored	Not stated

FMTC = familial medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Linked evidence

Summary of effectiveness

The accuracy of RET genetic testing could not be determined as no long-term clinical data were available to use as the reference standard. There were 6 uncontrolled studies that reported cases where total thyroidectomy had been recommended, based on calcitonin testing, in patients who were later found to have no RET mutations. Biochemical screening as a means of diagnosis is therefore known to have false positive results. From the data available it is impossible to determine whether mutation testing of the *RET* gene is associated with false positive test results.

There were uncontrolled data suggesting that many patients undergo prophylactic surgery based on knowledge of a RET mutation, and comparative data suggesting that prophylactic surgery is associated with much better health outcomes than curative surgery (surgery performed after clinical signs of disease).

As it was not clear when the systematic review was initiated that direct evidence was available for the assessment of RET mutation testing, a linkage of evidence was also undertaken.

Is it accurate?

It was initially decided that the accuracy of RET mutation testing would be determined using the reference standard of long-term clinical health outcomes (i.e. whether RET mutation status corresponds to MEN2 features over the patient's lifetime). However, given the age of the technology, long-term clinical data were not available in those who were RET-mutation-positive, and very little clinical data were available for those patients or family members who tested negative for RET mutations.

Histological data were available for those who had RET mutations and subsequently underwent total thyroidectomy. These data are shown in the section on the incidence and severity of MTC (Table 26; Table 74 and Table 75, Appendix C). Although this short-term data could be used as an imperfect reference standard to calculate the positive predictive value of RET mutation testing, compared with the positive predictive value of pentagastrin-stimulated calcitonin levels, this comparison may be misleading. RET mutation testing allows earlier detection of those at risk of developing an MTC, allowing surgery to be performed at an earlier stage of disease development. A different spectrum of patients would therefore be operated on. In patients with a RET mutation who undergo a total thyroidectomy, a histopathology result that shows no MTC may be thought of as a clinical success due to early intervention, rather than as a lack of accuracy of RET mutation testing. The positive predictive value of RET mutation testing, compared with pentagastrin-stimulated calcitonin testing, has therefore not been presented.

A further difficulty in determining the accuracy of RET mutation testing is that treatment bias may occur. Between RET mutation testing and long-term clinical outcomes, treatment (i.e. total thyroidectomy) occurs in those who are RET-mutation-positive. In those patients who have RET mutations corresponding to FMTC, the only clinical characteristic that patients are at risk of developing is an MTC. If patients have a prophylactic total thyroidectomy, they are unlikely to develop an MTC, so there is no way of confirming whether the results of RET mutation testing correspond to clinical outcomes in these patients.

Diagnostic yield

Diagnostic yield informs the question of what proportion of patients who are tested are found to have pathology RET mutations. As the results of this will be largely determined by the population tested, the results for diagnostic yield have been separated into the following different populations: those with an MTC; those with an apparently sporadic MTC; those presenting with phaeochromocytoma; and family members of someone with a confirmed RET mutation.

Due to the volume of small case series reporting diagnostic yield, which do not provide much additional information, studies on diagnostic yield were only included if they tested a minimum of 20 patients. Some results are available for numbers smaller than this, if the article includes several subpopulations.

Medullary thyroid carcinoma (MTC)

The diagnostic yield of RET mutation testing among patients presenting with an MTC is presented in Table 81 (hereditary MTC), Table 82 (apparently sporadic MTC) and Table 83 (unspecified MTC) in Appendix G. In the 17 uncontrolled studies that reported the diagnostic yield from patients with hereditary MTC, a median of 95.5% of index cases had a detectable RET mutation, ranging between 25% and 100% in individual studies. Thus, only 4.5% of families with inherited MTC did not have a detectable RET mutation in these studies. In some cases the study design precluded the detection of certain known RET mutations, such as the RET mutations on exons 15 and 16 responsible for MEN2B (Hedayati et al. 2006; Kimura et al. 1995). Additionally, the number of index cases with undetectable RET mutations would be expected to continue to decrease as RET mutation testing methodologies continue to improve.

From the 25 studies that reported the diagnostic yield of RET mutation testing of patients with apparently sporadic MTC, without any family history or additional features of MEN2, the prevalence of germline RET mutations ranged from zero to 55.6% with a median of 6.5% (Table 81, Appendix G). In this population RET mutation testing would be able to rule out 93.5% of patients from additional biochemical and surveillance for MEN2 features. In the 6 studies that presented diagnostic yield from unspecified cases of MTC, the diagnostic yield

of RET mutations ranged from 21.5% to 45.7% with a median of 30.6% (Table 82, Appendix G).

Phaeochromocytoma

In patients presenting with phaeochromocytoma, the diagnostic yield depended on whether the patients had other indicators of MEN2 or not. In 14 uncontrolled studies that reported diagnostic yield for patients who had apparently sporadic phaeochromocytoma, the proportion of patients who had germline RET mutations ranged from zero to 18.3% with a median of 0.45% (Table 84, Appendix G). Thus, up to 200 patients with apparently sporadic phaeochromocytoma would need to be tested in order to identify one MEN2 case.

In 4 studies with familial phaeochromocytoma, a median of 24.4% of patients had a RET mutation (Table 84, Appendix G), such that four patients would be tested for every case detected. In the 5 studies that reported the diagnostic yield for patients with unspecified phaeochromocytoma, a median of 7.8% of patients had a germline RET mutation.

Hyperparathyroidism

There was no evidence on the diagnostic yield of RET mutation testing in patients presenting with hyperparathyroidism, plus a diagnosis of MTC or phaeochromocytoma in a close relative. It is therefore unknown what proportion of patients with these characteristics would be positive for RET mutations.

Relatives of patients with a known RET mutation

Biologically, half of all first-degree relatives of someone with a confirmed RET mutation can be expected to also have a RET mutation, given the pattern of inheritance. However, the rates differ largely between studies (Table 85, Appendix G). This is likely due to different proportions of first-degree, versus more distant, relatives and whether symptomatic relatives are included. In families with a higher risk phenotype, those with RET mutations may be likely to already have clinical features of disease, so if the figures shown are only those who are still asymptomatic, the chances of having a RET mutation are much lower.

In the 11 studies that tested only first-degree relatives of someone with a RET mutation, the diagnostic yield ranged between 20% and 57.1% with a median of 37.5% (Table 85, Appendix G). This is lower than the 50% expected biologically, as several studies excluded family members with clinical signs of disease. In fact, 1 study found that, while 57.1% (36/63) of all first-degree relatives from 9 MEN2A families were RET-mutation-positive, only 33.3% (10/30) of those with no clinical signs of disease carried the RET mutation (McMahon et al. 1994). The 48 studies that included second-degree relatives or did not specify the relationship to the index patient had a similar median diagnostic yield of 39.4% (range 15.9–65.5%).

Does it change patient management?

A diagnostic test is only useful if the results then influence clinical management. In the case of RET mutation testing, knowledge of the *RET* gene is useful in determining whether patients require ongoing surveillance for features of MEN2 and would benefit from a total thyroidectomy (if they don't already have clinical signs of MTC, in which case they would have a thyroidectomy regardless of RET status).

In 1996 Learoyd et al. (1997) performed a survey of clinicians managing MEN2 families in Australia, and reported that 27/28 clinicians surveyed used RET mutation test results to assist in the management of MEN2. Given that mutation testing had only been available for this condition for 3 years, the authors concluded that there was a high level of acceptance by clinicians.

Table 86 (Appendix H) outlines the results of 28 uncontrolled studies that provide information on treatments received by patients. In total, 24 studies reported on the treatment of patients who were RET-mutation-positive. A median of 60.9% (range 0–100%) of patients underwent a total thyroidectomy, many of which were prophylactic. Additionally, a median of 3.9% (range 0–60%) of patients were scheduled for surgery and 17.1% (range 0–100%) were being monitored. However, a median of 8.5% (range 0–33.3%) of patients refused a thyroidectomy and/or further monitoring. Treatment decisions for patients who did not undergo a thyroidectomy were not reported in 10 studies (median 8.3% of patients, range 0–49.2%).

Ten studies listed in Table 86 (Appendix H) included anecdotal evidence that RET mutation testing influenced management of RET-mutation-negative family members. Three studies reported that RET-mutation-negative family members were released from further clinical and biochemical screening (Alvares Da Silva et al. 2003; Karga et al. 1998; Lindskog et al. 2004). Six studies reported that before RET mutation testing was available, some patients were classified as being clinically affected based on raised pentagastrin-stimulated calcitonin levels, warranting a total thyroidectomy, and were subsequently found to be free of RET mutations (Decker et al. 1995; Frank-Raue et al. 1996; Gagel et al. 1995; Halling et al. 1997; Hernandez et al. 1997; Lips et al. 1994; Marsh et al. 1996). In these patients it is clear that RET mutation testing would have avoided unnecessary surgery. This is illustrated in 3 studies in which patients with raised pentagastrin-stimulated calcitonin levels were spared from having a thyroidectomy when found to be RET-mutation-negative (Decker et al. 1995; Gagel et al. 1995; Hernandez et al. 1997).

Halling et al. (1997) reported on individuals from one FMTC kindred who underwent total thyroidectomy, and in whom both RET mutation status and pre-operative pentagastrin-stimulated calcitonin levels were recorded. This study shows that C-cell hyperplasia is common in both those with and without RET mutations regardless of whether or not

pentagastrin-stimulated calcitonin levels were elevated. It was present in 5/7 RET-mutation-negative patients with raised pentagastrin-stimulated calcitonin levels, and in 3/3 with normal levels. Of note is that one RET-mutation-negative patient with raised pentagastrin-stimulated calcitonin levels showed clinical signs of disease on biochemical screening, and was subsequently found on histopathology to have an MTC. From this family it is also clear that some patients had a prophylactic total thyroidectomy prior to RET mutation testing being available, even when pentagastrin-stimulated calcitonin levels were not raised.

Does change in management improve patient outcomes?

One key change in management that has occurred in patients with RET mutations is that those at risk of MTC are more likely to be operated on before clinical signs of disease develop.

One small cohort study of patients who underwent total thyroidectomies for MEN2 between 1995 and 2007 in Italy compared the outcomes between those who had a thyroidectomy after clinical signs developed (i.e. a curative thyroidectomy) and those who had a thyroidectomy before clinical signs developed, on the basis of a *RET* gene point mutation (i.e. a prophylactic thyroidectomy) (Table 33). This study is considered to have a high risk of bias as there were confounding factors (such as risk level) that were not evenly distributed between the treatment groups. The health outcomes resulting from these treatments (either curative or prophylactic thyroidectomies) may potentially be the result of risk level of their particular RET codonic mutations rather than due to the management strategy.

The patients who already showed clinical signs of an MTC or MEN2 at the time of thyroidectomy showed more-developed MTC on histology. Lymph node metastases were found in two patients with MEN2B. These patients died 6 and 7 years after diagnosis due to disease progression. Tumour stage at presentation is a key prognostic factor for outcomes.

It is expected that RET mutation testing has increased the proportion of patients having prophylactic thyroidectomies rather than curative thyroidectomies, and it is expected that patient health outcomes are improved.

Table 33 Prophylactic thyroidectomy versus thyroidectomy based on clinical signs/symptoms

Study and location	Level of evidence	Study population	Histology results	Prophylactic thyroidectomy	Curative thyroidectomy
(Spinelli et al. 2010) Italy	III-2 interventional evidence High risk of bias (16/26)	N=13 juvenile patients (8–17 years of age) with MEN2 who underwent surgery for MTC: 7 (54%) MEN2A 4 (31%) FMTC 2 (15%) MEN2B		n=6	n=7
			C-cell hyperplasia	6/6 (100%)	7/7 (100%)
			Monolateral MTC	4/6 (66.7%)	2/7 (28.6%)
			Bilateral MTC	0/6 (0%)	5/7 (71.4%)
			Extrathyroid invasion	0/6 (0%)	2/7 (28.6%)
			Central lymph node involvement	0/6 (0%)	2/7 (28.6%)
			No. of organs affected by distal metastases	0	2
			Stage	4 x T1N0M0 2 x no MTC	5 x T1N0M0 2 x T4N1M1 (2 MEN2B)

FMTC = familial medullary thyroid carcinoma

Other relevant considerations

RET mutation testing considered best practice

Despite the limitations in the direct evidence identified in this systematic review, there is unlikely to be any improvement in the evidence base in the future, with the possible exception of longer term follow-up being reported for the historical controlled studies. This is because RET mutation testing and prophylactic surgery are now considered the 'gold standard' in the diagnosis of patients with MEN2 and prediction of MEN2 risk in family members (Learoyd & Robinson 2005). It would therefore be considered unethical to perform a controlled trial examining the direct impact of RET mutation testing on the health outcomes of these people because the control arm would not, therefore, have access to the 'gold standard' method of determining MEN2.

Implications to the consumer

Public comment was sought during the development of the final DAP, which was released for public comment on 7 October 2011 and closed for comments on 4 November 2011. No comments from the public were received.

Ethical considerations

Introduction

Genetic testing of the *RET* gene in children and siblings of individuals with MEN2 is the only effective route to prevention and treatment for hereditary MTC, and is now considered standard care (Burke, Pinsky & Press 2001). Early identification permits the use of prophylactic thyroidectomy, which has been demonstrated to improve life expectancy and quality of life (Raue & Frank-Raue 2012). Yet a significant number of individuals choose not to undergo Ret mutation testing, indicating that there are barriers to understanding and appreciating its benefits for hereditary MTC (Rosenthal & Diekema 2011).

The aim of this assessment report is to synthesise the available evidence in order to inform a public funding decision. In the case of ethical issues, such synthesis equates to reviewing the relevant literature and assessing the balance of the arguments. The synthesis is descriptive but it is also normative insofar as it seeks to identify ethical ideals for framing policy on how medical professionals should conduct themselves.

Methods of evidence synthesis

Seven core papers (Burke & Press 2006; Giarelli 2001; Green & Botkin 2003; Kinder 1998; Korf 1999; Offit & Thom 2007; Winslow, Kodner & Dietz 2005) were selected from the 249 articles identified as potentially relevant in a literature search that obtained papers

addressing the linking of ethical theory to genetic testing. These constituted the main body of evidence. Where possible they were supported by additional articles that presented (i) material from an Australian perspective and (ii) issues relating specifically to RET mutation testing. Some of these additional articles were identified in the 'ethics' literature search, while others were identified in the systematic literature review searches conducted to assess the clinical safety and effectiveness of RET mutation testing. Additional key texts in medical ethics (Beauchamp & Childress 2001; Munson 2000; Rogers & Braunack-Mayer 2004) and web resources (ALRC 2003; HGSA 2008) were also sourced.

Genetic exceptionalism

The 'exceptionalism' debate began in 1991, with respect to HIV infection, during the evolution of HIV/AIDS policy (Bayer 1991). Genetic exceptionalism, which propounds that genetic information is special and distinct from other forms of information, resulted in development of gene-specific privacy and discrimination policies in many countries (Gostin & Hodge 1999). Several features of genetic testing support the concept of genetic exceptionalism:

1. Genetic testing for heritable conditions provides information that is private and personal but is also relevant to individuals other than just the tested individual. Test results have implications for family members, who may or may not wish to know their risk of suffering from a given disease.

2. Many tests are used to predict disease development that may occur many years into the future. Because of this, the psychological ramifications can be very different from the situation where the test is being used for a symptomatic patient.

3. There is concern that genetic information can be used to discriminate against individuals, as illustrated by a few incidences of discrimination by insurers and employers. Under Australian law an insurance company is unable to require that a genetic test is undertaken as a condition of insurance; however if a person has been genetically tested, they are obliged to inform the insurer of the outcomes of that test. Additionally, Australian law does not prevent an employer requiring a new or potential employee to provide a DNA sample.

4. Most patients have only a limited knowledge of genetics. Because of this, an informed consent process requires adequate counselling on an extensive array of issues. These include both standard considerations and those particular to individual and/or family circumstances (Kinder 1998).

Genetic exceptionalism is not universally accepted. Some argue that genetic and non-genetic diagnostic and predictive testing feature more similarities than differences (Diergaarde et al. 2007; Green & Botkin 2003), and that the introduction of genetic testing into medical

practice does not fundamentally alter the ethical obligations of physicians to their patients. Results from non-genetic tests such as cholesterol levels, HIV status, alcohol or narcotic addiction, blood pressure and a family history of inheritable disease can be used in the same way as genetic test results to discriminate against and/or stigmatise individuals. Likewise, some of these results have the potential to either affect family members or cause psychological harm in the same way as genetic test results can. Thus, they raise the same ethical dilemmas concerning the preservation of autonomous choice, privacy and confidentiality as genetic testing, and should all be handled in the same way (Green & Botkin 2003; Lazzarini 2001; Suthers 2008a). Some contend that the clinical integration of genetic risk assessment for common malignancies such as colon and breast cancer has negated the need for treating genetic information as special. This belief incorporates the view that many medical interventions are now reliant on genetic information in order to offer the best possible clinical care (Offit & Thom 2007).

Despite these counterarguments to genetic exceptionalism, there is no broad acceptance that genetic information is the same as other kinds of medical information. The United Nations Educational, Scientific and Cultural Organization, which is concerned with moral issues in relation to science, developed normative international standards for the use of biomedical applications using the genetic exceptionalism approach (Soini 2012). It also forms the basis of genetic-specific legislation governing privacy and discrimination in various countries. As a consequence, for this review we have adopted the conservative view of genetic exceptionalism—that genetic information is indeed unique and particularly vulnerable to misuse.[10]

Ethical framework

The philosophical approach adopted by this assessment is principlism because it is predominant within the field of biomedical ethics (Beauchamp & Childress 2001; Munson 2000; Rogers & Braunack-Mayer 2004). Furthermore, no alternative approach was used in any of the papers included in this assessment. Recently it has been recommended that health technology assessments should incorporate a comprehensive ethical analysis (Duthie & Bond 2011). Although a philosophical defence of principlism has not been possible within the confines of this assessment, it does not unduly undermine the assessment's capacity to report on the main ethical issues as identified in the literature search. Nor does it preclude a reasonable understanding of the main issues identified.

[10] For further discussion see the Australian Law Reform Commission President's preliminary account of the 2003 joint inquiry into the protection of human genetic information (Weisbrot 2003).

The 'four principles' approach

Principlism outlines four main principles—autonomy, non-maleficence, beneficence and justice—which are used to assess the ethical issues associated with genetic testing, as briefly described below.

Autonomy

Autonomy refers to self-rule. Individual autonomy is the governing of oneself and the directing of one's own life, free from coercive interference on the part of others and from limitations that might prevent one from making meaningful choices. A respect for individuals' autonomy entails that they have a right to self-determination or to act freely in accordance with a self-chosen plan. Such respect underpins the process of informed consent and education in medical care and research, and provides the basis for privacy of medical records (Burke & Press 2006; Giarelli 2001). Most theories of autonomy agree that two conditions are required for autonomous choice—liberty, or independence from controlling influences; and agency, or the capacity for intentional action (Beauchamp & Childress 2001; Winslow, Kodner & Dietz 2005).

Non-maleficence

Non-maleficence refers to not inflicting harm or injury to others, and is associated with the dictum *Primum non nocere*: 'Above all (first) do no harm'. The principle also finds expression in the modern Hippocratic oath: 'I will use treatment to help the sick according to my ability and judgement, but I will never use it to injure or wrong them'. In clinical practice the principle of non-maleficence is often combined with, and sometimes balanced against, the principle of beneficence, a version of which is expressed in the first half of the above Hippocratic oath (Beauchamp & Childress 2001; Giarelli 2001). For instance, even the best diagnostic tests and treatments can carry certain risks of harm, and it is practically impossible for medical professionals to act without ever causing harm. Indeed, causing some harms may be warranted in the light of greater potential benefits. Hence, the avoidance of unwarranted or unnecessary harm, even if unintentional, is paramount to the non-maleficent conduct of health professionals. Inextricably linked to the concept of non-maleficence is the obligation to exercise 'due care', which is not always explicitly defined but rather implied in many professional codes of clinical practice. Aspects of non-maleficent practice that are implied in the clinician's duty of care are neither more nor less important than those explicitly defined (Munson 2000).

Beneficence

The principle of beneficence asserts that it is not enough to respect the autonomy of patients and to avoid causing them harm; in addition, clinicians and providers of health services should act in ways that actively promote the welfare of patients (Kinder 1998). Just as there are standards of due care that explicitly and implicitly define appropriate conduct in

the protection of patients from harm, so too are there explicit and implicit standards of beneficence. For example, an obvious expectation in medical care is the physician's duty to help patients by providing appropriate treatment. More implicit is the wider societal expectation that physicians should make reasonable sacrifices for the sake of their patients. In the absence of a reasonable cause to act otherwise, a physician's neglect of a patient requiring medical intervention understandably warrants the disapproval of that patient and of the physician's colleagues, placing the ethical conduct of the physician in serious question even before potential legal ramifications are considered.

Practical constraints must be applied in acting beneficently. There are countless ways to promote the welfare of a patient, but the majority of people will distinguish between expectations that are reasonable and those that are not. In this way, whether or not clinicians fulfil their duty of beneficence relies on judgement and is constrained by various practical considerations. It is also constrained by the duty to act in accordance with other, sometimes conflicting, ethical principles (Munson 2000). Ethical dilemmas arise precisely when one is torn when acting in accordance with two or more ethical principles that commend different courses of action.

Justice

Justice refers to treating individuals equally. In medical ethics the principle of justice finds expression in the belief that everyone deserves equal access to advances in medicine, and in the importance of fairness in the treatment of patients, particularly in the distribution of scarce resources. Different theories of justice focus on conditions of entitlement, fair and equal treatment, and concerns that the distribution of social goods such as healthcare occurs on the basis of relevant factors, for example degree of need, capacity to benefit and/or particular rights. Distributive justice concerns how resources are distributed, to whom and for what reasons. For instance, difficult choices are sometimes made between greatly benefiting the few (those with rare diseases) and benefiting to a lesser degree the many (Giarelli 2001; Winslow, Kodner & Dietz 2005).

The main ethical issues raised by RET mutation testing for MEN2

Questions relevant to ethical inquiry when assessing a health technology have been listed previously (Hofmann 2005) and have provided valuable guidance for this assessment. However, the questions proposed by Hofmann have not been used as a 'checklist' on a question-by-question basis, as individual concepts cannot be logically separated. The emergent themes or issues are most comprehensibly captured when discussed in a collective manner.

The main ethical issues associated with genetic testing and their most relevant ethical principles are listed in Table 34 and discussed below.

Table 34 Main ethical issues for RET mutation testing and their most relevant principles

Issue	Most relevant principle(s)
Informed consent	Autonomy, non-maleficence, beneficence
Privacy and confidentiality	Autonomy
Balancing risks and benefits	Non-maleficence, beneficence
Potential for discrimination	Justice
Access	Justice
Direct-to-consumer genetic testing	Non-maleficence, beneficence, autonomy

Informed consent

Many people do not have a good understanding of genetics, and seeking informed consent for genetic testing poses particular challenges for clinicians and counsellors. Emphasis is placed on the need for an explicit agreement between the health provider and the patient. The basic elements of informed consent, adapted from guidelines of the American Society of Clinical Oncology (Kinder 1998), are listed in Table 35. In line with the principle of respect for autonomy, clinicians and counsellors should stress that testing for a genetic mutation is completely voluntary and optional. The competence of the individual to be tested would need to be assessed, with information provided in a format that the patient can understand. Particular emphasis should be on the likely accuracy of the diagnosis or prediction and the fact that test results will not always provide definitive information about whether the development of disease will ensue. The limits of other methods for predictive testing, if applicable, would also need to be discussed.

Table 35 Elements to be considered when obtaining informed consent for genetic testing

Element
Autonomy provisions • information on the specific test being performed • implications of a positive and a negative test result • possibility that the test will be inconclusive or not informative • options for risk estimation without genetic testing • risk of passing mutation to children • options to withdraw from study (in the case of genetic tests conducted for research)
Beneficence provisions • options for medical surveillance, risk reduction and screening following testing
Non-maleficence provisions • technical accuracy of the test • risks of psychological distress • risk of insurance or employer discrimination
Paternity provisions • procedures if relatedness (i.e. paternity/maternity) is not as expected • procedures governing notification of family
Privacy—professional responsibilities • confidentiality issues • fees involved in testing, counselling and follow-up care
Special considerations • ownership and research uses of DNA remaining after testing • reproductive uses of genetic information

Source: adapted from (Offit & Thom 2007)

In the context of hereditary MTC, information regarding an individual's disease subtype (MEN2A, MEN2B or FMTC) and the tumours to which they are predisposed, along with the clinical implications of a specific RET mutation, should form an integral part of the pre-test counselling. Individuals should also be given information on non-genetic periodic screening or surveillance methods and their limitations and benefits. Although genetic testing of close relatives of individuals with RET mutations is now considered part of standard clinical care (Burke, Pinsky & Press 2001), these individuals still need to be informed of the likelihood of tumour development, the mean age of onset of disease, available treatments and the long-term prognosis (both with and without genetic testing) associated with a familial RET mutation, so that they may make an informed decision.

Special concerns have arisen with regard to the situation where the intended recipients of genetic tests are unable to give informed consent, specifically children and embryos (Offit et al. 2004). In Australia the genetic testing of children for clinical purposes is not regulated by legislation. However, the World Health Organization, the Nuffield Council on Bioethics and the American Society of Human Genetics have developed guidelines on the genetic testing of children (ALRC 2003), and the Human Genetics Society of Australasia (HGSA) has published a position statement, *Pre-symptomatic and predictive testing in children and*

young people (HGSA 2008). In essence these guidelines and statements affirm that the predictive genetic testing of minors should only be conducted when there is an availability of treatment options that directly benefit the child. A study investigating the attitudes of clinical geneticists to predictive genetic testing in minors showed support for testing young children when it provides a clear medical benefit, such as in the case of MEN2 (Borry et al. 2008). The elevated potential for developing MTC in early childhood and the curative nature of a thyroidectomy suggests that the issue of informed consent cannot safely be deferred until the child is of age. This means that it is important that parents with children at risk of having a RET mutation are well informed about the nature of the disease, the screening procedures that may be avoided if mutations are ruled out by the testing of family members, and disease treatment regimes, with an unbiased presentation of the risks and benefits.

There has been some discussion about the ethical justification for RET mutation screening to be added to existing mandated newborn screening programs, which allow parents to opt out but does not require consent (Rosenthal & Diekema 2011; Shuman et al. 2012). RET mutation screening meets the ethical criteria for newborn screening proposed by the President's Council on Bioethics and the traditional Wilson-Jungner criteria (The President's Council on Bioethics 2008; Wilson & Jungner 1968). It was estimated that approximately 1,000 children born each year will develop hereditary MTC in their lifetime, and that 90% of at-risk newborns could be identified for early treatment with a newborn screening program (5% of individuals with MEN2A or MEN2B and 12% of those with FMTC have no identifiable RET mutation). The principles of beneficence support mandatory newborn screening for RET mutations as it removes decision-making from the parent. However, parental consent for a prophylactic thyroidectomy to prevent MTC would still be required. There have been many well-documented cases of parents refusing consent to medical procedures or treatment for their children (Rosenthal & Diekema 2011).

An ethical dilemma arises when parents refuse to provide consent for genetic testing for children at risk of hereditary MTC. Parents have the right to make decisions with regard to their children's health on the basis of their individual autonomy and beneficence (Shuman et al. 2012). However, the non-maleficence principle only allows an individual complete autonomy over his/her own beliefs and actions as long as they do not cause harm to others. If a parent's or guardian's actions or decisions places a child in harm's way, intervention is potentially justified (Rosenthal & Diekema 2011). Theoretically, in cases where a delay in genetic testing could result in a delay in undergoing a prophylactic thyroidectomy to prevent disseminated cancer, legal intervention to overturn the parental decision could be sought. However, as the risk of harm is not immediate and does not constitute a medical emergency, repeated discussions and mediation should be attempted before overriding parental decision-making rights with regard to genetic testing for RET mutations. It should be noted that there is no legal precedent for overriding parental rights in hereditary MTC

even though this may violate the ethical principles of beneficence and justice in certain cases (Shuman et al. 2012).

The issue of prenatal testing introduces particular ethical considerations insofar as definitions of personhood are contentious. Ethical guidelines for Australian practice in the area of genetic testing at the embryonic stage of human development appear to be lacking; however, various medical associations in the USA and Europe have developed similar positions. The main message is that, while prenatal testing is usually considered acceptable in instances of increased risk of foetal genetic disorders, embryo selection to avoid genetic disease is not appropriate in all circumstances. It depends on the gestational period at which selection would occur, as well as other factors including the disease's severity, probability of occurrence and age of onset (Offit & Thom 2007). Testing for RET mutations in embryos would need to be considered relative to the best available guidelines. Information about the nature of the disease, screening procedures and disease treatment regimens should be provided to RET mutation carriers who are contemplating prenatal testing. Out of continuing respect for autonomy, these individuals should also be informed of the risk of conceiving affected offspring.

Privacy and confidentiality

The principle of autonomy affirms the right to voluntary genetic testing, entailing access to the best available evidence of risks and benefits. Furthermore, it affirms the individual's right to privacy. While a patient may choose to reveal information, genetic test results must usually be kept confidential by medical personnel. In the case of inherited genetic conditions, keeping the results of a genetic test confidential protects an individual's right to privacy; however, it also limits the ability of other family members to make informed choices with respect to their own health. Given that inheritable genetic disorders are both an individual and a family matter, ethical dilemmas can arise when a clinician is torn between maintaining the confidentiality of a patient's test results and informing family members of their own corollary predisposition to disease (Giarelli 2001).

Although healthcare professionals recognise the need to maintain confidentiality in most clinical scenarios, some circumstances exist in which disclosure may be permissible, even required. From a legal perspective, the courts have ruled that the duty to protect confidentiality is not absolute when a threat to a third party is considered 'imminent' and 'serious' (Rosenthal & Pierce 2005). Judging which specific clinical situations warrant a breach of confidentiality remains one of the most difficult ethical issues raised by genetic testing.

Some authors have identified that the 'duty to warn' family members of genetic risk may be justified on the grounds that the clinician regards the entire family as the patient and, in this sense, revealing genetic information among family members does not represent a breach of confidentiality (Rogers & Braunack-Mayer 2004). However, as per considerations of informed

consent, counselling is required before test results can be disclosed. Counselling helps the initial test recipient understand and deal with information, but also to consider and state how much information they are prepared to share. Counselling the initial test recipient on the benefits of sharing information with close relatives, and in turn providing counselling to those relatives whether they are directly affected or not, is likely to require considerable skill and sensitivity.

Professional societies differ in their positions on confidentiality (Shuman et al. 2012). The American Medical Association and the American Society of Clinical Oncology do not support violating privacy in any manner in order to notify family members of genetic risks. In contrast, the World Health Organization, the National Human Genome Research Institute, the American Society of Human Genetics and the US Institute of Medicine's Committee on Assessing Genetic Risks, in addition to other national and international groups, support the ethical justification for disclosure in selected cases. It has been proposed that the following criteria must be met before a clinician contemplates any disclosure of genetic information (Winslow, Kodner & Dietz 2005):

1. All attempts to bring about voluntary disclosure must be exhausted.
2. The seriousness of the harms posed by the genetic mutation must be imminent and certain.
3. Effective means of preventive or therapeutic intervention must be available.

These criteria would likely apply in instances where index cases with a RET mutation resist disclosure to their relatives. The harms risked by maintaining confidentiality are substantial and certain, as close relatives carrying the familial RET mutation will develop MTC if left untreated, and there is an effective treatment available by undergoing a prophylactic thyroidectomy. Consequently, in the case of hereditary MTC, a breach in confidentiality could be considered ethically justifiable.

While best practice is represented by striving to avoid breaking confidentiality, the Australian Government has enabled genetic counsellors to legally do so in serious cases through its 2006 amendment to the *Privacy Act 1988*[11]. This amendment allows disclosure of genetic information, without consent, to relatives provided such disclosure is 'necessary to lessen or prevent a serious threat to the life, health or safety whether or not the threat is imminent' (Suthers, McCusker & Wake 2011). In 2009 the NHMRC developed guidelines that provide the formal mechanism for implementation of the new provisions under section 95AA of the Act, which practitioners must comply with (NHMRC 2009b).

[11] Website: Privacy Legislation Amendment Bill 2006 (cited 12/03/13)

Individuals who undergo genetic testing are also likely to have concerns about who will have access to their test results, and how the information will be used and for what purposes. They may be particularly concerned about disclosure to third parties—the potential for health and life insurance companies, employers and financial institutions to use genetic information in order to discriminate against them. Confidentiality and privacy are of particular importance in this respect (Beauchamp & Childress 2001). Some commentators have argued that the underwriting of health insurance premiums on the basis of genetic test results should not be an issue for Australian patients. Community rating dictates that all people pay the same rates for the same level of health cover, regardless of their health status and family history (Delatycki 2008). Life insurance, on the other hand, is not afforded the same level of protection against genetic discrimination. This may be defensible if healthcare is considered a basic or fundamental good or right, whereas death benefits are considered a commodity. The reduction of life insurance to a commodity, and thus the perspective that it is very different from health insurance, is contestable.

Legislation has sought to deal with this problem—under Australian law, no applicant for insurance is required to undergo genetic testing. However, results of tests already taken must be disclosed and employers can request a DNA sample from new or potential employees. This has practical implications and the fear of insurance or employer actions does deter some people from being tested (Wilcken 2011).

Weighing risks and benefits

Incorporating the principles of beneficence and non-maleficence means that risks should be minimised and benefits maximised before a genetic test is accepted into clinical practice. Thus, factors pertaining to the predictive value of the test, the benefit provided by interventions that are associated with a positive test result, the availability and acceptability of the interventions, and the possible harms posed by the knowledge of risk or by the interventions used to reduce risk must be evaluated (Burke & Press 2006).

Risks associated with genetic testing are generally psychological and social, but by no means should risks to physical health be neglected. For example, tests that erroneously present an individual as a non-carrier of a mutationin the *RET* gene may result in substantial physical harms to that individual, especially in the case of pre-symptomatic testing. Pre-symptomatic individuals will be afforded a false sense of security about their risk and will almost certainly miss the opportunity for screening or surveillance procedures that offer the potential for early detection and intervention against disease.

On the other hand the likely ramifications of a false *negative* test in a symptomatic individual seeking to confirm a clinical diagnosis are that the patient will experience some level of anxiety about symptoms that are not supported by a genetic diagnosis. In this case the potential for harm could be extended to include close relatives who are at risk of carrying an

inherited mutation but will not be offered predictive testing due to the false negative test result of the index case.

When it comes to the psychologically harmful effects of a positive genetic test result, the risk must be weighed against the potential benefit of information that can lead to targeted surveillance, preventive measures and/or more-specific and -effective treatment (Offit & Thom 2007). Respecting a patient's wish to keep genetic information private minimises the risk, however small to begin with, that disclosed information will lead to the patient being discriminated against and to attendant psychological, social and economic harms.

Historically, genetic information has been used by civilizations to discriminate against individuals and groups, such as Jewish people, African Americans and other ethnic minorities. Hence, there is considerable concern that individuals with positive genetic test results, and therefore known to suffer from conditions of a hereditary nature, may be stigmatised by society, as seen for AIDS patients in the 1980s (Green & Botkin 2003). Societal acceptance of genetic disorders cannot be regulated by law; it can only be ameliorated through education of the public (Winslow, Kodner & Dietz 2005).

Access issues

Individuals who undergo genetic testing deserve justice in resource allocation. Also, access to treatment should be provided in the event that results provide evidence of a RET mutation. In Australia we are lucky that there is fairly equitable access to medical services, with perhaps the exception of rural and remote communities (Wilcken 2011). Nevertheless, the allocation of resources and the resolution of other access issues may create ethical problems for policy makers. They must consider issues such as how cost-effective the treatment should be before publicly funding the RET mutation testing, and to what extent genetic counselling should be offered as part of the testing process (Wilcken 2011). Currently, most molecular tests are expensive and are not included in the MBS. For this reason suspected MEN2 syndrome patients from both rural and remote areas and metropolitan centres would be encouraged to have their blood sample collected through a public hospital so that this facility is charged for the testing. When patients are referred by a private facility they are billed directly and must cover the entire cost themselves. Moreover, no subsidies are offered for pre-implantation genetic testing, which is often preferred by families to avoid the risk of having to abort an affected foetus and which is prohibitively expensive for most people (Wilcken 2011).

Five accredited laboratories offer RET mutation testing in Australia (Suthers 2008b), although the Royal College of Pathologists of Australasia's genetic testing website currently lists only three of these[12]. It is expected that referral overseas would not be a common

[12] Website: The Royal College of Pathologists of Australasia (cited 12/09/12)

occurrence given the relatively low demand for this service. With current funding for RET mutation testing being provided either by the state/territory governments, where testing may be limited by budgetary constraints, or at a personal cost to the patient, it is probable that not all symptomatic patients or at-risk relatives are being tested, and this raises questions of justice. Listing RET mutation testing on the MBS should increase access to the test for all individuals who require it.

It should be noted that the quality of genetic testing varies significantly (Offit & Thom 2007) and that RET mutation testing is no exception. The test available from the Cancer Genetics Diagnostic Laboratory of PaLMS, Royal North Shore Hospital in Sydney, New South Wales, offers PCR and sequencing of RET exons 10, 11 and 13–16; the Molecular Pathology Division of the Peter MacCallum Cancer Centre, Victoria, offers targeted RET mutation analysis; and the Molecular Pathology Division of the IMVS in Adelaide, South Australia, offers a gene screen for all exons and associated splice junctions by direct sequencing. Thus, the range of RET mutations that can be detected may differ between laboratories due to differences in detection methods. This may result in a number of patients with rarer types of RET mutations that cannot be detected by some laboratories being misdiagnosed and receiving a false negative test result.

Direct-to-consumer testing

There is no clear requirement for direct-to-consumer (DTC) genetic testing companies to offer proper and effective pre-test genetic counselling or psychiatric evaluation, and they lack appropriate regulatory oversight to prevent deceptive practices and ensure a quality product (Caulfield & McGuire 2012; Wilcken 2011). Hence, ethical concerns have been raised about the potential harm of DTC services to consumers, implications for the health system and privacy issues related to the commercial storage of genomic data (Caulfield & McGuire 2012).

According to the principle of non-maleficence, the potential for psychological and physical harm caused by DTC genetic testing should be minimised. However, in the absence of appropriate genetic counselling, the results could cause anxiety or lead to an inappropriate behavioural response, either because individuals overinterpret the significance of a positive result or gain a false sense of security from a negative result. There is also potential for psychological and physical harm caused by inappropriate clinical decisions based on an inaccurate performance or interpretation of genetic tests (Caulfield & McGuire 2012; Offit & Thom 2007).

The principle of autonomy advocates that informed consent be obtained from each individual tested and guarantees their right to privacy. It is disconcerting that very few DTC companies have reasonably comprehensive privacy policies for protection of personal information and DNA samples (Caulfield & McGuire 2012). There is no guarantee of

continued privacy if a DTC company is sold, goes out of business or becomes bankrupt. The new owner of the stored genetic information and/or DNA samples may not feel bound by previous privacy arrangements, raising numerous concerns about what could happen to private information in a commercial setting (Wilcken 2011).

In Australia symptomatic patients and their at-risk relatives are unlikely to use DTC genetic testing as most would have testing funded through a public hospital. However, a non-symptomatic individual using such a service may be identified by chance as a RET mutation carrier. These individuals would then require further medical treatment and/or counselling to deal with this finding. The medical practitioner would also need to determine the likely accuracy of the genetic testing that was conducted by the DTC company as well as any further treatment, surveillance or repeat genetic testing that is needed for their ongoing clinical management.

Summary

The consensus standpoints for a range of ethical issues and, in some cases, dilemmas raised by genetic testing are summarised as follows:

- On balance, RET mutation testing appears ethically acceptable provided that it is both preceded and followed by adequate counselling on, among other things, the limitations and significance of test results, including the possible ramifications for family members and the possible courses of effective treatment should a test result be positive:
    - RET mutations identify MEN2, for which there is an effective prophylactic treatment option.
    - Counselling is necessary in order to ensure informed consent and minimise risks of harm, both psychological and, in the longer term, physical.
- Test results should remain confidential, although the patient should be counselled on the benefit of sharing information with family members who may benefit.
- As always, confidentiality should be broken only if risks to others are serious, imminent, certain and avoidable, and attempts at encouraging voluntary disclosure have been exhausted.
- Testing should be available, and not overly financially burdensome, to all who might benefit from it.
- Direct-to-consumer genetic testing appears to carry substantial risks.

Conclusion

With respect to RET mutation testing, the above ethical analysis would suggest that the test should only be offered on the MBS if it is performed in conjunction with genetic counselling from accredited counsellors with familiarity in both interpretation of RET mutation test results and management of the implications for the index case and family members.

What are the economic considerations?

In assessment of a new service MSAC is required to consider not only the comparative effectiveness and safety of the service but also the comparative cost and cost-effectiveness of the service. Thus, an economic evaluation is required, which is based on the clinical evidence for this service when added to or substituted for the main comparator in the relevant setting.

However, because RET mutation testing is *normal practice* for patients who currently present with symptoms that are potentially associated with MEN2 disease or hereditary RET mutations, and is currently funded through state hospitals/governments, an economic comparison between MBS-listed RET mutation testing and current clinical practice would not be expected to show incremental effects in either clinical outcomes or resource expenditure. Rather, it would simply highlight the transfer of costs from one healthcare funder to another. This does not provide information to MSAC on the inherent economic value of RET mutation testing.

The purpose of an economic evaluation is to inform MSAC as to the additional costs and additional gains (health or other socially relevant outcomes) of the proposed service over the comparator when used in the Australian healthcare system. In this context, where clinical practice has already adopted the technology irrespective of the funding source, the comparator has been nominated as the 'historical clinical scenario' (i.e. when RET mutation testing was not available). The following analysis is therefore intended to assist MSAC to ensure that society's health resources are allocated to those activities from which it will get the most value.

Two sets of economic analysis were undertaken to consider the two distinct applications of RET mutation testing (diagnostic and familial screening). With consideration of diagnostic testing of a patient presenting as an index case, a cost-minimisation approach is undertaken; and with respect to familial screening, a cost-utility model is undertaken.

Since the publication of the DAP

Updated information on the current pricing of RET mutation testing in Australia has been obtained (see Table 6 on page xxxiv). In this report the base-case economic evaluations have assumed a lower revised fee associated with testing, based on current prices.

The evidence base identified in the assessment provides little directly comparative and/or non-confounded data. The economic modelling is therefore undertaken on the basis of the available data with various assumptions. Where assumptions are made, these will be explicit and, in the base case, tend toward a conservative approach. Given the limitations of the

available data, all quantification within the economic evaluation may be considered indicative but uncertain.

Economic evaluation

Background literature

A literature search identified four published economic evaluations of RET mutation testing (Table 36).

Table 36 Published economic evaluations of RET mutation testing

Publication	Setting	Model & results
Delbridge L and Robinson B. (1998) Genetic and biochemical screening for endocrine disease: III. Costs and logistics (Delbridge & Robinson 1998)	RET mutation testing of family members of known MEN2A families in Australia	Cost-effectiveness model consideration of pentagastrin (biochemical screening) vs. no screening, and RET mutation testing vs. no testing Results: No testing: $0/lives saved; risk of dying 117/1,000 Pentagastrin testing: $76,315/life saved; risk of dying 22/1,000 RET mutation testing $5,175/life saved; risk of dying 3/1,000
Erlic Z, Rybicki L, Peczkowska M, et al. (2009) Clinical predictors and algorithm for the genetic diagnosis of pheochromocytoma patients (Erlic et al. 2009)	Genetic screening of index patients presenting with phaeochromocytoma	The model considers the costs of screening all potential index cases presenting with phaeochromocytoma for genetically relevant germline mutations to diagnose Von Hippel-Lindau Disease (VHL), MEN2 (RET), paraganglioma syndromes 1, 3 and 4 (SDHD) and type 1 neurofibromatosis (NF1). Taking into account expected diagnostic yields, the model determines the most cost-effective order for sequential testing, and also considers further cost reductions obtainable by pre-selection of at-risk patients.
Gilchrist DM, Morrish DW, et al. (2004) Cost Analysis of DNA-based testing in a large Candian family with multiple endocrine neoplasia type 2 (Gilchrist et al. 2004)	RET mutation testing of a specific family group with MEN2 history, in Canada	The authors conduct a cost analysis of RET mutation testing vs. clinical surveillance in the specific scenario of a family with a history of MTC. In this case the family was large and 58 family members were tested for germline RET mutations, in which only four members were identified as carrying RET mutations. It is concluded that RET mutation testing has resulted in significant cost savings in comparison with biochemical screening in this example.
Pigny P, Cardot-Bauters C, Do Cao C, et al. (2009) Should genetic testing be performed in each patient with sporadic pheochromocytoma at presentation? (Pigny et al. 2009)	Patients presenting with phaeochromocytoma, with no family history or concomitant disease, in France	This cost analysis of genetic testing for RET, VHL and NF1 (vs. alternative clinical/biological/imaging investigations) in 100 patients presenting with sporadic phaeochromocytoma concludes that cost-savings can be achieved without negative clinical outcomes if routine genetic screening for hereditary phaeochromocytoma is excluded in patients with unilateral adrenal tumour diagnosed after the age of 50 years.

The Delbridge and Robinson model is based on Australian clinical practice and is relevant to the proposed listing of RET mutation testing for family members, although the resource prices from the 1990s may require updating. This model uses a decision analytic to demonstrate that, where a family history has been established, RET mutation testing appears to be both more effective (i.e. it decreases the risk of dying) and less expensive (per life saved) than biochemical surveillance. However, the model does not consider initial

RET mutation testing in potential MEN2 index cases and does not present results in the form of an incremental cost-utility analysis with an estimate of QALYs or life-years gained.

The Gilchrist et al. model is specific to a single, particularly large family and the Canadian healthcare system, and is not generally applicable to Australia at a population level. While the Pigny et al. analysis supports the proposed age restriction on RET mutation testing in phaeochromocytoma, it does not provide cost-effectiveness information on the use of RET mutation testing in the proposed listings.

Although consistent in their conclusion that RET mutation testing is cost-effective in the respective setting of analysis, none of the published models are adequate to confirm the cost-effectiveness of the proposed MBS listings of RET mutation testing in both potential index cases and family members in the Australian population. Therefore, further economic modelling of costs and outcomes based on the proposed listings in the intended Australian population is required.

Structure

Economic analyses of costs and outcomes associated with RET mutation testing are undertaken for the four following scenarios (as requested in the DAP):

1. index patients presenting with MTC
2. index patients younger than 50 years of age presenting with phaeochromocytoma
3. first- (or second-)degree family members of patients presenting with MTC
4. first- (or second-)degree family members of patients younger than 50 years of age presenting with phaeochromocytoma

Scenarios 1 and 2 – Cost-minimisation analysis

In the case of diagnostic RET mutation testing in potential index patients (Scenarios 1 & 2), there is no evidence available to suggest a direct benefit in health outcomes to the index patient who is already presenting with symptoms. Equally, because no harms are associated with testing, it is assumed that there will be no change in clinical outcomes in either direction for the index patient. Therefore, the approach taken for these scenarios is a cost-comparison (cost-minimisation). In these scenarios the costs of RET mutation testing all potential index cases younger than 50 years of age presenting with either MTC or phaeochromocytoma, and subsequently commencing routine annual surveillance for additional MEN2 complications in those patients identified to have a RET mutation, are compared with the costs of providing routine annual surveillance for MEN2 complications in all patients younger than 50 years of age presenting with MTC or a phaeochromocytoma, because MEN2 could not be ruled out.

A diagrammatic representation of the structure of the cost-minimisation analysis of RET mutation testing in potential index patients *presenting with MTC* is shown in Figure 9.

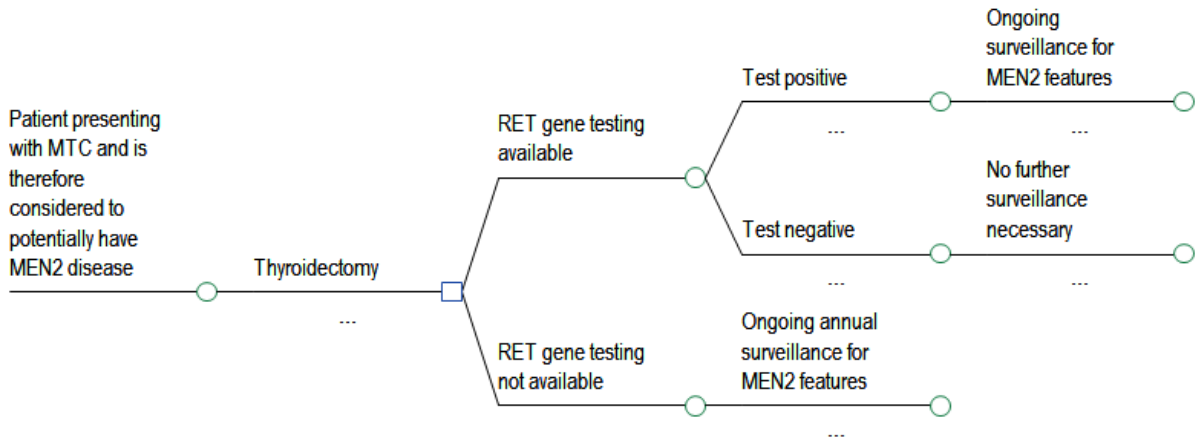

Figure 9 Structure of cost-minimisation comparisons of RET mutation testing and selective surveillance versus non-selective surveillance for MEN2 complications, in patients presenting as potential index cases (Scenarios 1 and 2)

A diagrammatic representation of the structure of the cost-minimisation analysis of RET mutation testing in potential index patients younger than 50 years of age *presenting with phaeochromocytoma* is shown in Figure 10.

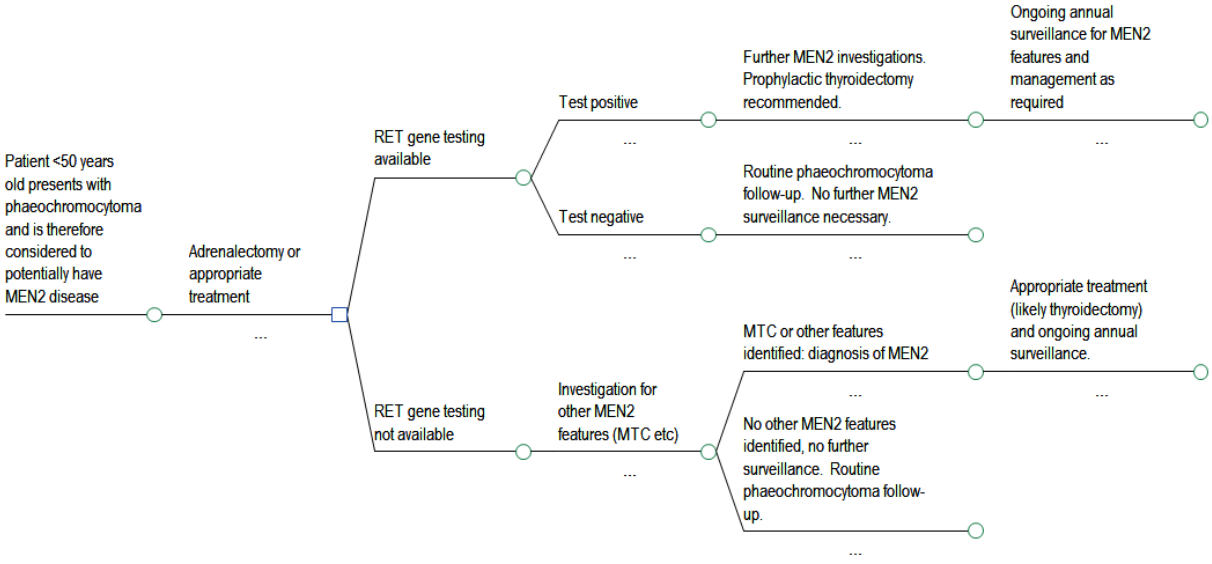

Figure 10 Structure of cost-minimisation comparisons of RET mutation testing and selective surveillance versus non-selective surveillance for MEN2 complications, in patients younger than 50 years of age presenting with phaeochromocytoma (Scenarios 1 and 2)

The cost-minimisation analysis considers only the costs and downstream costs of the proposed diagnostic test (full screen of relevant exons) for RET mutations, and ongoing clinical investigation/surveillance for other MEN2-related conditions in the patient presenting with potential MEN2A.

The incidence of MEN2A and the extent and costs of downstream *treatments* associated with the management of the presenting complaint (MTC or phaeochromocytoma) in the potential index case (confirmed or not as MEN2) will occur identically, irrespective of the availability of RET mutation testing; if these were to be included in the economic evaluation, they would 'cancel out' in each arm. Therefore, they have not been included in the cost-minimisation analyses of Scenarios 1 and 2.

The cost-minimisation models for Scenarios 1 and 2 run over 30 years.

Scenarios 3 and 4 – Cost-utility analysis of the availability of diagnostic RET mutation testing in suspected index cases and subsequent prognostic familial screening in first- and second-degree relatives of affected cases, versus non-availability of RET mutation testing.

The third and fourth economic analyses are cost-utility models that attempt to measure the incremental difference in clinical outcomes and resource use across the broader population cohort relevant to the proposed listing of the prognostic screen for family members. The modelled cohorts are the suspected index cases *and their potentially at-risk family members* because, given the serious and known hereditary nature of MEN2 diseases, historical clinical practice advised that all family members of a potential MEN2 patient should also receive ongoing medical surveillance for symptoms of disease (MTC, phaeochromocytoma, hyperparathyroidism). The second proposed MBS listing is for RET mutation testing in first- and second-degree family members of patients identified as RET-mutation-positive.

The comparison modelled (with index cases being (i) MTC or (ii) phaeochromocytoma at younger than 50 years of age) is:

RET mutation testing (index and family) available—index cases are tested; RET-mutation-negative index cases and their families require no further surveillance for MEN2/RET associated diseases. RET-mutation-positive index cases maintain ongoing annual surveillance for other MEN2 complications and their first- and second-degree family members are also tested for hereditary RET mutations. RET-mutation-negative family members require no surveillance for MEN2, while RET-mutation-positive family members are recommended prophylactic thyroidectomy where appropriate and ongoing annual surveillance.

versus No RET mutation testing available—all potential index cases and family members of potential index cases are advised to receive ongoing annual surveillance for MEN2 complications, but a MEN2 diagnosis would only be made with the development of additional clinical symptoms or positive biochemical test results or imaging studies. Prophylactic/treatment thyroidectomy occurs as indicated.

The key assumptions that drive the model are:

- that identification of RET-mutation-negative cases (index and/or family members) will substantially decrease the resource use and patient anxiety associated with what can subsequently be identified as unnecessary patient surveillance.

- that RET mutation testing enables earlier detection of MEN2 disease and, once identified as RET-mutation-positive, the risk–benefit profile of prophylactic thyroidectomy becomes more favourable. Therefore, prophylactic surgeries can be undertaken more often and sooner. Surgery at a younger age increases the likelihood that the thyroidectomy will more effectively cure or prevent future MTC, thereby increasing both survival and quality of life.

The health states associated with RET mutation testing and MTC included in the model are shown in Figure 11.

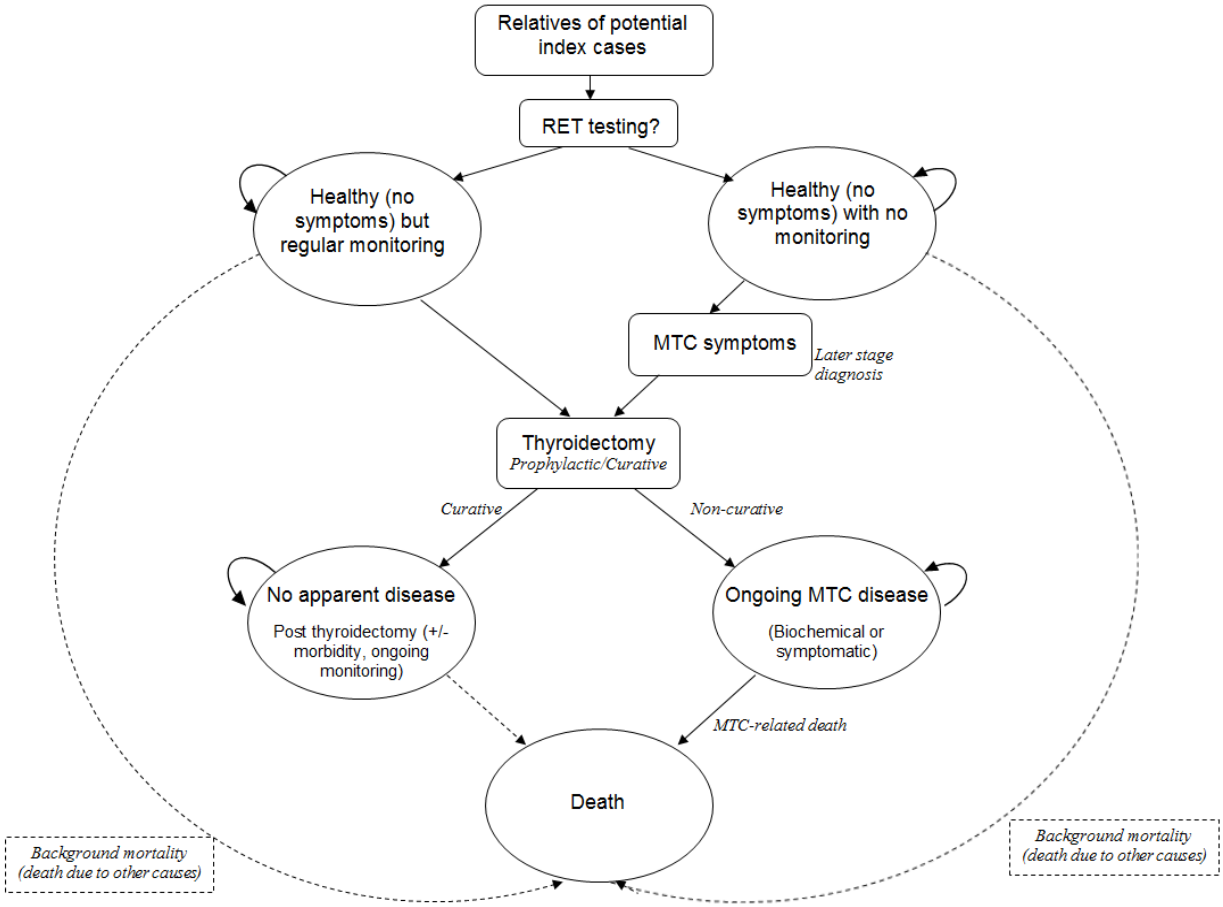

Figure 11 Health state transition diagram for potential MEN2 patients (i.e. family members of known MEN2 patient)

While other health states including phaeochromocytoma, hyperparathyroidism etc. may also be associated with MEN2, there is inadequate evidence to model any effect of RET mutation testing on the prevalence or pattern of these conditions; therefore, they do not need to be included in this model, which is intended to identify incremental effects only.

The cost-utility model runs over a 'lifetime' time horizon (70 years); however, results for shorter time horizons (10, 20 and 50 years) are also presented.

Discounting at a rate of 5% *per annum* is applied to costs and outcomes in all models.

Each model is calculated beginning with a cohort of 100 cases presenting with MTC/phaeochromocytoma, and assumes that these patients are an average age of 40 years.

Sensitivity analyses also consider the inclusion of familial screening in the cost-minimisation, although there is an argument that clinical outcomes may change (be improved) with familial screening; therefore, this is more thoroughly investigated with a cost-utility analysis.

Inputs

The inputs used in the economic models are from various sources which are detailed in Table 37 to Table 43.

The demographic and diagnostic parameters relating to the patient and family populations are detailed in Table 37. Transition probabilities between health states are detailed in Table 38. With respect to population and disease characteristics the median of results compiled from multiple sources in the systematic review generally informed the base-case inputs, unless an alternative justification is given. Where the data from studies was inconsistent or wide ranging, alternative inputs are tested in the sensitivity analyses. Where possible, inputs based on assumptions are also tested in sensitivity analyses.

Table 37 Inputs used in the economic models relating to population, disease and test characteristics

Variable	Base case	Source and discussion	Sensitivity analyses
Discount rate	5%	Convention used by Department of Health and Ageing	Nil
Diagnostic yield of RET mutation test in patients with MTC	30.6%	Median based on 6 studies presenting diagnostic yield from unspecified cases of MTC (Table 83)	
Diagnostic yield of RET mutation test in patients younger than 50 years of age with phaeochromocytoma	18.3%	The proportion of people with phaeochromocytoma identified as RET M+ in Krawczyk et al. (2010). This estimate is highly uncertain. There is no data identifying the prevalence of RET mutations that specifically considers patients younger than 50 years of age as a single risk factor. Alterative estimates based on the findings in this report on the incidence of mutation in phaeochromocytoma include a median of 7.8% in unspecified phaeochromocytoma cases (not defined by age) and 24.4% in cases with familial history (Table 84). These are tested in sensitivity analyses.	7–25%
Diagnostic yield of predictive RET mutation test in first- and second-degree relatives of known RET M+ patients	39.4%	Median based on 48 studies including second-degree relatives / not specifying relationship (Table 85); similar to median of studies of first-degree relatives only (37.5%, n=11). Biologically, 50% inheritance rate, but expect less due to *de novo* cases and some affected patients already diagnosed/dead	50%
Number of family members per index patient	11.4	Familial Cancer Unit of South Australia	5–15
Compliance rate for family-member surveillance in untested population	40%	Assumed that surveillance rate would be approximately equal to test uptake rate	10–90%
Familial screening uptake rate	40%	Suthers et al. (2006)	10–90%

RET M+ = RET-mutation-positive

Table 38 Transition probabilities used in cost-utility analysis (applicable to family members only)

From health state	To health state	Probability	Discussion
Healthy and undertaking either RET mutation test and/or biochemical investigation and surveillance	Post thyroidectomy	Where RET testing available: 100% of RET M+ patients are assumed to receive prophylactic thyroidectomy in the following year. Where RET testing is not available: 70% of RET M+ patients are assumed to receive prophylactic thyroidectomy. In the scenario of the index patient presenting with MTC, this is assumed to occur over the following 3 years (30%, 20%, 20% in each year). In the scenario where the index presentation is phaeochromocytoma, all prophylactic thyroidectomies are assumed to occur in the following year (i.e. 70%).	The assumption that all patients identified as RET M+ receive prophylactic thyroidectomy is based on current recommendations (Brandi et al. 2001). However, less than 100% is commonly reported and thyroidectomy uptake rates vary considerably between studies (Table 98), such that modelling 100% thyroidectomy results in a conservative estimate of the maximum likely additional surgical costs associated with testing. The assumption that only 70% of RET M+ patients receive early thyroidectomy where RET mutation testing is not available is based on the symptomatic penetrance rate of MTC. This is conservative with respect to the allocation of costs of thyroidectomy but does not bias the results with respect to outcomes, as the 30% of people who do not receive the thyroidectomy are assumed to have normal survival. In the MTC index case scenario the assumption that most surgery is performed over the 3 years following initial investigations and monitoring is consistent with the average age of surgery being a few years later in the pre-RET mutation testing era (Table 29). In the model where index patients present with phaeochromocytoma, identification of a RET mutation or signs of MTC in family members is assumed to require management in the year immediately following diagnosis, as (relatively) early manifestation of phaeochromoytoma suggests a patient group with a higher risk mutation (Moline & Eng 2011)
Healthy with no surveillance	Symptomatic / late-stage disease	Occurs in RET M+ people only: 70% at year 15	Year 15: (symptoms, thyroidectomy) – Post-thyroidectomy penetrance of symptomatic MTC is ~70%. Undetected MTCs in non-thyroidectomised patients have been assumed to become apparent at year 15.
Healthy (no surveillance) OR post-thyroidectomy	Death	Background mortality rate less people developing symptomatic disease.	Based on average mortality of people (men + women), starting at 40 years of age in year 1, from the Australian Bureau of Statistics (ABS 2012). In patients who have had a thyroidectomy: in the RET era 90% are assumed to be successful (i.e. long-term cure); in the pre-RET era 60% are assumed to be successful (based on the findings in Rohmer et al (2011) and the overall RR estimate derived in Results - Figure 7). 'Non-successful' thyroidectomies are not apparent until year 15.
Symptomatic / late-stage disease	Death	Kaplan Meir post-thyroidectomy survival curve from Kakudo et al. (1985); with linear extrapolation beyond 30 years.	Kakudo et al. (1985) (stage I, n=19; and stage III, n=27) case series (Kakudo, Carney & Sizemore 1985)

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive

Resource costs in the model are described in Table 39 to Table 43. These tables describe the application of the costs as well as their derivation and source. With the exception of pentagastrin ampoules (for which a current local cost could not be obtained), all resources costs used in the economic model are from current published Australian sources.

Table 39 Genetic testing costs applied to index patients and / or family members of known index cases in the 'RET mutation testing available' arms (all models)

	Costs	Derivation	Application
RET mutation testing in potential index cases	$436.30	Comprises of: RET mutation test (full set of relevant exons) $400 Genetic counselling $36.30	One-off cost in year 1 for each patient presenting with MTC or phaeochromocytoma.
RET mutation testing in first- and second-degree family members of known index cases	$236.30	Comprises of: RET mutation test (known mutation) $200 Genetic counselling $36.30	One-off cost in year 1 for each family member of confirmed index case who undergoes genetic testing

MTC = medullary thyroid carcinoma

Table 40 Initial investigations and ongoing annual surveillance costs for index patients presenting with MTC, with unknown (untested) or positive RET mutation status.

	Costs	Derivation
Investigation for phaeochromocytoma	$83.35	Plasma/urine catecholamines: (described in MBS Item 66695), but combined with ≥5 tests, therefore MBS item 66707 used
Investigations for hyperparathyroidism	$9.70	Serum calcium (MBS Item 66500) +/- parathyroid hormone (described in MBS 66695) but this is combined with ≥5 tests, included in MBS 66707 above

MBS = Medicare Benefits Schedule

Table 41 Initial investigations and ongoing annual surveillance costs for index patients presenting with phaeochromocytoma, with unknown or positive RET mutation status

	Costs	Derivation
Investigations for MTC	$152.80	Calcitonin (described in MBS Item 66695, but combined with parathyroid below), 2 tests, MBS 66698: $43.70 Neck (thyroid) ultrasound (MBS 55032) $109.10.
Additional costs for pentagastrin-stimulated calcitonin test (no RET mutation testing available arm)	$302.00	Pentagastrin ampoule $241, from Gilchrist et al. (2004) Calcitonin; baseline (included in sample above) and at 3 minutes and 5 minutes: (MBS 66695) $30.50 x2
Investigate for hyperparathyroidism	$0	Serum calcium (MBS Item 66500) assumed to be included in patient episode cone and not reimbursed +/- parathyroid hormone (described in MBS 66695) but as it is combined with 2 tests, included in MBS 66698 above

MBS = Medicare Benefits Schedule; MTC = medullary thyroid carcinoma

Table 42 Initial investigations and ongoing annual surveillance costs for first- or second-degree family members of index patients, with unknown or positive RET mutation status, compliant with testing/surveillance recommendations

	Costs	Derivation
Consultation	$36.30	*MBS Item 23*
Investigate for phaeochromocytoma	$83.35	Plasma/urine catecholamines: (described in MBS Item 66695), but combined with ≥5 tests, therefore MBS item 66707 used
Investigate for hyperparathyroidism	$9.70	Serum calcium (MBS Item 66500) +/- parathyroid hormone (MBS 66695) but as it is combined with ≥5 tests, included in MBS 66707 above
Investigate for MTC	$109.10	Neck (thyroid) ultrasound (MBS 55032) $109.10 Calcitonin (MBS 66707), but ≥5 tests required, therefore included in MBS 66695 above
Additional costs for pentagastrin-stimulated calcitonin test (no RET mutation testing available arm only.)	$271.50	Pentagastrin ampoule $241, from Gilchrist et al. (2004) Calcitonin; baseline (included in sample above) and at 3 minutes and 5 minutes: (MBS 66695) $30.50 (additional test assumed to be included in patient episode cone).

MBS = Medicare Benefits Schedule; MTC = medullary thyroid carcinoma

Table 43 Thyroidectomy and post-thyroidectomy costs in family members of index patients receiving prophylactic thyroidectomy (following positive genetic or biochemical test) or treatment thyroidectomy (after detection of MTC).

	Costs	Derivation
Thyroidectomy (one-off)	$5,491.59	Includes: Thyroidectomy (MBS Item 30296) $1,023.70 Pre-anaesthetic consultation (MBS Item 17615) $85.55 Anaesthesia (MBS Item 20320) $118.80 Age modifier for anaesthesia (MBS item 25015) $19.80 Assistant (MBS item 51303) $204.74 Hospital accommodation for thyroid operation (Private hospital Inpatient NHCDC[a] cost weights for K06Z: ALOS 2.04 days) $4,039.00
Post-thyroidectomy (annual)	$54.22	Thyroxine tablets (PBS Item 2173J $27.11, 200mcg x200 tabs), required 2x prescriptions/year (assumed)
Ongoing surveillance for other MEN2 symptoms following development of MTC, *in patients previously non-compliant with testing/surveillance recommendations*	$129.35	Includes: Consultation (Item 23) $36.30 Investigation for phaeochromocytoma (Plasma/urine catecholamines (≥5 tests described in MBS 66695) (MBS Item 66707): $83.35 Investigation for hyperparathyroidism (Serum calcium, MBS Item 66500, $9.70 +/- parathyroid hormone, included in MBS 66707).

ALOS = average length of stay; MBS = Medicare Benefits Schedule; MTC = medullary thyroid carcinoma

[a] National Hospital Cost Data Collection private hospital costs, AR-DRG version 5.1 round 13 (2008–2009) Round 13 (2008-09) Cost Report

The survival curve following thyroidectomy as presented by Kakudo et al. (1985) is shown in Figure 12.

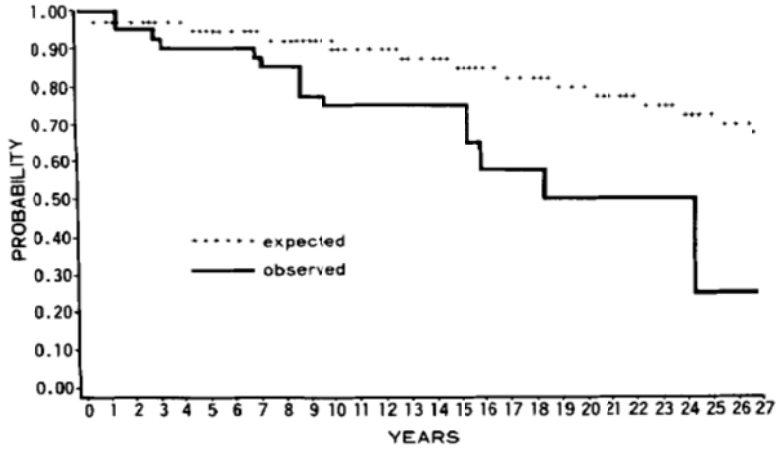

Figure 12 Cumulative survival curve for 46 patients following thyroidectomy for MTC by Kaplan-Meier method from Kakudo et al. (1985)

Pentagastrin ampoules

The injectable drug pentagastrin used in the calcitonin stimulation tests to identify early MTC prior to availability of RET mutation testing is not routinely available in Australia. No current pricing of this drug was available through routine wholesaler enquiries; therefore, the estimated cost of $241 per biochemical test (including injection-associated consumables) is based on the unadjusted value (in Canadian dollars) used in the published study by Gilchrist et al. (2004). While the currency exchange rates are similar, given inflation, differing healthcare systems and importation costs, this value is uncertain and may be an underestimate. If the cost of pentagastrin is underestimated, this will result in the model underestimating both the costs of biochemical surveillance and the cost savings associated with RET mutation testing.

Test accuracy

Test accuracy data is not included in the model and this is a significant shortcoming. Although the RET mutation test is assumed to be relatively accurate, there are reports of false positives, and the negatives do not necessarily rule out MEN2 disease. Given the robustness of the model to identifying both cost savings and health benefits, it is unlikely that the incorporation of sensitivity/specificity data—if available—would change the overall conclusion of economic effectiveness, although it is theoretically possible if the test performance is poor enough. While this assumption of 100% test accuracy does not completely reflect reality, it does not necessarily introduce a bias favouring RET mutation testing into the economic model—equally, the accuracy of biochemical surveillance is excluded from the model, and this too is imperfect. Learoyd et al. (1997) and other publications report on a number of known cases of false positives associated with biochemical monitoring, with estimations that the rate of false positives associated with pentagastrin stimulation may be as high as 10% (Costante et al 2009). False positives

associated with biochemical monitoring would increase the costs and decrease the health outcomes associated with the comparator. If, in reality, the rate of false positives is lower with RET mutation testing than with biochemical monitoring, then the omission of this information from the model is a source of bias against RET mutation testing and a further area where the model is conservative with respect to estimates of the incremental benefits associated with RET mutation testing.

Palliative care

An accurate estimation of palliative care costs associated with advanced MTC in the Australian setting was not obtained and these costs have not been included in the model. Although palliative care costs, if included, would occur late in the time horizon of the model and be substantially discounted, the omission of these costs results in a likely underestimate of the cost savings associated with RET mutation testing. In the cohort where RET mutation testing is available, relative to where it is not available, the increased prophylactic and early surgery associated with identification of RET mutations would decrease the incidence of advanced MTC requiring palliation.

Utility values

A literature search for utility values associated with MEN2A, thyroidectomy (including complication of surgery) and MTC was undertaken. While many sources of published utility values were identified, only one source (Li et al. 2011) was identified that included 'watchful waiting'—equivalent to surveillance—which has a significant role in the economic model. In general it appeared that the utilities reported by the various sources were generally consistent with each other and not wide-ranging; however, for improved consistency, a single source was selected for the model. The utility values reported by Li et al. (2011) are derived from time-trade-off methodology with a panel of thyroid experts, and also supported by alternative literature where available. The utility values used are described in detail in Table 44, in addition to the weightings applied to them. The actual thyroidectomy procedure has not been allocated a unique utility value; rather, the entire post-thyroidectomy value is assumed for the entire year of the procedure.

Table 44 Health state utilities used in cost-utility analysis

Health state	Utility	Source	Discussion
Healthy with no symptoms and not undergoing any surveillance	1	Assumed	This utility applies to family members of index patients who are RET mutation tested and found to be RET M–, and to family members of index patients who do not participate in surveillance who may be either RET M– or RET M+ but who are not yet symptomatic of MEN2 disease.
Healthy and undergoing surveillance	0.98	(Li et al. 2011)	Described as 'watchful waiting', this utility was determined using time-trade-off methodology with a panel of thyroid experts. This appears reasonable at face value and allows for the anxiety of being 'at risk' and requiring regular monitoring.
Post-thyroidectomy	0.944	Weighted combination from (Li et al. 2011)	Weighting of utilities from Li et al. (2011) for post-thyroidectomy (no complications): 0.97; and post-thyroidectomy (permanent complications): 0.65. Weighted at 92% and 8%, respectively, based on 8% rate of permanent complications (hypoparathyroidism/hypocalcaemia, 6.7%; and recurrent nerve injury, 1.3%) reported following surgery in asymptomatic family members (Dralle et al. 1998).
Advanced MTC / recurrence	0.60	(Li et al. 2011)	Estimated to be a reasonable average utility across all time points (from diagnosis to progression/death) for patients diagnosed with MTC who have not been tested or monitored, and present late due to symptoms. Disease is assumed to be at a later stage (e.g. commonly stage III) compared with cases of MTC identified/predicted through screening/surveillance (generally precancerous CCH/stage I).

MTC = medullary thyroid carcinoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Assumptions

The model does not attempt to replicate all potential health outcomes and pathways that may occur in clinical practice. There is potentially an infinite number of combinations of age, genetic risk, disease stage and treatment outcomes that could exist for both index cases and family members, and there is little reliable data on the distribution of these in RET-mutation-positive populations generally, let alone within Australia. Therefore, the model rather crudely predicts costs and outcomes where times to events, patient age, survival, disease development and treatment outcomes are point estimates. The following assumptions are made:

- *RET mutation testing does not impact MTC or phaeochromocytoma treatment or outcomes (costs, utilities or survival) in index cases* presenting with symptoms of disease. (These will be identical in each arm of the model and would cancel out of any incremental calculation; *therefore, these parameters are not calculated or included in the model*.) This may be a conservative assumption as patients with an identified RET mutation may be more diligent in routine monitoring for additional symptoms and subsequently have better clinical outcomes. Also, index patients identified as RET-mutation-negative may experience a utility benefit associated with the knowledge that they are not at increased risk of other MEN2-associated conditions.

- *RET mutation testing does not impact the incidence or treatment of phaeochromocytoma (costs, utilities or survival) in familial RET-mutation-positive patients*. This is based on the fact that, unlike MTC, no prophylactic measures are taken to prevent development of

phaeochromocytoma in RET-mutation-positive patients, and the RET mutation test does not alter the natural pathway of disease. If RET mutation testing is associated with earlier detection of phaeochromocytoma and this is associated with improved outcomes, then this approach is conservative.

- The average age of patients entering the model is 40 years, based on the mean age of diagnosis of index patients in the pre-RET mutation testing era (Table 28). The background mortality rate is then based on ABS data on the average Australian mortality rates from age beginning at 40 years. Although uncertain in reality, for simplicity it is assumed in the model that this is also the average age / annual background mortality rate of family members also. The higher the background mortality rate, the more conservative the model is with respect to RET mutation testing, as the majority of the costs for testing occur in the first few years of the model, whereas costs associated with biochemical screening are spread more evenly over a person's lifetime.

- Patients identified as RET-mutation-positive through genetic familial screening (non-symptomatic) are all recommended to have prophylactic thyroidectomy. The model assumes 100% of RET-mutation-positive patients receive a prophylactic thyroidectomy in the year immediately after they are tested for a RET mutation. This is a conservative approach with respect to estimating costs associated with RET mutation testing, as in practice some of these thyroidectomies will be delayed a few years (in low risk patients) and therefore costs should be discounted, and some RET-mutation-positive patients refuse prophylactic treatment and/or ongoing surveillance and do not incur these further costs (Alvares Da Silva et al. 2003; Kinlaw et al. 2005; Romei et al. 2011).

- In untested true RET-mutation-positive patients it is estimated that only 70% would develop a *symptomatic* MTC and subsequent thyroidectomy before the age of 70 years (Frank-Raue et al. 2011). This assumption has also been assumed to apply to patients undgoing monitoring in the pre-RET mutation testing era. While actual lifetime penetrance rates of any MTC (including microscopic and those identifiable only through biochemical/pathology testing) are estimated at close to 100%, sometimes the MTC would not develop to be apparent or symptomatic before the patient has died of other conditions or reaches an age where active treatment is inappropriate.

- In the historical comparison arm, of the family members who participate in biochemical screening and are true RET-mutation-positive, the first 5 years of biochemical screening detects all 70% of cases who would develop symptomatic MTC in their lifetime, and these patients then undergo prophylactic/early thyroidectomy. Of these cases, 30% are assumed to be identified in the first year of screening and an additional 20% in each of the second and third years after screening commences.

- The 'cure rate' for prophylactic thyroidectomy in RET-mutation-positive family members is assumed to be 90% (i.e. the rate of patients developing symptomatic advanced stage relapse is assumed to be 10%). This assumption approximates the the median estimate of the rate of recurrence in patients for whom surgery is considered prophylactic (9%). Where family members do not have access to RET mutation testing, but have early surgery on the basis of family history and biochemical surveillance, the cure rate is estimated at 60% and the relapse rate at 40%. These estimates are based on the results published by Rohmer et al. (2011) and the meta-analysis presented in Figure 7. These estimates are based on limited data and it is acknowledged that they are highly uncertain and likely to be biased. Alternative RRs, up to 1 are tested in the sensitivity analyses.

- Where prophylactic/early MTC surgery is curative and successful, life-expectancy is equivalent to the normal population. This is supported by literature reports that patients with stage I MTC have a life expectancy similar to the general population (de Groot et al. 2006) and that long term mortality in MEN2A patients alive 10-15 years after surgery is similar to the population.(Szinnai et al. 2003)

- Where prophylactic/early MTC surgery is not successful and relapse occurs, this is assumed to present at year 15 of the model (11-14 years after thyroidectomy). This estimate is highly uncertain; but approximates the recurrence time of 10.5 years reported in Skinner et al. (1996) and is consistent with the estimate of symptomatic presentation of MTC in untreated patients.

- Where late stage disease develops (i.e. in true RET-mutation-positive patients *not* undergoing medical surveillance), presentation is assumed to occur at year 15 in the model, at which time these patients require a thyroidectomy and will be subsequently tested and monitored. There is no applicable data available on the time to presentation of symptoms and so this estimate is highly uncertain. This estimate is considered plausible given it represents the development of symptomatic MTC in family members at similar ages to the presentation in index patients, if it is assumed that family members equally comprise people of the same generation and one generation younger than index patients, and a generation approximates 30 years. Furthermore, the estimate has face validity with respect to allowing for the development of late stage disease in patients who may be free of disease initially, given that:

    - patients with clinical disease are on average 8 years older than those without clinical disease (Punales et al. 2003)

    - the average interval between primary tumour and metastases is 6.6 years (Fialkowski & Moley 2006)

Nevertheless, this estimate of the time to the development of symptomatic MTC in non-monitored family members is reasonably arbitrary.

- Equivalent surveillance for MTC, phaeochromocytoma and hyperparathyroidism (and the recommendation for prophylactic thyroidectomy) is undertaken on all patients with a known RET mutation, or suspected of possibly having MEN2, irrespective of the specific codon mutation or predicted risk level (e.g. from low-risk FMTC through to high-risk MEN2B). In reality, increasing research is being undertaken stratifying risk levels on the basis of the specific mutation identified. While some papers suggest that this is too uncertain to yet influence clinical practice, other guidelines suggest that the range of risk profiles justifies varying clinical approaches with respect to prophylactic thyriodectomy etc. There is currently inadequate data to incorporate various mutation specific risk profiles into the economic models. Such a model would be highly complex but, if achievable, such an approach would be likely to improve the accuracy of the model results.

While it can be seen that, to generate quantitative results, the economic models contain numerous assumptions that will not necessarily replicate reality in all circumstances, an attempt has been made to ensure that the base-case assumptions are generally conservative and do not favour economic benefit toward RET mutation testing, such that in reality, RET mutation testing is likely to be of greater economic value than predicted in the economic models presented here.

Results

Scenarios 1 and 2 – Cost-minimisation analysis

The results of the cost-minimisation comparing RET mutation testing (and selective biochemical surveillance) with biochemical investigation and surveillance in patients presenting with MTC or phaeochromocytoma younger than 50 years of age are shown below (Table 45 and Table 46).

Table 45 Results of cost-minimisation analysis or RET mutation testing vs. biochemical screening in a cohort of 100 patients presenting with MTC, over varying time horizons (1, 10, 20 and 30 years)

Time horizon	Costs associated with RET mutation testing and selective surveillance	Costs associated with non-selective biochemical surveillance	Increment
1 years	$46,477.33	$9,305.00	$37,172.33
10 years	$66,436.89	$74,532.32	–$8,095.43
20 years	$80,006.73	$118,878.19	–$38,871.47
30 years	$88,036.99	$145,120.88	–$57,083.89

Table 46 Results of cost-minimisation analysis or RET mutation testing vs biochemical screening in a cohort of 100 patients younger than 50 years of age presenting with phaeochromocytoma, over varying time horizons (1, 10, 20 and 30 years)

Time Horizon	Costs associated with RET mutation testing and selective surveillance	Costs associated with non-selective biochemical surveillance	Increment
1 year	$51,953	$45,480	$6,473

The cost analysis clearly demonstrates that, over the long term, substantial savings would be expected following the use of RET mutation testing to identify RET-mutation-positive patients among those presenting with MTC where target biochemical surveillance for other MEN2 symptoms could subsequently be used.

These results show that, in the case of potential index patients presenting with MTC, while in the year of RET mutation testing there is an increased cost per patient associated with testing, over time the savings in screening costs accumulate and an overall saving is achieved. Savings begin to accrue within 10 years due to the subsequent reduction in the extent of biochemical surveillance required within a genetically tested cohort. Over an extended time period (e.g. 30 years) net savings of around $535 per MTC patient tested would be expected, despite the initial outlay of $400 per RET mutation test per patient.

In the case of phaeochromocytoma patients, assuming no change in patient management on the basis of the test, and on the grounds that ongoing annual surveillance for MEN2 is not undertaken regularly in phaeochromocytoma patients unless the initial investigation shows a further symptom, a marginal increase in costs is observed (approximately $65 per patient), because the diagnostic RET mutation test costs slightly more than biochemical pentagastrin testing and imaging.

Scenarios 3 and 4 – Cost-utility analysis of the availability of diagnostic RET mutation testing in suspected index cases and subsequent prognostic familial screening in first- and second-degree relatives of affected cases, versus non-availability of RET mutation testing

MTC

The results of the cost utility analysis in a cohort of 100 index cases presenting with MTC (Scenario 3) are presented in Table 47 to Table 50, which show disaggregated costs and outcomes. These are combined in Table 51 to estimate an incremental cost-effectiveness ratio.

Table 47 Results: discounted cumulative societal healthcare costs associated with a cohort of 100 potential index cases presenting with MTC and family members where RET mutation testing is available

Time horizon (years)	Costs in index patients	Costs in family members	Total costs
1	$46,477	$46,687	$93,165
10	$66,437	$452,866	$519,303
20	$80,007	$713,164	$793,171
30	$88,037	$789,352	$877,389
60	$96,724	$869,615	$966,339

Table 48 Results: discounted cumulative societal healthcare costs associated with a cohort of 100 potential index cases presenting with MTC and family members where RET mutation testing is not available

Time horizon (years)	Costs in index patients	Costs in family members	Total costs
1	$9,305	$234,577	$243,882
10	$74,532	$2,020,587	$2,095,119
20	$118,878	$3,265,563	$3,384,441
30	$145,121	$3,923,320	$4,068,441
60	$173,511	$4,610,136	$4,783,646

Table 49 Results: incremental difference in cumulative societal healthcare costs between where RET mutation testing is available and where RET mutation testing is not available for a cohort of 100 potential index cases presenting with MTC and their family members

Time horizon (years)	Total costs where RET mutation testing available	Total costs where RET mutation testing is not available	Incremental cost difference
1	$93,165	$243,882	–$150,717
10	$519,303	$2,095,119	–$1,575,816
20	$793,171	$3,384,441	–$2,591,271
30	$877,389	$4,068,441	–$3,191,052
60	$966,339	$4,783,646	–$3,817,307

Table 50 Results: discounted cumulative quality-adjusted life-years (QALYs) in the family members of 100 potential index cases presenting with MTC

Time horizon (years)	Where RET mutation testing is available	Where RET mutation testing is not available	Increment
1	1,149	1,141	8
10	9,188	9,129	59
20	14,600	14,476	124
30	17,760	17,557	203
60	21,171	20,822	349

Table 51 Overall results: incremental costs, quality-adjusted life-years (QALYs) and incremental cost-effectiveness ratio (ICER) of RET mutation testing versus no RET mutation testing, over various time horizons for a cohort of 100 potential index cases presenting with MTC and their family members

Time horizon (years)	Incremental costs	Incremental QALYs	ICER
1	–$150,717	8	Dominant
10	–$1,575,816	59	Dominant
20	–$2,591,271	124	Dominant
30	–$3,191,052	203	Dominant
60	–$3,817,307	349	Dominant

The base-case analysis indicates that RET mutation testing is highly cost-effective, both saving costs and improving health outcomes, when made available to index cases presenting with MTC and their family members, who would otherwise be recommended long-term biochemical surveillance. Cost savings and utility gains are made in the first year and continue to increase when longer time horizons are considered.

Phaeochromocytoma

Similar analysis is conducted in the alternative scenario (4), a cohort of 100 potential index cases presenting with phaeochromocytoma, and the results of this scenario are shown in Table 52 to Table 56.

Table 52 Results: discounted cumulative societal healthcare costs associated with a cohort of 100 potential index cases younger than 50 years of age presenting with phaeochromocytoma and family members where RET mutation testing is available

Time horizon (years)	Costs in index patients	Costs in family members	Total costs
1	$51,953	$27,800	$79,753
10	$51,953	$267,068	$319,020
20	$51,953	$420,401	$472,354
30	$51,953	$465,281	$517,234
60	$51,953	$512,561	$564,514

Table 53 Results: discounted cumulative societal healthcare costs associated with a cohort of 100 potential index cases younger than 50 years of age presenting with phaeochromocytoma and family members where RET mutation testing is not available

Time horizon (years)	Costs in index patients	Costs in family members	Total costs
1	$45,480	$42,928	$88,408
10	$45,480	$428,340	$473,820
20	$45,480	$707,753	$753,233
30	$45,480	$826,573	$872,053
60	$45,480	$940,531	$986,011

Table 54 Time horizon (years) Total costs where RET mutation testing available Total costs where RET mutation testing is not available Incremental cost difference

Time horizon (years)	Total costs where RET mutation testing available	Total costs where RET mutation testing is not available	Incremental cost difference
1	$79,753	$88,408	-$8,654
10	$319,020	$473,820	-$154,800
20	$472,354	$753,233	-$280,879
30	$517,234	$872,053	-$354,819
60	$564,514	$986,011	-$421,497

Table 55 Results: discounted cumulative quality-adjusted life-years (QALYs) in family members of 100 potential index cases presenting with phaeochromocytoma

Time Horizon (years)	Where RET mutation testing is available	Where RET mutation testing is not available	Increment
1	1,149	1,148	1
10	9,198	9,200	–2
20	14,638	14,625	14
30	17,832	17,786	46
60	21,283	21,165	118

Table 56 Overall results: Incremental costs, quality-adjusted life-years (QALYs) and incremental cost-effectiveness ratio (ICER) of RET mutation testing vs. no RET mutation testing, over various time horizons, in potential index cases presenting with phaeochromocytoma and their family members

Time horizon (years)	Incremental costs	Incremental QALYs	Incremental ICER$/QALYs gained
1	-$8,654	1	Dominates
10	-$154,800	–2	$72,873 (cost savings per QALY lost)
20	-$280,879	14	Dominates
30	-$354,819	46	Dominates
60	-$421,497	118	Dominates

Overall, the cost-effectiveness of RET mutation testing in potential index cases presenting with phaeochromocytoma and their families is apparent. Cost savings are incurred every year, primarily due to the fact that RET mutation testing for family members is less expensive than comprehensive biochemical screening and imaging. A negative health outcome is associated with testing in the short term (to 17 years), associated with the increased rate of thyroidectomy (including a complication rate); however, once increased relapse of MTC is included in the model (occurring from 15 years), health outcome benefits associated with RET mutation testing and earlier thyroidectomy are apparent and high cost-effectiveness is demonstrated.

Sensitivity analyses

Sensitivity analyses are conducted on various parameters in the economic model to identify the importance of uncertainties and key assumptions. The sensitivity tests undertaken and the results of the analyses are presented in Table 57 to Table 60.

The sensitivity analysis of cost-utility of RET mutation testing in the cohort of index patients presenting with MTC and their family members indicates that, despite many uncertainties in the economic model, the cost-effectiveness of RET mutation testing in this cohort appears robust (Table 59). The only parameter that significantly altered cost-effectiveness in the sensitivity analysis was removing all clinical benefit attributed to using the test to achieve quicker, more successful prophylactic thyroidectomies (i.e. such that the RR of recurrence of MTC following thyroidectomy after RET versus thyroidectomy on the basis of biochemical screening was 1.0). However, further testing indicted that, for any RR less than 0.97, RET mutation testing remained dominant.

The sensitivity analysis of inputs used in the phaeochromocytoma model indicates that the conclusion of cost-effectiveness of RET mutation testing in this cohort of index patients and family members is sound, despite many uncertainties in the model (Table 60). The benefit in outcomes is maintained in all circumstances; however, where extreme uptake rates of testing or minimal uptake rates of surveillance are incorporated in the model, an increase in costs becomes apparent such that the ICER increases up to $2,600 per QALY. Where no change in RR of relapse is modelled, unsurprisingly, genetic testing is dominated as it results in both increased costs and quality of life decrements (due to costs and complications in additional thyroidectomies), with no clinical benefit.

Table 57 Sensitivity analyses in the economic model – Scenario 1: 100 index cases only considered, presenting with MTC

Parameter	Base-case value	Alternative values tested	Results: net cost at 30 years
Base case			–$57,083 (i.e. savings)
Proposed fee for diagnostic RET mutation tests	$400 for index case	$600–$1,150	–$37,083 (i.e. savings) to $17,916 (costs)
Discount rate (costs and outcomes)	5%	0%	–$148,097.10
Diagnostic yield in index cases	30.6%	5–95%	–$94,234.84 to $36,373.96

Table 58 Sensitivity analyses in the economic model – Scenario 2: 100 index cases only considered, patients younger than 50 years of age presenting with phaeochromocytoma

Parameter	Base-case value	Alternative values tested	Results: net cost at 30 years
Base case			$6,473
Proposed fee for diagnostic RET mutation tests	$400 for index case	$600–$1,150	$26,473–$81,473
Discount rate (costs and outcomes)	5%	0%	$6,473
Diagnostic yield in index cases	18.3%	7–25%	$1,334–$9,520

Table 59 Sensitivity analyses in the economic model – Scenario 3: 100 index cases presenting with MTC and their family members

Parameter	Base-case value	Alternative value or range tested	Results: ICER at 30 years (across range, respectively)
Base case			Dominates
Proposed fee for diagnostic RET mutation test	$400	$600–$1150	Dominates (either fee)
Proposed fee for predictive RET mutation test	$200 for family screen	$250	Dominates
Discount rate (costs and outcomes)	5%	0%	Dominates
Health outcome measure	Utility values	Life-years gained	Dominates
Diagnostic yield in index cases	30.6%	5–95%	Dominates (either yield)
Diagnostic yield in family members	40%	50%	Dominates
Predictive RET mutation testing uptake rate	40%	15–100%	Dominates (either uptake)
Family surveillance uptake rate (in the absence of testing)	40%	15–100%	Dominates (either uptake)
RR of relapse (based on likelihood of MTC at time of surgery) in RET mutation testing era vs. no RET mutation testing	Absolute risk of relapse: 10% in RET mutation testing arm and 40% in non-RET mutation testing arm (RR 0.25)	Absolute risk of relapse = 10% in both arms (RR 1.0) Values of RR ≤ 0.975 (Relapse in no test arm ≥ 10.25%)	Dominated (from year 29) Dominates

Table 60 Sensitivity analyses in the economic model – Scenario 4: 100 index cases, patients younger than 50 years of age presenting with phaeochromocytoma, and their family members

Parameter	Base-case assumption	Alternative value or range tested	Results: ICER at 30 years (across range, respectively)
Base case			Dominates
Proposed fee for diagnostic RET mutation tests	$400 for index case	$600–$1,150	Dominates (either price)
Proposed fee for family screening *RET* gene test	$200 for family screen	$480	Dominates
Discount rate (costs and outcomes)	5%	0%	Dominates
Health outcome measure	Utility values	Life-years gained	Dominates

Parameter	Base-case assumption	Alternative value or range tested	Results: ICER at 30 years (across range, respectively)
Diagnostic yield in index cases	18.3%	7–25%	Dominates (either yield)
Diagnostic yield in family members	40%	50%	Dominates
Predictive RET mutation testing uptake rate	40%	15–100%	Dominates–$485/QALY
Family surveillance uptake rate (in the absence of testing)	40%	15–100%	$1,123/QALY—dominates
Relative risk of relapse (based on likelihood of MTC at time of surgery) in RET mutation testing era vs. no RET mutation testing	Absolute risk of relapse in RET = 10%; risk of relapse in non-RET mutation testing era = 40% (RR 0.25)	Absolute risk of relapse = 10% in both arms (RR 1.0) RR 0.25-0.43 (Relapse in no test arm 23%-40%)	$4,721 saved/QALY lost Dominates

The sensitivity analyses show that the assumption of a clinical benefit in terms of a reduced MTC development or relapse in patients who receive genetic testing (and earlier thyroidectomy) is critical to demonstrate cost-effectiveness in each cost-utility model. Although the estimate of the RR of MTC relapse in patients treated prophylactically is uncertain, the evidence consistently indicates that improved outcomes are expected with earlier prophylactic thyroidectomies undertaken on the basis of genetic test results. Therefore it is reasonable to assume that a RR less than one would be realised in practice, such that testing would be cost-effective.

Cost analysis

Financial / budgetary impacts

Sources of data

Table 61 describes the sources of the data and parameters used in the estimation of the financial and budgetary impact of listing RET mutation testing on the MBS.

Table 61 Sources of data and parameters used to estimate the financial and budgetary impact of listing RET mutation testing on the MBS

Data or parameter	Value used	Source
Incidence of thyroid cancer in Australia, 2005–09	2005: 1,617 cases 2006: 1,664 cases 2007: 1,789 cases 2008: 1,995 cases 2009: 2,039 cases *See reference for access to full data set.*	Australian Institute of Health and Welfare website, *Thyroid cancer* workbook (AIHW 2010)
Incidence of MTC 1982–2009	5–10% of total thyroid cancers	Keatts & Itano (2006)
Projected growth in thyroid cancer and MTC 2010–15	6.3%	Calculated based on average annual increase in thyroid cancer 2005–09

Data or parameter	Value used	Source
Percentage of MTC patients found to have hereditary mutation	25–30%	Raue & Frank-Raue (2010)
Number of first- and second-degree family members to be tested per proband case	11.5	Suthers et al. (2006)
Uptake rate of testing in family members	40%	Suthers et al. (2006)
Cost of RET mutation testing (base case)	*RET* gene screen (diagnostic): $500.00 Known RET mutation test (familial): $200.00	Calculated, based on the quoted price for RET mutation testing of the 6 most common exons (Table 6 on page xxxv)

In the base-case scenario the costs of RET mutation testing have been based on the average cost of RET mutation testing (see Table 6 on page xxxv). The financial and budgetary impact of testing based on the lowest sourced quote (*RET* gene screen: $400; known RET mutation test: $200) is presented in Table 64 and Table 65; and that based on the costs proposed in the final DAP (diagnostic RET mutation test: $1,150; known RET mutation test: $480) is presented in Appendix K.

Genetic counselling may or may not be covered by the MBS, depending on the setting in which the service is provided. The state and territory systems currently provide services through accredited genetic counsellors and medical specialists (clinical geneticists). Genetic counselling may alternatively be provided by the treating practitioner in consultation and, as such, would be covered by the MBS. The cost of genetic counselling has not been accounted for in the financial and budgetary estimates, as the current distribution of services is unlikely to change, with little impact expected to the overall health budget, MBS, and state and territory systems.

Likely volume of diagnostic RET mutation tests per year

RET mutation testing (diagnostic testing) is expected to occur in all patients who are considered to *potentially* have a diagnosis of MEN2. As 95–100% of patients with MEN2 are at risk of developing a thyroid tumour, all patients presenting with MTCs are considered potential cases of MEN2 that require further confirmation (Margraf et al. 2009). As described, diagnostic RET mutation testing is standard practice, such that the number of newly diagnosed MTCs each year is a useful proxy for the estimated number of diagnostic RET mutation tests performed annually. In routine practice, patients would only require one diagnostic RET mutation test per lifetime, with repeat testing required only on possibly erroneous results; however, it is uncertain how often these may arise, due to a non-obligatory RCPA recommendation to conduct predictive RET mutation testing in duplicate (Ravine & Suthers 2012).

The likely volume of diagnostic RET mutation testing performed per year, presented in Table 62, is based on epidemiological estimates of the proportion of MTC within total thyroid cancer data during 2007–09. Projected numbers of MTC and tests performed beyond 2007 are based on an assumed ongoing annual increase of 6.3% in the incidence of new thyroid cancers. This was the average annual growth rate for the period 2005–09 and was only marginally less than the average growth rate of 6.7% seen over the 27 years of available data.

Table 62 Estimated number of diagnostic RET mutation tests during 2007–15, with or without MBS listing

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015
Incidence of thyroid cancer[a]	1,789	1,995	2,039	2,168	2,304	2,449	2,604	2,768	2,942
Estimated number of diagnostic RET mutation tests[b]	89–179	100–200	102–204	108–217	115–230	122–245	130–260	138–277	147–294
Reported number of tests (Suthers 2008b)	150								

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] incidence of thyroid cancer from 2010 onwards projected based on the average annual incidence during 2005–09 of 6.3%

[b] estimated number of diagnostic RET mutation tests performed based on the reported incidence of MTC in all thyroid cancers of 5–10% (Keatts & Itano 2006)

Inaccuracies in the estimates may occur as a result of the following factors:

- Some patients with newly diagnosed MTCs may have had RET mutation testing previously performed as a result of known familial RET mutations and therefore do not require testing upon MTC diagnosis. These patients will be overrepresented in the MTC estimate.
- Some diagnostic RET mutation testing will be initiated in patients suspected of having MEN2 due to other symptoms (e.g. phaeochromocytoma + family history) and will subsequently be underrepresented in the MTC estimate.

However, overall, these estimates are considered reasonable and are consistent with the reported 150 diagnostic RET mutation tests performed in 2007 (and 107 in 2006), the only available quantitative data on the extent of RET mutation testing in Australia (Suthers 2008b).

It is considered that*, should listing occur,* these *estimates of potential MBS diagnostic test use and expenditure equate to* estimates of *diagnostic test use and approximate expenditure for state health budgets under the current scenario where, although not MBS-*

listed, RET mutation testing in these patients is already considered standard practice in Australia[13].

It is also noted that the incidence of thyroid cancer has increased every year since 1982, except in three individual years (see Appendix J) and therefore, an ongoing annual increase at a similar rate in the numbers of tests ordered, might also be anticipated beyond the years projected in the report.

Likely volume of known RET mutation testing per year

Only 25–30% of MTC patients who undergo diagnostic RET mutation testing are expected to have positive hereditary mutations (Raue & Frank-Raue 2010) that warrant known RET mutation testing in their families (familial screening). Across index patients with a mutation, it is assumed that there will be an average of 11.5 first- or second-degree relatives eligible for familial screening. Of these, it is expected that approximately 40% will accept an offer of familial screening within 2 years (Suthers et al. 2006). Therefore, to estimate the number of familial screens undertaken if MBS listing occurred, it is assumed that an average of 4.6 relatives per index case of MEN2 would be tested. Again, it is assumed that this testing would normally occur only once in a person's lifetime. The calculations and projections are presented in Table 63.

Table 63 Estimated number of familial screens during 2007–15, with an MBS listing

Year	2007	2008	2009	2010	2011	2012	2013	2014	2015
Estimated number of proband screens[a]	89–179	100—200	102–204	108–217	115–230	122–245	130–260	138–277	147–294
Positive results[b]	22–54	25–60	25–61	27–65	29–69	31–73	33–78	35–83	37–88
Number of relatives *eligible* for a familial screen[c]	257–617	287–688	293–703	312–748	331–795	352–845	374–898	398–955	423–1,015
Anticipated number of familial screens performed, assuming 40% uptake rate	103–247	115–275	117–281	125–299	132–318	141–338	150–359	159–382	169–406

MBS = Medicare Benefits Schedule

[a] estimated based on a 5–10% incidence of MTC in all thyroid cancers; incidence of thyroid cancer beyond 2010 projected based on the average annual incidence during 2005–09 of 6.3%

[b] assuming positive hereditary RET mutations identified in 25–30% of MTC patients tested

[c] assuming 11.5 relatives per proband

In 2007 there were 49 presymptomatic familial RET mutation tests performed in Australia (up from 29 the year before) (Suthers 2008b). This represents only one or two relatives screened per index patient identified (assuming a 25% diagnostic yield in the initial test) and is a lower volume of familial screening than would be expected. While the reasons for

[13] MESP member and endocrinologist, R Clifton-Bligh, email received on 20 June 2011

the low uptake of familial screening seen historically are unknown, potentially increased rates of familial screening would occur following an MBS listing—both directly as a result of MBS funding improving current access/funding-related issues (if they are present); and if patient attitudes toward RET mutation testing become more positive and MBS funding is perceived as government endorsement of the practice.

While the extent to which potentially affected families will uptake familial screening in the future is highly uncertain, it would be prudent to anticipate that predictive RET mutation testing may increase from previously reported levels and retain the estimated 40% uptake rate as a base estimate.

Total cost to the Australian healthcare system overall

The MBS listing of the diagnostic RET mutation testing in patients with MTC considered to potentially have MEN2 disease is not expected to have any impact on the costs of the overall Australian healthcare system considered in its entirety. The practice of RET mutation testing and counselling already occurs routinely in these patients in a manner unchanged by the proposed listing and at a similar cost, which is currently borne by state government hospital budgets.

Likewise, familial screening and counselling for known RET mutations in family members of identified proband cases is currently available as in the proposed listing and at a similar cost, which is currently borne by the state government. However, the extent of familial screening that has been reported in the past (2006–07) is lower than would have been expected, based on the reported incidence of RET mutations and the number of index cases anticipated.

Total costs to the MBS

Table 64 and Table 65, respectively, present the estimated annual costs of listing diagnostic and predictive RET mutation testing on the MBS between 2007 and 2015, assuming that all services are provided in an outpatient setting where the MBS covers 85% of the cost of services. Based on an estimated number of 130–260 diagnostic and 150–359 predictive RET mutation tests performed in 2013, the estimated median cost to the MBS is $109,654. This would increase to $123,906 in 2015 based on 147–294 diagnostic and 169–406 predictive tests performed (Table 66). However, an unknown proportion of patients may qualify for the Medicare Safety Net, in which case 100% of the scheduled fee would be paid by the MBS. Allowing for application of the Medicare Safety Net, the overall true costs to the Commonwealth health budget would lie between the total costs to the MBS and the total combined costs of RET mutation testing, i.e. up to $129,005 in 2013 and $145,772 in 2015.

Sensitivity analyses assuming upper estimates around disease incidence (positive results in 30% of patients with MTCs tested) and a 100% uptake rate of familial screening are

presented in Table 67 to provide an extreme upper limit of the predictable financial costs. The cost of RET mutation testing to the MBS under these extreme upper limits increases from $241,217 in 2013 to $272,568 in 2015.

Table 64 Estimated cost of diagnostic RET mutation tests during 2007–15, with or without MBS listing

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Number of diagnostic RET mutation tests[b]	89–179	100–200	102–204	108–217	115–230	122–245	130–260	138–277	147–294
Estimated expenditure on diagnostic RET mutation testing[c]	$44,725 – $89,450	$49,875 – $99,750	$50,976 – $101,953	$54,188 – $108,376	$57,602 – $115,204	$61,231 – $122,461	$65,088 – $130,176	$69,189 – $138,378	$73,548 – $147,095
Patient co–payment[d]	$6,709 – $13,418	$7,481 – $14,963	$7,646 – $15,293	$8,128 – $16,256	$8,640 – $17,281	$9,185 – $18,369	$9,763 – $19,526	$10,378 – $20,757	$11,032 – $22,064
Estimated MBS expenditure[e]	$30,413 – $60,826	$33,915 – $67,830	$34,664 – $69,328	$36,848 – $73,696	$39,169 – $78,338	$41,637 – $83,274	$44,260 – $88,520	$47,048 – $94,097	$50,012 – $100,025

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] estimated based on a 5–10% incidence of medullary thyroid cancer in all thyroid cancers

[c] assuming the cost of the diagnostic RET mutation test is $400 (see Table 6 on page xxxv)

[d] assuming most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[e] assuming all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Table 65 Estimated cost of predictive RET mutation tests during 2007–15, with an MBS listing

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Relatives eligible for screening[b]	257–617	287–688	293–703	312–748	331–795	352–845	374–898	398–955	423–1,015
Number of relatives screened[c]	103–247	115–275	117–281	125–299	132–318	141–338	150–359	159–382	169–406
Estimated expenditure on predictive RET mutation testing[d]	$20,574 – $49,376	$22,943 – $55,062	$23,449 – $56,278	$24,926 – $59,823	$26,497 – $63,592	$28,166 – $67,599	$29,941 – $71,857	$31,827 – $76,384	$33,832 – $81,197
Patient co-payment[e]	$3,086 – $7,406	$3,441 – $8,259	$3,517 – $8,442	$3,739 – $8,974	$3,975 – $9,539	$4,225 – $10,140	$4,491 – $10,779	$4,774 – $11,458	$5,075 – $12,179
Estimated MBS expenditure[f]	$17,487 – $41,970	$19,501 – $46,803	$19,932 – $47,836	$21,187 – $50,850	$22,522 – $54,054	$23,941 – $57,459	$25,450 – $61,079	$27,053 – $64,927	$28,757 – $69,017

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] estimated based on the identification of a positive hereditary mutation in the *RET* gene in 25–30% of tests performed; each patient was assumed to have, on average, 11.5 first- or second-degree relatives eligible for familial screening

[c] assuming an uptake rate of 40% in eligible family members

[d] assuming the cost of the familial predictive RET mutation test is $200 (see Table 6 on page xxxv)

[e] assuming most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[f] assuming all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Table 66 Total MBS costs associated with predictive RET mutation testing (combined costs of listing for diagnostic purposes and listing for familial screening), with or without the safety net

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Total combined cost of RET mutation testing[b]	$88,645	$98,852	$101,035	$107,401	$114,167	$121,359	$129,005	$137,132	$145,772
Lower limit	$56,354	$62,843	$64,230	$68,277	$72,578	$77,151	$82,011	$87,178	$92,670
Upper limit	$120,936	$134,862	$137,840	$146,524	$155,755	$165,568	$175,999	$187,087	$198,873
Total patient co–payment[c]	$13,297	$14,828	$15,155	$16,110	$17,125	$18,204	$19,351	$20,570	$21,866
Lower limit	$8,453	$9,426	$9,635	$10,242	$10,887	$11,573	$12,302	$13,077	$13,901
Upper limit	$18,140	$20,229	$20,676	$21,979	$23,363	$24,835	$26,400	$28,063	$29,831
Total cost to the MBS[d]	$75,348	$84,024	$85,880	$91,290	$97,042	$103,155	$109,654	$116,562	$123,906
Lower limit	$47,900	$53,416	$54,596	$58,035	$61,692	$65,578	$69,710	$74,101	$78,770
Upper limit	$102,796	$114,633	$117,164	$124,546	$132,392	$140,733	$149,599	$159,024	$169,042

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] assuming all patients qualify for the Medicare Safety Net, the total cost to the MBS would equate to the total combined cost of RET mutation testing

[c] assuming most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[d] assuming all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Table 67 Sensitivity analyses

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Number of diagnostic RET mutation tests[b]	179	200	204	217	230	245	260	277	294
Total cost of diagnostic RET mutation tests[c]	$71,560	$79,800	$81,562	$86,701	$92,163	$97,969	$104,141	$110,702	$117,676
Total number of relatives screened[d]	617	688	703	748	795	845	898	955	1015
Total cost of predictive RET mutation tests[e]	$123,441	$137,655	$140,695	$149,559	$158,981	$168,997	$179,644	$190,961	$202,992
Combined cost of RET mutation testing[f]	$195,001	$217,455	$222,257	$236,259	$251,144	$266,966	$283,785	$301,663	$320,668
Patient contribution[g]	$29,250	$32,618	$33,339	$35,439	$37,672	$40,045	$42,568	$45,249	$48,100
Total cost to the MBS[h]	$165,751	$184,837	$188,919	$200,821	$213,472	$226,921	$241,217	$256,414	$272,568

MBS: Medicare Benefits Schedule; RET: rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence 2005–09 of 6.3%

[b] based on 10% incidence of medullary thyroid cancer in all thyroid cancers

[c] assuming the cost of the diagnostic RET mutation test is $400 (see Table 6 on page xxxv)

[d] based on 11.5 relatives per proband and assuming 100% uptake of familial screen

[e] assuming the cost of the predictive RET mutation test is $200 (see Table 6 on page xxxv)

[f] assuming all patients qualify for the Medicare Safety Net, then the total cost to the MBS would equate to the combined cost of RET mutation testing

[g] assuming most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[h] assuming all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees. No allowance for additional MBS if some patients qualify for the Medicare Safety Net

Costs to the State and Territory health systems

The costs of RET mutation testing would shift from the state and territory systems to the MBS; however, the costs of genetic counselling services provided in hospitals would continue as per current arrangements. Thus, the overall financial and budgetary impact to the state and territory systems would be a cost saving.

Costs to the private health insurer and/or patient

Under current arrangements patients who are referred to RET mutation testing by a private facility are billed directly, as private health insurance generally subsidises testing only on MBS items (PaLMS 2011). Currently, some patients who are referred through the public system receive genetic counselling services and testing at no direct cost (Centre for Genetics Education 2013). With the listing of RET mutation testing on the MBS, assuming that most patients would receive testing as outpatients, Medicare would pay 85% of the scheduled fee and a patient contribution of 15% of the scheduled fee would apply ($75 per patient for a diagnostic RET mutation test or $30 per predictive RET mutation test, in addition to any 'gap' charges or out-of-pocket expenses). Private health insurance may assist with these patient costs. Patients who may be eligible for the Medicare Safety Net, and those whose pathology service bulk-bills tests listed on the MBS (SA Health 2013), may not be required to contribute a co-payment.

Discussion

Is it safe?

No studies were identified that reported on adverse events directly related to RET mutation testing or due to surveillance for MEN2 features. The RET mutation test itself is considered to have minimal risk as it only involves a peripheral blood sample being taken. However, with any diagnostic test there may be psychological harms that arise from informed knowledge regarding being at risk of a disease. In the absence of RET mutation testing, psychological distress is likely to still be a problem, as first-degree family members would know that they have a 50% chance of having MEN2. Therefore, any psychological harms from RET mutation testing are offset by the reassurance given to relatives who are found to be free from the mutation.

It is considered that the best strategy to cure an MTC is to prevent it from developing (Spinelli et al. 2010). Since the introduction of RET mutation testing, those at risk of developing an MTC have been able to be identified with a high degree of accuracy, and more gene carriers have been undergoing surgery prior to clinical evidence of an MTC. A comparison of the safety of prophylactic total thyroidectomy versus thyroidectomy performed upon clinical evidence of disease was thought relevant.

One historical controlled study with a high risk of bias (level III-3 interventional evidence) and 6 uncontrolled studies (level IV interventional evidence) reported on adverse events related to total thyroidectomies performed after RET mutation testing (both those with MTC and asymptomatic family members).

The historical controlled study reported that two patients died from surgical complications, one who was diagnosed clinically and one genetically. However, no information regarding confounding factors, such as stage of disease or surgical technique, was provided. No conclusions regarding the comparative risk of death from surgery can therefore be made. This comparative study did not report on other forms of adverse events after surgery.

From the 12 case series the two common types of complications were hypoparathyroidism and laryngeal nerve palsy. In the majority of cases these were temporary. However, hypoparathyroidism was permanent in a small percentage of patients (5.9–13.6% in 3 studies), requiring ongoing calcium supplementation. In one case (out of 75 patients in 1 study) laryngeal nerve palsy was also permanent. The only other permanent complication was a case of unilateral Horner's syndrome in a 63-year-old patient. Two further complications reported were 1 case of arterial bleeding requiring re-operation (5.9% of 1 study) and a paediatric case with fluctuating thyroid function test results, despite good thyroxine replacement compliance at 1-year follow-up.

Due to the uncontrolled nature of the studies identified, it is unknown how these rates of complications compare with those who have surgery at a later stage of disease progression. However, surgery performed prior to the development of MTC allows less aggressive surgical procedures to be performed, which reduces the risks associated with neck surgery in children. When performed prophylactically, the chances of local infiltration or involvement of cervical lymph nodes are reduced, such that wide neck dissections may be avoided. Wide neck dissections are associated with higher rates of permanent hypoparathyroidism than removal of the thyroid by itself; therefore, the rates of this adverse outcome are hypothesised to be lower when surgery is performed prophylactically (Spinelli et al. 2010).

One concern about operating at an earlier stage of disease is that patients are operated on at an earlier age, which may be difficult for parents to accept. Although no evidence was identified that directly assessed rates of complication from surgery based on age, some authors mentioned that, in their experience, there is no direct relationship between patient age and the risk of complications (Schellhaas et al. 2009).

For those with a RET mutation associated with MEN2, the penetrance of MTCs is over 90% (Raue & Frank-Raue 2012). Although high, this does mean that, for every 10 patients for whom prophylactic surgery is performed, up to 1 patient may be having the surgery unnecessarily. Although prophylactic total thyroidectomies may use less aggressive surgical techniques and it is therefore logical that they are safer than total thyroidectomies performed for the purposes of treatment rather than prevention, the risks of operating on gene carriers who potentially do not require it need to be considered. However, this situation is not unique to the scenario in which RET mutation testing is available. Prior to RET mutation testing becoming available, total thyroidectomies were often performed if an MTC was suspected due to raised calcitonin levels. This measure is not specific to those at risk of developing an MTC, and there were cases reported in our systematic review who had undergone a total thyroidectomy based on clinical signs and were later found to have wildtype RET, and thus were not at risk of MTC at all (Algun et al. 2002; Decker et al. 1996; Frank-Raue et al. 1996).

An overall summary of the body of evidence on the safety of total thyroidectomies after RET mutation testing is shown in Table 68. The evidence is rated as poor overall due to the high risk of bias and the uncontrolled nature of the studies identified.

Table 68 Body of evidence matrix for the safety of surgery performed on asymptomatic RET M+ gene carriers

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]				Level IV studies, or level I to III studies/SRs with a high risk of bias
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact				Slight or restricted
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability			Probably applicable to Australian healthcare context with some caveats	

SR = systematic review

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

Source: adapted from NHMRC (2008)

Is it effective?

The use of RET mutation testing in the diagnosis of patients with MEN2 was quickly introduced across the world, with no formal trials to assess whether patients who are diagnosed using the test have better health outcomes than those who do not have access to RET mutation testing.

The evidence on the comparative effectiveness of RET mutation testing was 9 historical controlled studies (level III-3 interventional studies). These studies are inherently at high risk of bias due to potential differences that may have occurred over the same time period, e.g. in the surgical techniques and screening procedures used. There are also large differences in the length of follow-up between the intervention (with RET mutation testing) and comparator (without RET mutation testing) arms, such that longer term follow-up data should be interpreted with caution.

Six historical controlled studies all reported that patients were, on average, having surgery at an earlier stage of disease development since the introduction of RET mutation testing. Overall, patients had almost half the risk of having an MTC (rather than C-cell hyperplasia or no disease) at the time of total thyroidectomy compared with those treated prior to RET mutation testing (RR=0.52, 0.32, 0.90). The full range of plausible outcomes includes only a small difference, so the clinical impact is considered 'substantial' rather than 'very large' in the body of evidence matrix (Table 69).

The risk of persistence or recurrence was also reported in 6 historical controlled studies; however, the length of follow-up in the cohorts diagnosed with the addition of RET mutation testing was too short for this outcome to be clinically meaningful. There were significantly more cases of persistence or recurrence noted in the historical cohorts, prior to the use of RET mutation testing, than in the RET-mutation-tested cohorts (RR=0.28, 0.17, 0.45); however, it is unknown to what degree these health outcomes differ due to the longer time interval since surgery. It is also possible that other historical effects may have influenced the differential health outcomes over time.

One study reported that, in patients who all underwent RET mutation testing, age at time of surgery was a significant predictor of residual or recurrent disease (Schreinemakers et al. 2010). Since the introduction of RET mutation testing, the age of patients at the time of thyroidectomy has dramatically decreased. One Australian historical controlled study reported that the mean age of thyroidectomy based on calcitonin testing was 32 years, whereas the mean age of thyroidectomy after RET mutation testing was 16 years. Five other historical controlled studies also reported a reduction in the age at time of surgery.

Although all the identified studies had a high risk of bias, they were consistent that the introduction of RET mutation testing has allowed treatment of those at risk of MTC to occur at a younger age, when it is more likely to be prophylactic, and to be associated with a greater likelihood of the surgery being considered curative.

Overall findings from the body of evidence on the effectiveness of RET mutation testing in improving health outcomes are summarised in the Table 69.

Table 69 Body of evidence matrix for the effectiveness of RET mutation testing on improving health outcomes

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]				Level III-3 studies with a high risk of bias
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact		Substantial		
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

Source: adapted from NHMRC (2008)

Diagnostic accuracy

No evidence was identified that provided a measure of the true diagnostic accuracy of RET mutation testing against an appropriate reference standard. Diagnostic yield studies were provided. The summary of the body of evidence on accuracy is shown in Table 70. Diagnostic yield studies are level IV diagnostic evidene and they provided very limited information on the accuracy of RET mutation testing.

Table 70 Body of evidence matrix showing the body of evidence on the accuracy of RET mutation testing

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]				Level IV studies
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact	N/A			
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

N/A = not applicable

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

Source: adapted from NHMRC (2008)

Impact on patient management

Studies assessing whether there has been a change in management since the introduction of RET mutation testing were uncontrolled. There was evidence that asymptomatic gene carriers would often undergo prophylactic total thyroidectomy, and there was evidence that some patients had been inappropriately treated prior to RET mutation testing becoming available. There was evidence that family members who were found to have mutations were recommended to undergo surveillance for MEN2, and that those who were found to be free from mutations were able to cease surveillance and their descendants did not require any testing. These studies had good generalisability and applicability although the level of evidence was poor (Table 71).

Table 71 Body of evidence matrix for the assessment of a change in management due to RET mutation testing

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]				Level IV studies
Consistency[b]		Most studies consistent and inconsistency may be explained		
Clinical impact		Substantial		
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

Source: adapted from NHMRC (2008)

Impact on health outcomes

One study assessed the difference in severity of disease at the time of surgery between those who had prophylactic surgery and those who had curative surgery. This cohort study had a high risk of bias. It showed a moderate difference in the severity of MTC (as determined by histopathology results) and was generalisable to the Australian population and applicable to the healthcare system (Table 72).

Table 72 Body of evidence matrix for the assessment of a whether the change in management due to RET mutation testing results in differential health outcomes

Component	A	B	C	D
	Excellent	Good	Satisfactory	Poor
Evidence base[a]				Level II study with a high risk of bias
Consistency[b]	N/A			
Clinical impact			Moderate	
Generalisability		Population(s) studied in the body of evidence are similar to the target population		
Applicability		Applicable to Australian healthcare context with few caveats		

N/A = not applicable

[a] Level of evidence determined from the NHMRC evidence hierarchy—Table 19

[b] If there is only 1 study, rank this component as 'not applicable'.

Source: adapted from NHMRC (2008)

What are other relevant considerations?

With any genetic test there are ethical issues that should be considered, such as matters of informed consent, privacy and confidentiality, and the balance of risks and harms from information regarding genetic testing. Given that it has clear treatment and screening implications for either the individual or their family members, RET mutation testing would on balance appear useful, as long as patients are fully informed on the implications of testing and give true informed consent following accredited genetic counselling.

Controlled studies assessing the direct health impact of RET mutation testing are unlikely to be able to be performed in the current clinical environment, where RET mutation testing is considered best practice for those considered at risk of MEN2 (Learoyd & Robinson 2005). Withholding mutation testing for the purposes of further research would therefore be considered unethical.

What are the economic considerations?

The quantitative results derived from the economic model are highly uncertain, as many of the inputs were based on non-comparative data or data with a high risk of bias, including those that quantify the extent of estimated clinical benefit in family members (i.e. the RR of developing advanced MTC; the penetrance of symptomatic MTC; and the expected timeframes of testing, prophylactic thyroidectomy and development of symptomatic MTC in at-risk patients). Nevertheless, while the quantification is uncertain, the directions of the effect of RET mutation testing on clinical practice and expected outcomes are consistently associated with a decrease in overall expenditure and an increase in overall health in the relevant cohort. Cost savings can be expected, although their extent ranges significantly depending on patient uptake rates and adherence to compliance. Likewise, a net benefit in health outcome benefits is consistently shown, although the rate of symptomatic MTC with and without prophylactic thyroidectomy, the accuracy of the translation into QALYs, and patient uptake rates influence the extent of these benefits, and accurate estimations of these are unknown. Therefore, the conclusion—that, in economic terms, the intervention of RET mutation testing dominates the alternative (historical) clinical practice—is robust.

Except where practically no clinical benefit (RR>0.97) is assumed for RET mutation testing, with respect to long-term relapse outcomes following thyroidectomy, all sensitivity analyses demonstrate that RET mutation testing across index patients and first- or second-degree family members is cost-effective. Although an unbiased estimate of the RR of MTC relapse following prophylactic thyroidectomy undertaken on the basis of RET mutation testing rather than biochemical screening is not available, the available data support the RR used in the base-case analysis (RR 0.56), such that the upper limit of the RR as tested in the sensitivity analysis is extreme, and cost-effectiveness is almost certain in clinical practice.

The number of patients likely to be treated requires estimations of both potential new index cases requiring full *RET* gene screening and of family members who may undertake familial screening. MEN2 is a relatively rare condition and the most common first-presenting symptom is MTC.

The estimate of the population suspected of having MEN2 and eligible for diagnostic RET mutation testing is 130–260 patients in 2013, increasing to 147–294 in 2015. This is based on the number of Australians diagnosed with thyroid cancer, and assuming 5–10% of cases are MTC (AIHW 2010; Keatts & Itano 2006). This approach is used as MTC is the most common first presentation of MEN2 (and while this will likely include patients diagnosed after familial screening, it omits patients whose initial presentation is phaeochromocytoma, thus providing a reasonable proxy overall). An annual increase in the incidence of thyroid cancer (and MTC) of 6.3% has been projected based on the average annual increase in thyroid cancer in Australia 2005-09. Uncertainty is likely contained within the estimated range.

The likely number of eligible family members who elect to have predictive RET mutation tests is estimated to be 150–359 in 2013, increasing to 169–406 in 2015. This estimate assumes that 25–30% of diagnostic RET mutation tests performed identify a patient with a hereditary RET mutation (Raue & Frank-Raue 2010) and that each index patient has 11.5 first- or second-degree relatives eligible for predictive RET mutation testing (Suthers et al. 2006). It is also assumed that only 40% of eligible relatives accept (i.e. uptake) familial screening (Suthers et al. 2006). The estimates of predictive RET mutation testing are more uncertain than the estimates of diagnostic testing given they are further dependent on family size and uptake rates and therefore familial screening rates are potentially underestimated, although non-MBS data does not suggest this (Suthers 2008b).

It is assumed that each patient will only require one test per lifetime, as the relevant germline RET mutations are stable. Therefore, the number of tests is equivalent to the number of patients. (While there would conceivably be cases of false positives/negatives that are at odds with clinical observations, or non-determinable results that may prompt re-testing, the expected re-test rate would be low and has not been considered).

The base case of the model assumes that uptake rates of RET mutation testing will remain the same as uptakes rates of biochemical/imaging surveillance. This is assumed to occur in all potential index cases presenting with potential symptoms of MEN2. For family members it is estimated that, in each comparison arm (and in each scenario), 40% of the eligible population would receive either testing or biochemical/imaging surveillance. The economic analysis is not highly sensitive to uptake rates, with extreme proportions (in the range 15–100%) being tested in the sensitivity analyses and cost-effectiveness still evident.

Conclusions

Safety

There was no evidence regarding the comparative safety of RET mutation testing and subsequent management, versus the historical comparator prior to RET mutation testing. However, it is likely that RET mutation testing has allowed total thyroidectomies to be performed at an earlier stage of disease, or prior to any clinical signs of MTC, which allows a less aggressive form of surgery to be performed. Although there was no direct comparative evidence identified to show this, it is highly likely that more conservative surgical techniques are safer for patients than surgical techniques performed once clinical signs of MTC exist.

Effectiveness

RET mutation testing was found to be associated with treatment at a younger age and lower severity of disease (less chance of having MTC or nodal metastases at time of surgery). These two factors have been found to greatly reduce the risk of having residual or recurrent disease. Although the direct evidence comparing the rates of residual or recurrent disease by era (pre- versus post-RET mutation testing) is at risk of bias due to differences in the length of follow-up between the cohorts, it is logical to expect that health outcomes following diagnosis with RET mutation testing have significantly improved.

An overall summary of the body of evidence is that it may be graded 'C' for 'satisfactory'. The studies themselves were at high risk of bias due to being historical controlled studies. However, they were all consistent both in the direction of effect and between different measures of effectiveness. The clinical impact is expected to be substantial, and the evidence is considered to be generalisable to the Australian population suspected to be at risk of having MEN2 and applicable to the Australian healthcare setting.

Diagnostic accuracy

The diagnostic accuracy of RET mutation testing was not performed, as no studies provided long-term data to enable true disease status to be known. In addition, the reference standard of 'long-term clinical data' is inherently flawed if patients have had a prophylactic total thyroidectomy for MTC—removal of the thyroid should prohibit the development of an MTC, which can be the only clinical presentation of MEN2A for some patients and all patients with FMTC.

Impact on patient management

Four different articles identified cases where patients who had either undergone or been referred for total thyroidectomy based on calcitonin levels were found not to have MTC (if

they had undergone surgery), and were later identified as being RET-mutation-negative. It is expected that RET mutation testing as part of the diagnostic strategy can prevent unnecessary surgery in those not at risk of MEN2.

Uncontrolled studies showed that a substantial proportion of patients who were gene carriers underwent prophylactic total thyroidectomy based on the results of mutation testing.

Impact on health outcomes

One cohort study (level III-2 interventional evidence) with a high risk of bias showed that a greater proportion of those who underwent total thyroidectomy on the basis of raised calcitonin, compared with those where surgery was considered prophylactic, had disease that had advanced into the lymph nodes or distant metastases. Consistent with the direct evidence, it can be expected that prophylactic surgery based on RET mutation testing allows surgery at an earlier stage of disease, which decreases the chances of residual or recurrent disease.

Other relevant considerations

RET mutation testing should always be accompanied by accredited genetic counselling to assist patients in understanding the implications of being tested and in the interpretation of test results.

RET mutation testing is already considered best practice, and *not* testing patients suspected of MEN2 for RET mutations would be considered negligent.

Economic considerations

Overall, RET mutation testing in both potential index cases and their family members, as described in the proposed listing, is almost certainly cost-effective, particularly where the index case presents with MTC but also in phaeochromocytoma where consideration of surveillance of family members is included. The economic model, although lacking in high-quality data, suggests that use of RET mutation testing almost certainly results in improved health outcomes and decreased costs compared with hypothetical scenarios where RET mutation testing is not available.

Costing

The expected uptake of RET mutation testing in proband patients is estimated at 35–85 tests annually (for 35-85 patients – ie 1 test per patient).

The expected uptake of predictive RET mutation testing in first- or second-degree family members is estimated to be 160–380 tests annually, for the same number of people.

The total cost to the MBS for RET mutation testing is estimated to be $75,000–$160,000 annually.

The total cost to the Australian healthcare system, including the MBS, for the intervention/procedure is not expected to change given that testing is routine clinical practice that is currently funded through state hospital budgets or private sources.

Appendix A Health Expert Standing Panel and Assessment Group

Health Expert Standing Panel (HESP)

Member	Expertise or affiliation
Dr Roderick Clifton-Bligh	Head (locum), Cancer Genetics Unit and Staff Specialist in Endocrinology Kolling Institute/ Royal North Shore Hospital Sydney, New South Wales

Assessment group

AHTA, University of Adelaide, South Australia

Name	Position
Ms Skye Newton	Team leader (Medical HTA)
Ms Camille Schubert	Senior Health Economist
Dr Judy Morona	Research Officer
Mr Paul Fitzgerald	Research Officer
Assoc Prof Tracy Merlin	Managing Director

Noted conflicts of interest

There were no conflicts of interest.

Appendix B Uncontrolled studies reporting persistence or recurrence of disease

Table 73 Persistence or recurrence following RET mutation testing and subsequent treatment

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
In patients with MTC or CCH					
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	N=30 RET M+ family members of patients with MTC reclassified from sporadic MTC to FMTC or MEN2A due to a RET mutation, who showed clinically and/or biochemical signs of disease on screening: 29 FMTC 1 MEN2A	RET mutation testing, method changed over 15 years Initially used direct DNA sequencing of exons 10,11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy	3/30 (10%) persistence or recurrence 27/30 (90%) disease free	Mean = 6.0 years
(Gonzalez et al. 2003) Mexico	IV Interventional evidence High quality (5/6)	N=17 RET M+ family members (plus probands) from 5 MEN2 families: 14 with MEN2A (14 x 634 mutations) 3 with MEN2B (3 x 918 mutations)	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15 Clinical screening Thyroidectomy	14/17 (82.4%) disease free 1/17 (5.9%) alive with disease 2/17 (11.8%) dead from MTC	Mean = 6.7 years (range 1–24 years)
(Dralle et al. 1998) Germany	IV Interventional evidence Moderate quality (4/6)	N=75 RET M+ patients <20 years of age identified retrospectively through a questionnaire	RET mutation testing (method not stated) Clinical screening Total thyroidectomy	3/75 (4%) persistent elevated calcitonin postoperatively	Postoperative
(Milos et al. 2008) Romania, Germany, Chile, Brazil, Argentina,	IV interventional evidence Moderate quality (4/6)	N=34 carriers of RET C634W mutation from 20 unrelated MEN2A families for whom postoperative data were available	RET mutation testing (method not stated) Thyroidectomy	9/34 (26.5%) MTC patients with postoperative data showed raised serum calcitonin levels	Postoperative

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
Hungary, Spain, The Netherlands, Czech Republic, Poland, USA					
(Quayle et al. 2004) USA	IV interventional evidence Moderate quality (3/6)	N=38 RET M+ patients with MEN2 or FMTC diagnosed when over 50 years of age who had a thyroidectomy	RET mutation testing (method not stated) Total thyroidectomy	17/30 (56.7%) patients had increased pentagastrin-stimulated calcitonin levels 5/38 (13.2%) patients had repeat neck exploration procedures for recurrent or persistent disease 3/38 (7.9%) patients had distant metastases detected during follow-up period	Median = 6.4 years
(Yoshida et al. 2009) Japan	IV interventional evidence Moderate quality (3/6)	N=12 adults who underwent total thyroidectomy for MTC and had MEN2	RET mutation testing (method not stated) and total thyroidectomy (unclear whether treatment decisions influenced by RET mutation)	1/12 (8.3%) patients had surgery that was not considered curative, and died of advanced metastatic MTC at 1 year postoperatively 11/12 (91.7%) patients had no recurrence of MTC	Median = 8.8 years
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Low quality (2/6)	N=6 index cases with a RET Y791F mutation who had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	1/6 (16.7%) had increased calcitonin levels 4/6 (66.7%) had normal calcitonin levels 1/6 (16.7%) died	Up to 15 years
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=5 members of an MTC family with a RET L790F mutation (including index patient), who had a thyroidectomy All had abnormal pentagastrin-stimulated calcitonin levels 3 had clinical signs of disease	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Direct DNA sequencing of exon 13 in family members Clinical screening Prophylactic thyroidectomy	0/5 (0%) had recurrent disease All patients were cured, with a mean follow-up time of 6.6 years	Mean = 6.6 years (range 6–8 years)

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
In asymptomatic family members					
(Skinner et al. 2005) USA	IV interventional evidence High quality (5/6)	N=50 RET M+ patients from MEN2A families, who were <20 years of age at time of thyroidectomy	RET mutation testing by restriction site polymorphism analysis and/or direct DNA sequencing of RET exons 10, 11, 13, 14 and 16 Total thyroidectomy	6/50 (12%) had persistent or recurrent disease detected *Post hoc analyses:* Thyroidectomy patients younger than 8 years of age: 0/22 had raised calcitonin levels postoperatively Thyroidectomy over age 8 years: 6/28 had raised calcitonin levels postoperatively No evidence that recurrence or persistence was dependent on codon (p=0.92)	Range = 5–10 years
(Lau et al. 2009) Hong Kong	IV interventional evidence High quality (5/6)	N=22 asymptomatic patients from 8 MEN2A families, who underwent prophylactic total thyroidectomy based on RET mutation status	RET mutation testing by restriction enzyme analysis and/or direct DNA sequencing (exons not specified) Prophylactic thyroidectomy with or without a unilateral central compartment neck dissection	5/22 (22.7%) patients were diagnosed with PCC and had adrenalectomy 2/22 (9.1%) patients had mild HPT 1/22 (4.5%) patient had fluctuating thyroid function test results, despite good thyroxine replacement compliance after 1 year 0/22 (0%) had clinical, biochemical or ultrasonographic evidence of MTC recurrence	Median = 49 months (range 13–128 months)
(Wells Jr & Skinner 1998) USA	IV interventional evidence High quality (5/6)	N=18 first-degree relatives aged 21 years or younger, from 7 MEN2A kindreds with no clinical symptoms, who were RET M+ and underwent a thyroidectomy	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 10 and 11 Prophylactic thyroidectomy with bilateral cervicocentral lymphadenectomy	18/18 (100%) had normal postoperative pentagastrin-stimulated calcitonin levels 13/13 (100%) had normal pentagastrin-stimulated calcitonin levels after 3 years	Range = 0–3 years
(Schellhaas et al. 2009) Germany	IV interventional evidence High quality	N=17 patients with a RET codon 634 mutation: 14 with MEN2A	RET mutation testing (method not stated) Prophylactic total thyroidectomy with bilateral cervicocentral lymphadenectomy	2/17 (11.8%) with increased calcitonin levels during follow-up 1 was a 36-year-old at operation who	Median = 13.3 years

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
	(5/6)	3 with apparent FMTC		presented with T2N1 neoplasm 1 was a 9-year-old at operation who presented with T1 MTC and lymph node metastases Lymph node status associated with probable recurrent/persistent MTC (p=0.024)	
(Alvares Da Silva et al. 2003) Brazil	IV Interventional evidence Moderate quality (4/6)	N=35 RET M+ members of an FMTC family with a RET G533C mutation All underwent thyroidectomy Data were not available for 7 (recent surgery)	RET mutation testing by direct DNA sequencing of exon 8 Prophylactic thyroidectomy	20/28 (71.4%) normal postoperative calcitonin levels (baseline <11.5 pg/mL) 8/28 (28.6%) elevated calcitonin (6 with lymph node metastases and 2 without)	Postoperative
(Franz & Wells Jr 1997) USA, Germany	IV Interventional evidence Moderate quality (4/6)	N=20 RET M+ patients: 19 MEN2A patients 1 FMTC patient	RET mutation testing by restriction site polymorphism analysis and/or DNA sequencing (exons not specified) Clinical screening Prophylactic thyroidectomy based on RET status	20/20 (100%) normal postoperative calcitonin levels	Postoperative
(Sanso et al. 2002) Argentina	IV interventional evidence Moderate quality (4/6)	N=18 RET M+ patients (aged 17 months – 21 years) had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, confirmed by restriction site polymorphism analysis Prophylactic thyroidectomy	17/18 (94.4%) had normal postoperative calcitonin levels In 1 juvenile patient with regional lymph node metastases calcitonin levels remained high after surgery	Postoperative
(Chiefari et al. 1998) Italy	IV Interventional evidence Moderate quality (4/6)	N= 16 RET M+ members of separate families with hereditary MTC, who had a thyroidectomy	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14 Clinical screening Total thyroidectomy	5/16 (31.3%) had elevated postoperative pentagastrin-stimulated calcitonin levels	Postoperative
(Decker et al. 1996) USA	IV Interventional evidence Moderate quality (4/6)	N=11 children from confirmed MEN2A patients who underwent prophylactic thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11 Clinical screening	11/11 (100%) normal postoperative calcitonin levels	Postoperative

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
			Prophylactic thyroidectomy		
(Frank-Raue et al. 1997) Germany	IV Interventional evidence Moderate quality (4/6)	N=11 asymptomatic children with a RET mutation from 8 MEN2A/FMTC families	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing or restriction site polymorphism analysis of exons 10, 11 and 13 Prophylactic thyroidectomy	11/11 (100%) normal postoperative calcitonin levels	Postoperative
(Frank-Raue et al. 1996) Germany	IV interventional evidence Moderate quality (4/6)	N=9 presymptomatic RET M+ patients from families clinically identified with hereditary MTC, who had prophylactic thyroidectomy Data available for 8 patients	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA sequencing or restriction site polymorphism analysis of exons 10 and 11 Direct DNA sequencing of exons 13 and 16 Clinical screening Prophylactic thyroidectomy	8/8 (100%) normal postoperative calcitonin levels	Postoperative
(Lombardo et al. 2002) France and Italy	IV interventional evidence Moderate quality (3/6)	N=31 patients with RET V804L mutations from 5 families, who underwent thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Clinical screening Total thyroidectomy	3/31 (9.7%) patients had elevated pentagastrin-stimulated calcitonin levels postoperatively With a median follow-up of 8.5 years, no biochemical or clinical recurrence of MTC was observed	Median = 8.5 years
(Rodriguez Gonzalez et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=22 patients at risk of MEN2A who were found to be RET M+ and received prophylactic thyroidectomy All had RET codon 634 mutations	RET mutation testing by denaturing gradient gel electrophoresis of exons 10, 11, 13, 14 and 16, confirmed by restriction site polymorphism analysis Clinical screening Prophylactic total thyroidectomy ± central neck dissection	1/22 (4.5%) slightly elevated calcitonin after pentagastrin stimulation after 2 years	Mean = 23 months (range 6–57 months)

Study and location	Level of evidence	Study population	Intervention	Incidence of persistence or recurrence	Follow-up
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=16 RET M+ family members of a MEN2A family with a RET codon 618 mutation, who had a thyroidectomy 15 were identified by biochemical screening	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central neck dissection	7/12 (58.3%) patients tested had elevated pentagastrin-stimulated calcitonin levels	Mean = 19±9 years
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	N=8 RET M+ members of a 3-generation FMTC family (including index case), who underwent total thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of index case Analysis of exon 10 in family members Total thyroidectomy with either central neck dissection or modified radical neck dissection	3/8 (37.5%) had elevated pentagastrin-stimulated calcitonin levels	Median = 10 years
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Low quality (2/6)	N=12 RET M+ family members who had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	1/12 (8.3%) patient had recurrent MTC 11/12 (91.7%) patients had normal calcitonin levels	Up to 15 years

CCH = C-cell hyperplasia; HPT = hyperparathyroidism; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Appendix C Uncontrolled studies reporting incidence and severity of MTC

Table 74 Incidence and severity of MTC in index cases and clinically affected relatives

Study and location	Level of evidence	Study population	Intervention	No disease	Medullary micro-carcinoma	C-cell hyperplasia (without MTC)	MTC (+/– C-cell hyperplasia)	Lymph node metastases
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	N=23 unrelated RET M+ index cases with MTC: 4 FMTC 9 MEN2A 4 MEN2B 6 sporadic MTCs Note: Data for 1 FMTC index case was not available	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Thyroidectomy Surgical decisions often made before RET mutation testing with no separation of data based on clinical or genetic diagnosis	0/22	Not stated	0/22	22/22 (100%): 2 x T1N0M0 3 x T2N0M0 2 x T2N2M0 3 x T3N0M0 1 x T3N0M1h 1 x T3N0M1hpp 1 x T3N2M0 1 x T3N2M1h 4 x T4N0M0 1 x T4N2M0 1 x T4N2M1h 1 s T4N2M1pp 1 x T4N2Mx	9/22 (40.9%)
(Neumann et al. 2002) Germany and Poland	IV interventional evidence High quality (5/6)	N=13 RET M+ patients with non-syndromic PCC without family history of disease	RET mutation testing by single-strand conformation polymorphisms and direct DNA sequencing of exons 13–16	Not stated (unclear if 1/13 is disease free or has CCH)	Not stated	Not stated	12/13 (92.3%)	Not stated
(Sanso et al. 2002) Argentina	IV Interventional evidence Moderate	N=17 index cases with MEN2A N=5 index cases with MEN2B	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, confirmed by restriction site	MEN2A: 0/17 MEN2B: 0/5	Not stated	MEN2A: 0/17 MEN2B: 0/5	MEN2A: 17/17 (100%) MEN2B: 5/5 (100%) with aggressive MTC	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	Medullary micro-carcinoma	C-cell hyperplasia (without MTC)	MTC (+/– C-cell hyperplasia)	Lymph node metastases
	quality (4/6)		polymorphism analysis Prophylactic thyroidectomy					
(Boer et al. 2003) Hungary	IV Interventional evidence Moderate quality (4/6)	N=14 consecutive unrelated patients with MTC admitted for genetic screening for MEN2A and FMTC, who were found to be RET M+ and underwent a thyroidectomy	RET mutation testing by direct DNA sequencing (exons not specified) Thyroidectomy	0/14 (0%)	Not stated	5/14 (35.7%)	9/14 (64.3%)	3/11 (27.3%) of those examined
(Gimm et al. 2002). Germany, Austria	IV interventional evidence Moderate quality (4/6)	N=13 index patients with a RET codon 790/791 mutation who underwent thyroid operations: 7 had RET L790F mutation 6 had RET Y791F mutation	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14 Total thyroidectomy with or without lymph node dissection	1/13 (7.7%) 0/7 (0%) L790F mutation 1/6 (16.7%) Y791F mutation	Not stated	1/13 (7.7%) 0/7 (0%) L790F mutation 1/6 (16.7%) Y791F mutation	11/13 (84.6%): 7/7 (100%) L790F mutation 4/6 (66.7%) Y791F mutation	5/13 (38.5%) 4/7 (57.1%) L790F mutation 1/4 with distant metastases 1/6 (16.7%) Y791F mutation
(Abdelhakim et al. 2009) Morocco	IV Interventional evidence Moderate quality (4/6)	N=9 index patients with diagnosed MTC: 3 were RET M+: 2 MEN2A 1 unclassified 6 were suspected sporadic MTC (RET M–)	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 Total thyroidectomy	0/9 (0%)	Not stated	Not stated	9/9 (100%) Severity not stated	Not stated
(Spinelli et al. 2010) Italy	IV interventional evidence High quality (4/6)	N=7 patients with MEN2 who underwent curative surgery for MTC	RET mutation testing by direct DNA sequencing (exons not specified) Curative or prophylactic total thyroidectomy	0/7 (0%)	Not stated	0/7 (0%)	7/7 (100%): 5 T1N0M0 2 T4N1M1	2/7 (28.6%)

Study and location	Level of evidence	Study population	Intervention	No disease	Medullary micro-carcinoma	C-cell hyperplasia (without MTC)	MTC (+/– C-cell hyperplasia)	Lymph node metastases
(Ameur et al. 2009) France	IV Interventional evidence Moderate Quality (3/6)	N=21 tissue samples collected from MTC, CCH, MCC or mixed MTC patients that were found to have a germline RET mutation	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 from normal and diseased tissue samples to determine germline and somatic RET status	0/21 (0%)	1/21 (4.7%)	2/21 (9.5%)	18/21 (85.7%): 7 x T1N0M0 5 x T1N1M0 2 x T1N1Mx 1 x T2N0M0 1 x T2N1M0 2 x T3N1M0	10/21 (47.6%)
(Bergant et al. 2006) Slovenia	IV interventional evidence Moderate quality (3/6)	N=13 patients with sporadic MTC found to be RET M+	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case Total thyroidectomy	0/13 (0%)	Not stated	Not stated	13/13 (100%): 1 x T1bN1aM0 2 x T1bN1bM0 1 x T2aN0M0 3 x T2aN1aM0 3 x T2bN0M0 2 x T2bN1bM0 1 x T3aN1bM0	9/13 (69.2%)
(Machens et al. 2001) Germany	IV interventional evidence Poor quality (2/6)	N=63 RET M+ patients with MTC who had a thyroidectomy 36 were index patients	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13 and 14 Thyroidectomy				63/63 (100%): 38/63 T1 18/63 T2 4/63 T3 3/63 T4	37/63 (58.7%) 6 with distant metastases
(Vaclavikova et al. 2009) Czech Republic	IV Interventional evidence Poor quality (2/6)	N=6 index cases with a RET Y791F mutation had a total thyroidectomy: 1 MEN2B case 1 MEN2A case 3 apparently sporadic MTCs 1 PCC case	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	0/6 (0%)	2/6 (33.3%)	2/6 (33.3%)	4/6 (66.7%): 1 T2NxMx 1 T1N1M0 2 T4N1M0	3/6 (50.0%)

Study and location	Level of evidence	Study population	Intervention	No disease	Medullary micro-carcinoma	C-cell hyperplasia (without MTC)	MTC (+/– C-cell hyperplasia)	Lymph node metastases
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=5 members of an MTC family with a RET L790F mutation (including index patient), who had a thyroidectomy All had abnormal pentagastrin-stimulated calcitonin levels 3 had clinical signs of disease	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–15, and restriction site polymorphism analysis for exon 16 Direct DNA sequencing of exon 13 in family members Clinical screening Prophylactic thyroidectomy	0/5 (0%)	Not stated	Not stated	5/5 (100%): 5 T1N0M0	0/5 (0%)

CCH = C-cell hyperplasia; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 75 Incidence and severity of MTC in family members

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Machens et al. 2005) Germany	IV interventional evidence High quality (5/6)	N=206 consecutive RET M+ patients who underwent surgery for CCH, MTC or PCC: 74 index cases 132 non-index cases (criteria for diagnosis and/or surgery not reported)	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 Clinical screening Thyroidectomy and/or adrenalectomy	Not stated	Not stated	Not stated	135/206 (65.5%): 18/18 (100%) highest risk (RET codon 918) 85/117 (72.6%) high risk (RET codons 609–634) 32/71(45.1%) less high risk (RET codons 768–891)	Not stated
(Nguyen et al. 2001) France	IV interventional evidence High quality	N=87 first-degree relatives of index cases in MEN2 families, who were diagnosed with MTC and found to be RET M+:	MEN2 diagnosed by linkage analysis between 1989 and 1994	0/87 (0%)	Not stated	0/87 (0%)	87/87 (100%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
	(5/6)	84 patients from 52 MEN2A families 3 patients from 3 MEN2B families	RET mutation testing by sequence analysis since 1994 (method not stated) Total thyroidectomy					
(Skinner et al. 2005) USA	IV interventional evidence High quality (5/6)	N=50 RET M+ patients from MEN2A families, who were <20 years of age at time of thyroidectomy	RET mutation testing by restriction site polymorphism analysis and/or direct DNA sequencing of RET exons 10, 11, 13, 14 and 16 Total thyroidectomy	4/50 (8%)	Not stated	13/50 (26%)	33/50 (66%)	Not stated
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	N=30 RET M+ family members of patients with MTC reclassified from spontaneous MTC to FMTC or MEN2A due to RET mutation, who showed clinically and/or biochemical signs of disease on screening and underwent a total thyroidectomy: 29 phenotype FMTC 1 phenotype MEN2A	RET mutation testing method changed over 15 years Initially used direct DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy	1/30 (3.3%)	Not stated	6/30 (20%)	23/30 (76.6%): 15 x T1N0M0 3 x T1mN0M0 1 x T1bN0M0 1 x T1AN0M0 1 x T1N1aM0 1 x T3mN0M0 1 x T2MN1M0	2/30 (6.7%)
(Lau et al. 2009) Hong Kong	IV interventional evidence High quality (5/6)	N=22 asymptomatic patients from 8 MEN2A families, who underwent prophylactic total thyroidectomy All had RET codon 634 mutations	RET mutation testing by restriction enzyme analysis and/or direct DNA sequencing (exons not specified) Prophylactic thyroidectomy with or without a unilateral central compartment	1/22 (4.5%) RET C634W mutation	Not stated	4/22 (18.2%) 3 RET C634Y 1 RET C634W	17/22 (77.3%): 9 RET C634Y 4 RET C634R 1 RET C634W 3 RET C634G	0/22 (0%)

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
			neck dissection					
(Wells Jr & Skinner 1998) USA	IV interventional evidence High quality (5/6)	N=18 RET M+ first-degree relatives aged 21 years or younger from 7 MEN2A kindreds with no clinical symptoms, who underwent a thyroidectomy	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 10 and 11 Prophylactic thyroidectomy with bilateral cervicocentral lymphadenectomy	0/18 (0%)	13/18 (72.2%)		5/18 (27.7%)	0/18 (0%)
(Schellhaas et al. 2009) Germany	IV interventional evidence High quality (5/6)	N=17 patients with mutation in codon 634: 14 from MEN2A 3 with apparent FMTC	RET mutation testing (method not stated) Prophylactic thyroidectomy with bilateral cervicocentral lymphadenectomy	0/17 (0%)	Not stated	3/17 (17.6%) Aged 5, 6 and 9 years	14/17 (82.4%): 12/17 T1 MTC 2/17 T2 MTC (according to 1997 TNM classification, or 14/17 T1 by 2002 classification)	2/17 (11.7%) Aged 9 and 36 years
(Pinna et al. 2007) Italy	IV interventional evidence High quality (5/6)	N=14 RET M+ family members who have undergone prophylactic total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 8–16	1/14 (7.1%)	Not stated	1/14 (7.1%)	12/14 (85.7%)	Not stated
(Frank-Raue et al. 2011) Germany	IV Interventional evidence Moderate quality (4/6)	N=340 patients proven to be carriers of a germline mutation in exon 10 of the *RET* gene Identified through: 47% symptomatic 53% screening	RET mutation testing method not disclosed Analysis of clinical and demographic data	Not stated	Not stated	Not stated	263/340 (77%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Milos et al. 2008) Romania, Germany, Chile, Brazil, Argentina, Hungary, Spain, The Netherlands, Czech Republic, Poland, USA	IV interventional evidence Moderate quality (4/6)	N=81 carriers of RET C634W mutation from 20 unrelated MEN2A families, who underwent thyroidectomy	RET mutation testing (no methods stated) Thyroidectomy.	6/81 (7.4%)	Not stated	7/81 (8.6%)	68/81 (88%): 52% by age 30 years 83% by age 50 years Distant metastases 4/61 cases (aged 28–69 years)	16/61 (26%) had lymph node metastases (aged 20–72 years)
(Dralle et al. 1998) Germany	IV Interventional evidence Moderate quality (4/6)	N=75 RET M+ patients <20 years of age who underwent prophylactic thyroidectomy	RET mutation testing (method not stated) Clinical screening Total thyroidectomy Retrospectively identified through questionnaire	0/75 (0%)	Not stated	29/75 (38.6%)	46/75 (61.3%)	Not stated
(Learoyd et al. 2005) Australia and New Zealand	IV interventional evidence Moderate quality (4/6)	N=57 members of 2 families: Family 1: RET M+ 22 family members from 4 generations with RET V804L mutation Family 2: 5 RET M+ family members from 3 generations with RET V804M mutation	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 From 1998, analysis also of exons 13–15 for probands Family members screened for family RET mutation Prophylactic thyroidectomy	Family 1: 2/22 (9.1%) Family 2: 0/3 (0%)	Not stated	Family 1: 11/22 (50%) Family 2: 2/3 (66.7%)	Family 1: 9/22 (40.9%) Family 2: 1/3 (33.3%)	Family 1: 1/22 (4.5%) (proband) Family 2: 1/3 (33.3%)

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Alvares Da Silva et al. 2003) Brazil	IV Interventional evidence Moderate quality (4/6)	N=35 RET M+ members of a large extended FMTC family with a RET G533C mutation, who underwent a total thyroidectomy	RET mutation testing by direct DNA sequencing of exon 8 Prophylactic thyroidectomy	0/35 (0%)	Not stated	6/35 (17.1%)	29/35 (82.8%)	11/35 (31.4%)
(Etit et al. 2008) USA	IV Interventional evidence Moderate quality (4/6)	N=42 specimens from patients retrospectively identified from hospital records, who underwent a prophylactic thyroidectomy for possible MTC 32 underwent RET mutation testing 31 with family history: 22 MEN2A 1 MEN2B 8 non-MEN 27 were RET M+	RET mutation testing by direct DNA sequencing analysis of exons 10, 11 and 13–16	3/42 (7.1%) RET M+ 3/27 (11.1%)	29/42 (71.4%)	9/42 (21.4%) RET M+ 8/27 (29.6%)	30/42 (71.4%) RET M+ 16/27 (59.3%)	22/42 (52.4%)
(Gimm et al. 2002). Germany, Austria	IV interventional evidence Moderate quality (4/6)	N = 27 patients identified during RET mutation screening with a RET codon 790/791 mutation who underwent thyroid operations. 16 had RET L790F mutation 11 had RET Y791F mutation	RET mutation testing by single strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14. Total thyroidectomy.with or without lymph node dissection	5/27 (18.5%) 2/16 (12.5%) RET L790F mutation 3/11 (27.3%) RET Y791F mutation	Not stated	14/27 (51.9%) 6/16 (37.5%) RET L790F mutation 8/11 (72.7%) RET Y791F mutation	8/27 (29.6%) 8/16 (50%) RET L790F mutation 0/11 (0%) RET Y791F mutation	2/27 (7.4%) 2/16 (12.5%) RET L790F mutation 0/11 (0%) RET Y791F mutation
(Gosnell et al. 2006) Australia	IV Interventional evidence Moderate	N=22 RET M+ members of a single MEN2A kindred with a RET codon 804 mutation, who underwent thyroidectomy	RET mutation testing (method not stated) Clinical screening Prophylactic	2/22 (9.1%)	2/22 (9.1%)	9/22 (40.9%)	9/22 (40.9%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
	quality (4/6)		thyroidectomy					
(Franz & Wells Jr 1997) USA, Germany	IV Interventional evidence Moderate quality (4/6)	N=20 RET M+ patients: 19 MEN2A 1 FMTC	RET mutation testing by restriction site polymorphism analysis and/or DNA sequencing (exons not specified) Clinical screening Prophylactic thyroidectomy based on RET status	0/20 (0%)	Not stated	20/20 (100%)		0/20 (0%)
(Feldman et al. 2000) UK, USA, France	IV Interventional evidence Moderate quality (4/6)	N=20 members from 2 FMTC families who have a RET V804M mutation	RET mutation testing by restriction analysis of exon 14 Clinical screening. Thyroidectomy	1/20 (5%)	Not stated	6/20 (30%)	13/20 (65%)	Not stated
(Sanso et al. 2002) Argentina	IV interventional evidence Moderate quality (4/6)	N=18 RET M+ juvenile patients from MEN2 families who had a total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, confirmed by restriction site polymorphism analysis Prophylactic thyroidectomy	0/18 (0%)	Not stated	3/18 (16.7%)	15/18 (83.3%): 7 unilaterally 8 bilaterally	1/18 (5.6%)
(Decker et al. 1995) USA	IV Interventional evidence Moderate quality (4/6)	N=17 members of 10 MEN2A or FMTC kindreds with known RET mutations who had a thyroidectomy	RET mutation testing by denaturing gradient gel analysis of exons 10 and 11, with confirmatory direct DNA sequencing	0/17 (0%)	Not stated	10/17 (58.8%)	7/17 (41.2%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Calva et al. 2009) USA	IV Interventional evidence Moderate quality (4/6)	N=16 RET M+ members of a MEN2 family with a RET C609Y mutation, who underwent a thyroidectomy	RET mutation testing (method not stated) Clinical screening Total thyroidectomy for treatment or prophylaxis	5/16 (31.3%)	Not stated	2/16 (12.5%)	9/16 (56.3%)	6/16 (37.5%)
(Chiefari et al. 1998) Italy	IV interventional evidence Moderate quality (4/6)	N=16 RET M+ members from 10 families who had available data, and who underwent total thyroidectomy: 11 MEN2A 2 MEN2B 2 FMTC 1 other	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14 Clinical screening Total thyroidectomy	0/16 (0%)	Not stated	1/16 (6.3%)	15/16 (93.8%)	Not stated
(Frohnauer et al. 2000) USA	IV Interventional evidence Moderate quality (4/6)	N=14 members of 5 MEN2A kindreds who had a RET codon 804 mutation and a thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis, confirmed by direct DNA sequencing of exon 14 Thyroidectomy	1/14 (7.1%)	Not stated	6/14 (42.9%)	7/14 (50%)	3/14 (21.4%) 1/14 (7.1%) had distant metastases
(Guyetant et al. 2003) France	IV interventional evidence High quality (5/6)	N=14 potential MEN2 carriers belonging to 9 families who had been operated on for CCH or MTC 3 MEN2A 14 FMTC 7 were children aged <15 years	RET mutation testing of exons 8, 10, 11 and 13–16 (method not stated)	0/14 (0%)	Not stated	3/14 (21.4%) 3/7 (42.9%) children	11/14 (78.6%) 4/7 (57.1%) children	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Heizmann et al. 2006) Switzerland	IV interventional evidence Moderate quality (4/6)	N=14 RET M+ patients who were presymptomatic, from 2 MEN2A kindreds: 3 RET C634Y mutations 11 RET C618G mutations	RET mutation testing by single-strand conformation polymorphism analysis, denaturing gradient gel electrophoresis and direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central compartment dissection in those older than 6 years of age	0/14 (0%)	Not stated	3/14 (21.4%)	11/14 (78.5%) 4/14 bilateral MTCs 9 x pT1 pN0 2 x pT1 pN1a	2/14 (14.3%) (<2 mm)
(Donis-Keller 1995) USA	IV Interventional evidence Moderate quality (4/6)	N=13 RET M+ family members from 7 MEN2A kindreds, who had a thyroidectomy 7 had elevated calcitonin levels	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of affected exon Thyroidectomy	0/13 (0%)	Not stated	13/13 (100%) had evidence of CCH with or without MTC		Not stated
(Algun et al. 2002) Turkey	IV interventional evidence Moderate quality (4/6)	N=12 RET M+ members from 4 generations of an extended family with MEN2A, who had a thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exon 11 Confirmation with clinical tests Total thyroidectomy with central lymph node dissection	3/12 (25%)	4/12 (33.3%)	3/12 (25%)	5/12 (41.7%)	6/12 (50%) 1 with bone metastases
(Vestergaard et al. 2007) Denmark	IV interventional evidence Moderate	N=12 first-degree RET M+ family members of index case with a RET Y791F mutation	RET mutation testing by direct DNA sequencing of exon 13 No thyroidectomy	12/12 had normal pentagastrin-stimulated	Not stated	Not stated	Not stated	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
	quality (4/6)			calcitonin levels				
(Frank-Raue et al. 1997) Germany	IV Interventional evidence Moderate quality (4/6)	N=11 asymptomatic RET M+ children from 8 MEN2A/FMTC families	RET mutation testing by single-strand conformation polymorphism analysis or restriction site polymorphism analysis, and direct DNA sequencing of exons 10, 11 and 13 Prophylactic thyroidectomy	0/11 (0%)	Not stated	5/11 (45.5%)	6/11 (54.5%)	Not stated
(Decker et al. 1996) USA	IV interventional evidence Moderate quality (4/6)	N=11 RET M+ children of confirmed MEN2A patients from 4 distinct families who underwent prophylactic surgery	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11 Clinical screening Prophylactic thyroidectomy	1/11(9.1%)	Not stated	9/11 (81.8%)	1/11 (9.1%)	Not stated
(Frank-Raue et al. 1996) Germany	IV Interventional evidence Moderate quality (4/6)	N=9 presymptomatic RET M+ patients who underwent prophylactic thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA sequencing or restriction site polymorphism analysis of exons 10 and 11 Direct DNA sequencing of exons 13 and 16 Clinical screening	0/9 (0%)	5/9 (55.6%)	9/9 (100%)	0/9 (0%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
			Prophylactic thyroidectomy					
(Spinelli et al. 2010) Italy	IV interventional evidence High quality (4/6)	N=6 patients with MEN2 who underwent prophylactic surgery for MTC	RET mutation testing by direct DNA sequencing (exons not specified) Curative or prophylactic total thyroidectomy	0/6 (0%)	Not stated	2/6 (33.3%)	4/6 (66.7%) T1N0M0	0/7 (0%)
(Marsh et al. 1996) Australia and New Zealand	IV interventional evidence Moderate quality (4/6)	N=5 RET M+ asymptomatic members from 2 MEN2A families	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11	1/5 (20%)had raised pentagastrin-stimulated calcitonin levels	Not stated	1/5 (20%)	3/5 (60%)	Not stated
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=50 RET M+ index cases and family members with 634 mutation, who underwent surgery: 43 had clinical disease 7 were clinically asymptomatic gene carriers	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15 Total thyroidectomy, with a central cervical lymph node dissection in those with increased calcitonin levels	0/50 (0%)	Not stated	50/50 (100%) CCH or MTC		22/43 (51.2%) with clinical disease 1/7 (14.3%) asymptomatic gene mutation carriers 14/43 (32.6%) with clinical disease had distant metastases
(Quayle et al. 2004) USA	IV interventional evidence Moderate quality (3/6)	N=39 patients with MEN2 or FMTC diagnosed when over 50 years of age 36 patients from MEN2A families	RET mutation testing (method not stated) Total thyroidectomy	0/39 (0%)	Not stated	2/39 (5.1%)	37/39 (94.9%) AJCC staging: 12 (34%) stage I 11 (31%) stage II 11 (31%) stage III	3/7 who underwent central node dissection had N1 disease

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
		3 from FMTC families 38 with known RET mutation: 5 with codon 609 mutation 15 with codon 618 mutation 6 with codon 620 mutation 12 with codon 634 mutation 1 with unknown mutation					1 (3%) stage IV	
(Lombardo et al. 2002) France and Italy	IV interventional evidence Moderate quality (3/6)	N=31 patients with RET V804L mutations from 5 families, who underwent thyroidectomy: 3 index cases with MTC 1 with follicular tumour 14 with detectable basal calcitonin levels 13 with significant increase in pentagastrin-stimulated calcitonin levels	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Clinical screening Total thyroidectomy	1/31 (3.2%)	Not stated	12/31 (38.7%)	18/31 (58.1%): Before age 40 years: 2/11 (18.2%) After age 40 years: 16/20 (80%)	6/31 (19.4%)
(Erdogan et al. 2007) Turkey	IV Interventional evidence Moderate quality (3/6)	N=30 RET M+ patients identified from 15 pedigrees, who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–15, and restriction enzyme analysis of exon 16 Total thyroidectomy	0/30 (0%)	Not stated	30/30 (100%) had multifocal MTC and/or CCH		Not stated
(Halling et al. 1997) USA	IV interventional evidence Moderate quality (3/6)	N=28 RET M+ family members from 1 large FMTC kindred with a RET C609Y mutation, who had thyroidectomy before prior to testing	RET mutation testing by direct DNA sequencing of exon 10 Clinical screening Thyroidectomy	RET M+ 5/28 (17.9%) RET M– 1/10 (10%)	Not stated	RET M+ 8/28 (28.6%) RET M– 8/10 (80%)	RET M+ 15/28 (53.6%) RET M– 1/10 (10%)	Not stated

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
		19 with elevated pentagastrin-stimulated calcitonin levels N=10 RET M– family members who had thyroidectomy prior to genetic testing, with elevated pentagastrin-stimulated calcitonin levels						
(Rodriguez Gonzalez et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=22 RET M+ patients with normal basal and pentagastrin-stimulated calcitonin levels who received a prophylactic thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis of exons 10, 11, 13, 14 and 16, confirmed by restriction site polymorphism analysis Clinical screening Prophylactic total thyroidectomy ± central neck dissection	0/22 (0%)	Not stated	7/22 (31.8%)	15/22 (68.2%) 14 x T1N0M0 1 x T1N1aM0	1/22 (4.5%)
(Bergant et al. 2006) Slovenia	IV interventional evidence Moderate quality (3/6)	N=16 RET M+ family members screened from index patients	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case Total thyroidectomy with central neck dissection	3/16 (18.8%) T0N0M0	Not stated	2/16 (12.5%)	13/16 (81.3%): 3 x T1bN0M0 2 x T1bN1bM0 2 x T2bN0M0 4 x T2bN1bM0 1 x T3bN1bM0 1 x T4bN1bM0	8/16 (50.0%)

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=16 RET M+ family members of a MEN2A family with a RET codon 618 mutation, who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central neck dissection	0/16 (0%)	Not stated	Not stated	16/16 (100%): 1 T1NXM0 3 T1N0M0 4 T1N1M0 6 T2N1M0 2 T4N1M1	12/16 (75%)
(Shifrin et al. 2009) USA	IV interventional evidence Moderate quality (3/6)	N=15 family members from family with RET V804M mutation, who had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (not clear if relatives tested for specific mutation or all) Total thyroidectomy with central and ipsilateral lateral neck dissection	0/15 (0%)	Not stated	5/15 (33%)	10/15 (67%)	
(Wu et al. 1998) Taiwan	IV interventional evidence Moderate quality (3/6)	N=13 RET M+ first- and second-degree relatives from 2 unrelated MEN2A families	RET mutation testing by direct DNA sequencing of exons 10 and 11	5/13 (38.5%) showed no clinical signs of disease	Not stated	Not stated	8/13 (61.5%)	Not stated
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	N=8 members of a 3-generation FMTC family, who underwent total thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of index case Analysis of exon 10 in family members	0/8 (0%)	Not stated	0/8 (0%)	8/8 (100%): 1 x T1N1M0 1 x T2N0M0 4 x T2N1M0 2 x T3N1M0	7/8 (87.5%)

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
			Total thyroidectomy with either central neck dissection or modified radical neck dissection					
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	N=8 asymptomatic RET M+ members of 4 large MEN2A families, who had a thyroidectomy	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy	0/8 (0%)	Not stated	8/8 (100%)	8/8 (100%) (scattered, generally small, irregular foci of MTC)	Not stated
(Gagel et al. 1995) USA	IV Interventional evidence Moderate quality (3/6)	N=4 RET M+ patients (children aged 3–12 years) who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16 Thyroidectomy based on genetic screening	0/4 (0%)	Not stated	3/4 (75%)	1/4 (25%) Unilateral microscopic MTC	Not stated
(Pacini et al. 1995) Italy	IV interventional evidence Moderate quality (3/6)	N=4 clinically unaffected RET M+ family members from 7 MEN2A and 2 MEN2B families, who had a thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	0/4 (0%)	Not stated	0/4 (0%)	4/4 (100%)	1/4 (25%)
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=12 family members with a RET Y791F mutation, who underwent a total thyroidectomy 1 MEN2B family 1 MEN2A family 1 FMTC family 1 apparently sporadic MTC family	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	1/12 (8.3%)	Not stated	9/12 (75.0%)	2/12 (16.7%) T1N1M0	2/12 (16.7%)

Study and location	Level of evidence	Study population	Intervention	No disease	MCC (medullary microcarcinoma)	C-cell hyperplasia (without MTC)	MTC	Lymph node metastases
(Hernandez et al. 1997) Spain	IV interventional evidence Poor quality (2/6)	N=6 RET M+ asymptomatic members of 3 MEN2A families, who had a thyroidectomy All had raised preoperative pentagastrin-stimulated calcitonin levels	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11 Clinical screening Total thyroidectomy	0/6 (0%)	Not stated	3/6 (50%)	3/6 (50%)	0/6 (0%)
(Kinlaw et al. 2005) USA	IV interventional evidence Poor quality (2/6)	N=6 asymptomatic RET M+ members of 3 MEN2A families, who had a thyroidectomy All 6 had raised pentagastrin-stimulated calcitonin levels and a thyroidectomy	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11 Clinical screening Total thyroidectomy	0/6 (0%)	Not stated	4/6 (66.7%)	2/6 (33.3%)	Not stated
(Uchino et al. 1999) Japan	IV interventional evidence Low quality (2/6)	N=6 clinically unaffected RET M+ members from 5 MEN2A families with mutations on codon 634	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11, 13, 14 and 16	1/6 (16.7%) Slightly increased tetragastrin-stimulated calcitonin levels	Not stated	Not stated	5/6 (83.3%) Diagnosis: 3 by pathology 3 by ultrasound	0/3 (0%) of those examined

CCH = C-cell hyperplasia; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Appendix D Uncontrolled studies reporting incidence of phaeochromocytoma and hyperparathyroidism

Table 76 Penetrance of phaeochromocytoma and hyperparathyroidism in RET-mutation-positive index cases

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	N=23 unrelated index cases with MTC clinically and biochemically characterised as: 82 sporadic MTCs 10 FMTC 10 MEN2A 4 MEN2B 23 were RET M+ Details provided on 22 cases	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	12/22 (54.5%) confirmed or suspected	Not stated
(Frank-Raue et al. 2011) Germany	IV Interventional evidence Moderate quality (4/6)	N=340 patients from 14 different countries who were proven carriers of germline mutation in exon 10 of the *RET* gene	RET mutation testing (method not stated) Clinical screening	54/319 (17%) Identified through: 54% symptomatic 46% screening	8/299 (2.7%) Identified through: 12.5% symptomatic 87.5% screening
(Sanso et al. 2002) Argentina	IV interventional evidence Moderate quality (4/6)	N=17 index cases with MEN2A (5 men, 12 women, aged 19–60 years) N=5 index cases with MEN2B (3 men, 2 women, aged 5–22 years)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, confirmed by restriction site polymorphism analysis	MEN2A: 13/17 (76.4%) (bilateral in 7 and malignant in 2) 4/17 had parathyroid adenoma MEN2B: 2/5 (bilateral)	Not stated
(Boer et al. 2003) Hungary	IV Interventional evidence Moderate quality (4/6)	N=14 consecutive unrelated patients with MTC admitted for genetic screening for MEN2A and FMTC, who were RET M+ and underwent a thyroidectomy	RET mutation testing by direct DNA sequencing (exons not specified) Thyroidectomy	Not stated	1/14 (7.1%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Etit et al. 2008) USA	IV Interventional evidence Moderate quality (4/6)	N=13 patients retrospectively identified from hospital records who had undergone a prophylactic thyroidectomy for possible MTC	RET mutation testing by direct DNA sequencing analysis of exons 10, 11 and 13–16	Not stated	5/13 (38.5%) hypercellular parathyroids were encountered on exploration
(Gimm et al. 2002). Germany, Austria	IV interventional evidence Moderate quality (4/6)	N = 13 index patients with a RET codon 790/791 mutation who underwent thyroid operations	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14 Thyroidectomy	2/13 (15.4%)	0/13 (0%)
(Abdelhakim et al. 2009) Morocco	IV Interventional evidence Moderate Quality (4/6)	N=9 patients with confirmed MTC 3 were RET M+: 2 MEN2A 1 unclassified 0/6 suspected sporadic MTC cases had a RET mutation	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 Total thyroidectomy	2/9 (22.2%)	0/9 (0%)
(Paszko et al. 2007) Poland	IV interventional evidence Moderate quality (3/6)	N=46 patients with MTC who were RET M+	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	19/46 (41.3%)	3/46 (6.5%) with parathyroid pathologies (adenoma and hyperplasia)
(Erdogan et al. 2007) Turkey	IV Interventional evidence Moderate quality (3/6)	N=41 RET M+ patients identified from 15 pedigrees: 12 MEN2A 2 MEN2B 1 FMTC 26 were asymptomatic	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–15, and restriction enzyme analysis of exon 16 Total thyroidectomy	19/41 (46.3%)	1/41 (2.4%)
(Patocs et al. 2006) Hungary	IV interventional evidence Moderate	N=40 patients from 18 families who had a thyroidectomy due to hereditary MTC or CCH: 33 MEN2A	RET mutation testing by single-strand conformation polymorphism analysis, restriction site polymorphism analysis, and direct	Total: 16/40 (40%) Exon 10: 2/5 Exon 11: 14/26	Total: 3/40 (7.5%) Exon 10: 0/5 Exon 11: 2/26

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
	quality (3/6)	1 MEN2B 6 from MTC families without PCC or HPT	DNA sequencing of exons 10, 11, 13, 14 and, in MEN2B phenotype, exons 15 and 16	Exon 14: 0/8 Exon 16: 0/1	Exon 14: 1/8 Exon 16: 0/1
(Bergant et al. 2006) Slovenia	IV interventional evidence Moderate quality (3/6)	N=13 RET mutation + patients out of 69 with 'sporadic' MTC	RET mutation teting by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction enzyme analysis to confirm mutations in FMTC, MEN2A and MEN2B families	5/13 (38.5%)	2/13 (15.4%)
(Machens et al. 2006) Germany	IV interventional evidence Poor quality (2/6)	N=219 patients with RET mutations divided into 3 categories: RET codon 918 mutations (highest risk) RET codons 609–634 (high risk) RET codons 768–891 (least high risk) (Machens et al. 2005) previously described 206 patients: 74 index cases 132 non-index cases	RET mutation testing (method not stated)	Highest risk (918): Penetrance at age: 30 years: 43% 35 years: 100% 50 years: 100% 70 years: 100% High risk (609–634): Penetrance at age: 30 years: 8% 35 years: 18% 50 years: 54% 70 years: 73% Least high risk (768–891): Penetrance at age: 30 years: 0% 35 years: 0% 50 years: 4% 70 years: 9%	Not stated
(Karga et al. 1998)	IV interventional	N=24 clinically affected RET M+ patients who had a thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA	10/24 (41.7%)	1/24 (4.2%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
Greece	evidence Poor quality (2/6)	for MTC prior to genetic testing	sequencing of exons 10, 11, 13, 14 and 16		
(Hernandez et al. 1997) Spain	IV interventional evidence Poor quality (2/6)	N=17 symptomatic RET M+ members of 3 MEN2A families	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11 Clinical screening Total thyroidectomy	6/17 (35.3%)	0/17 (0%)
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=10 index cases with RET Y791F mutation 1 MEN2B case 1 MEN2A case 1 FMTC case 3 apparently sporadic MTCs 1 with PCC 3 HSCR cases 6 underwent total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	2/10 (20%): 1 case both sides (aged 31 years) 1 case right (aged 38 years, malignant)	0/10 (0%)

CCH = C-cell hyperplasia; HPT = hyperparathyroidism; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Table 77 Penetrance of phaeochromocytoma and hyperparathyroidism in RET-mutation-positive family members

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Nguyen et al. 2001) France	IV interventional evidence High quality (5/6)	N=87 first-degree relatives of index cases in MEN2 families, who were diagnosed with MTC and found to be RET M+: 84 patients from 52 MEN2A families 3 patients from 3 MEN2B families	MEN2 diagnosed by linkage analysis between 1989 and 1994 RET mutation testing by sequence analysis since 1994 (method not stated)	14/87 (16.1%) 12/84 MEN2A 2/3 MEN2B	4/87 (4.6%)
(Romei et al. 2011)	IV interventional	N=30 RET M+ family members of patients with MTC reclassified from	RET mutation testing method changed over 15 years	0/30	0/30

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
Italy	evidence High quality (5/6)	spontaneous MTC to FMTC or MEN2A due to RET mutation, who showed clinical and/or biochemical signs of disease on screening and had a thyroidectomy: 29 phenotype FMTC 1 phenotype MEN2A	Initially used direct DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy		
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	N=14 RET M+ relatives of index cases with MTC, who had high calcitonin levels and a thyroidectomy Index cases: 1 x sporadic MTC 10 x MEN2A 1 x FMTC 2 x MEN2B	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Thyroidectomy Surgical decisions often made prior to RET mutation testing with no separation of data based on clinical or genetic diagnosis	7/14 (50%) suspected	Not stated
(Kameyama, Okinaga & Takami 2004) Japan	IV interventional evidence Moderate quality (4/6)	N=108 patients with histologically confirmed familial MTC: 83 MEN2A 14 FMTC 11 MEN2B 53 were symptomatic when diagnosed	RET mutation testing (method not stated)	MEN2A: 45% FMTC: 0% MEN2B: 0%	MEN2A: 11% FMTC: 0% MEN2B: 0%
(Schuffenecker et al. 1994) France	IV interventional evidence Moderate quality (4/6)	N=259 affected members from 53 families (including index cases) with RET codon 634 mutations N=60 affected members from 13 families (including index cases) with RET codon 618 or 620 mutations	RET mutation testing by direct DNA sequencing of exons 10 and 11	Codon 634: 151/259 (58%, 95% CI 52, 64) C634R: 70/110 (64%) C634Y: 53/95 (56%) Other: 28/54 (52%) Codon 618 or 620: 5/60 (8%, 95% CI 3, 18)	See below (Schuffenecker et al. 1998)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Schuffenecker et al. 1998) France	IV interventional evidence Moderate quality (4/6)	N=188 patients from 30 families with RET codon 634 mutation 10 C634R mutations 11 C634Y mutations 9 other 634 mutations	RET mutation testing by direct DNA sequencing of exons 10 and 11 for index cases, and by restriction site polymorphism analysis for relatives	Not stated	Total: 36/188 (19.1%): C634R: 15/65 (23.1%) C634Y: 14/80 (17.5%) C634F: 6/17 (35.3%) C634S: 0/11 (0%) C634G: 1/12 (8/3%) C634W: 0/3 (0%) Penetrance at age: 30 years: 14% 40 years: 26% 60 years: 48% 70 years: 81%
(Milos et al. 2008) Worldwide (Romania, Germany, Chile, Brazil, Argentina, Hungary, Spain, The Netherlands, Czech Republic, Poland, USA)	IV interventional evidence Moderate quality (4/6)	N=92 carriers of RET C634W mutation from 20 unrelated MEN2A families	RET mutation testing (method not stated)	41/92 (44.5%) Age-related penetrance: 20% by age 30 years 67% by age 50 years	6/64 (9.4%) Age-related penetrance: 3% by age 30 years 21% by age 50 years
(Dralle et al. 1998) Germany	IV Interventional evidence Moderate Quality (4/6)	N=75 RET M+ patients <20 years of age who have undergone a prophylactic total thyroidectomy	RET mutation testing (method not stated) Clinical screening Total thyroidectomy Retrospectively identified through questionnaire	2/75 (2.6%)	0/75 (0%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Algun et al. 2002) Turkey	IV interventional evidence Moderate quality (4/6)	N=18 members from a family with MEN2A who were RET M+ N=12 RET M+ family members who underwent total thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exon 11 Clinical screening Total thyroidectomy with central lymph node dissection	3/18 (16.7%) 3/12 (25%)	1/18 (5.6%) 1/12 (8.3%)
(Calva et al. 2009) USA	IV Interventional evidence Moderate quality (4/6)	N=16 RET M+ family members who underwent a thyroidectomy RET C609Y mutation	RET mutation testing (method not stated) Clinical screening Total thyroidectomy	0/16 (0%)	1/16 (6.3%)
(Learoyd et al. 2005) Australia, New Zealand	IV interventional evidence Moderate quality (4/6)	N=57 members of 2 families: Family 1: 22 RET M+ family members from 4 generations with a RET V804L mutation Family 2: 5 RET M+ family members from 3 generations with a RET V804M mutation	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 From 1998, analysis also of exons 13–15 for probands Family members screened for family RET mutation	Family 1: 1/22 (4.5%) Family 2: 0/5	Family 1: 6/22 (27.3%) Family 2: 0/5
(Gosnell et al. 2006) Australia	IV Interventional evidence Moderate quality (4/6)	N=22 RET M+ members from a MEN2A family with RET codon 804 mutation, who underwent total thyroidectomy	RET mutation testing (method not stated) Clinical screening Prophylactic thyroidectomy	1/22 (4.5%) (index patient)	3/22 (13.6%) with parathyroid involvement 1 with parathyroid hyperplasia 1 with parathyroid adenoma 1 with parathyroid cyst
(Chiefari et al. 1998) Italy	IV interventional evidence Moderate quality (4/6)	N=16 RET M+ patients from 8 families with hereditary MTC, who had a thyroidectomy: 11 x MEN2A 2 x MEN2B 2 x FMTC 1 x other	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14 Clinical screening Thyroidectomy	5/16 (31.3%)	1/16 (6.2%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Vestergaard et al. 2007) Denmark	IV interventional evidence Moderate quality (4/6)	N=12 first-degree family members who had a RET Y791F mutation	RET mutation testing by direct DNA sequencing of exon 13	0/12	0/12
(Marsh et al. 1996) Australia and New Zealand	IV interventional evidence Moderate quality (4/6)	N=5 asymptomatic RET M+ members from 2 MEN2A families	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11	2/5 (40%)	Not stated
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	N=80 MEN2A gene carriers (61 diagnosed by DNA sequence analysis) 14 were symptomatic	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11	39/80 (48.8%)	3/80 (3.8%)
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=69 RET M+ index cases and family members from 52 MEN2A and 3 MEN2B families with RET codon 634 mutations	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15	18/69 (26.1%)	9/69 (13.0%)
(Lecube et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=25 family members of a FMTC family who had the RET V804M mutation	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–16 Biochemical screening on those who were RET M+	0/25	0/25
(Rodriguez Gonzalez et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=22 RET M+ patients without clinical signs of disease who received prophylactic thyroidectomy All had mutations in RET codon 634	RET mutation testing by denaturing gradient gel electrophoresis, confirmed by restriction analysis Prophylactic total thyroidectomy +/– central neck dissection	2/22 (9.1%) had bilateral PCC detected prior to total thyroidectomy	1/22 (4.5%) had parathyroid hyperplasia diagnosed preoperatively
(Bergant et al.	IV interventional	N=16 RET M+ family members who had a total thyroidectomy	RET mutation testing by single-strand conformational analysis and	7/16 (43.8%)	3/16 (18.8%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
2006) Slovenia	evidence Moderate quality (3/6)		confirmatory direct DNA sequencing of exons 10, 11 and 13–16 or restriction site polymorphism analysis of exon affected in index case Total thyroidectomy with central neck dissection		
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=16 RET M+ family members of a MEN2A family with a RET codon 618 mutation, who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central neck dissection	1/16 (6.3%)	4/16 (25%)
(Shifrin et al. 2009) USA	IV interventional evidence Moderate quality (3/6)	N=15 members from a family with a RET V804M mutation, who had a total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (not clear if relatives tested only for specific mutation) Total thyroidectomy with central and ipsilateral lateral neck dissection	0/15 (0%) (95% CI 0, 22)	2/15 (13.3%) (95% CI 2, 40)
(Wu et al. 1998) Taiwan	IV interventional evidence Moderate quality (3/6)	N=13 RET M+ first- and second-degree relatives from 2 unrelated MEN2A families	RET mutation testing by direct DNA sequencing of exons 10 and 11	6/13 (46.2%)	3/13 (23.1%)
(Yoshida et al. 2009) Japan	IV interventional evidence Moderate quality (3/6)	N=12 adults who underwent total thyroidectomy for MTC and had MEN2 5 were symptomatic All had raised pentagastrin-stimulated calcitonin levels	RET mutation testing (method not stated) Total thyroidectomy; the parathyroid gland was also removed and autotransplanted (unclear whether treatment decisions influenced by RET mutation)	5/12 (41.7%): 1 had adrenalectomy before thyroidectomy 3 had adrenalectomy after thyroidectomy 1 had right adrenalectomy before thyroidectomy and left adrenalectomy after thyroidectomy	2/12 (16.7%)

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Pacini et al. 1995) Italy	IV interventional evidence Moderate quality (3/6)	N=5 clinically unaffected RET M+ family members from 7 MEN2A and 2 MEN2B families	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	1/5 (20%) One 23 year old patient has MTC with lymph node metastases, PCC and HPT	
(Quayle et al. 2007) USA	IV interventional evidence Poor quality (2/6)	N=323 patients from 65 MEN2A families who were RET M+	RET mutation testing (method not stated)	102/323 (31.5%): C609G: 1/1 (100%) C609Y: 0/23 C618F: 0/7 C618G: 5/21 (23.8%) C618R: 11/27 (40.7%) C618S: 7/41 (17.1%) C618Y: 0/9 C620F: 0/2 C620R: 2/23 (8.7%) C620S: 0/4 C620Y: 2/16 (12.5%) C634G: 0/3 C634R: 49/103 (47.5%) C634S: 4/4 (100%) C634W: 4/5 (80%) C634Y: 17/34 (50%)	Not stated
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=21 family members with RET Y791F mutation 1 MEN2B family 1 MEN2A family 1 FMTC family 3 apparently sporadic MTC families 3 HSCR families	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	1/21 confirmed (4.8%) (left, at 30 years of age) 1/21 suspected (at 17 years of age)	Not stated

Study and location	Level of evidence	Study population	Intervention	Incidence	
				Phaeochromocytoma	Hyperparathyroidism
(Kinlaw et al. 2005) USA	IV interventional evidence Poor quality (2/6)	N=11 RET M+ family members (including index case) of a MEN2A family with a RET C609S mutation, who were evaluated	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index case Restriction site polymorphism analysis to detect C609S mutation in family members	3/11 (27.3%)	Not stated
(Uchino et al. 1999) Japan	IV interventional evidence Low quality (2/6)	N=6 clinically unaffected RET M+ members from 5 MEN2A families with mutations on codon 634	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11, 13, 14 and 16	1/6 (16.7%)	Not stated
(Karga et al. 1998) Greece	IV interventional evidence Poor quality (2/6)	N=5 asymptomatic RET M+ children	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and 16	0/5 (0%)	1/5 (20%)

HPT = hyperparathyroidism; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Appendix E Uncontrolled studies reporting age at diagnosis

Table 78 Mean age at diagnosis (index cases)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
(Neumann et al. 2002) Germany and Poland	IV interventional evidence High quality (5/6)	N=271 patients with nonsyndromic PCC without family history of disease 13 were RET M+	RET mutation testing by single-strand conformation polymorphisms and direct DNA sequencing of exons 13-16. Also checked for mutations in SDHB, SDHD and VHL.	Mean=36.4 years (range 21 – 50)
(Kameyama, Okinaga & Takami 2004) Japan	IV interventional evidence Moderate quality (4/6)	N=271 patients with histologically confirmed MTC: 108 hereditary MTC: 83 MEN2A; 14 FMTC; 11 MEN2B 53 were symptomatic 55 were asymptomatic 163 were sporadic MTC	RET mutation testing (method not stated)	MEN2A: mean=35.6 years MEN2B: mean=30.5 years FMTC: mean=34.6 years Sporadic MTC: mean=47.6 years
(Abdelhakim et al. 2009) Morocco	IV Interventional evidence Moderate quality (4/6)	N=9 patients clinically diagnosed with MTC	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 Total thyroidectomy	Mean = 37.8±15.8 years Median age for: MEN2A = 20 years Sporadic MTC = 38 years
(Patocs et al. 2004) Hungary	IV interventional evidence Moderate quality (4/6)	N=7 RET M+ patients with PCCs	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 14	Mean = 49.4±10.9 years (range 33–63 years)
(Ameur et al. 2009) France	IV Interventional evidence Moderate quality (3/6)	N=46 tissue samples from patients diagnosed with MTC 21 had a germline RET mutation	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 from normal and diseased tissue samples to determine germline and somatic RET status	*Germline mutations:* Mean = 27.5 years (range 4–73 years) *Sporadic:* Mean = 50.8 years (range 21–74 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
(Patocs et al. 2006) Hungary	IV interventional evidence Moderate quality (3/6)	N=40 patients from 18 families who had had a thyroidectomy due to hereditary MTC or CCH: 33 MEN2A 1 MEN2B 6 from MTC families without PCC or HPT	RET mutation testing by single-strand conformation polymorphism analysis, restriction site polymorphism analysis, and direct DNA sequencing of exons 10, 11, 13, 14 and, in MEN2B phenotype, exons 15 and 16	C609S: 35 years (range 15–48 years) C609Y: 41 years (range 27–55 years) C634F: 43 years (range 27–55 years) C634Y: 27 years (range 16–51 years) C634S: 41 years (range 33–51 years) C634R: 28.5 years (range 22–35 years) C634W: 35 years (range 16–55 years) V804M: 38 years (range 34–45 years) V804L: 38 years (range 33–55 years) M918Y: 18 years
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=17 MEN2 index cases: 11 MEN2A 1 FMTC 4 MEN2B 1 other (fewer than 4 MTC cases in family)	RET mutation testing by single-strand conformational polymorphism analysis, restriction enzyme analysis and direct sequencing of exons 10, 11 and 13–15	Mean = 30.6±12.6 years (range 11–55 years) *MEN2A* Mean = 32.9±8.4 years (range 19–45 years) *MEN2B* Mean = 15.0±4.2 years (range 11–21 years)
(Bergant et al. 2006) Slovenia	IV Interventional evidence Moderate quality (3/6)	N=13 'sporadic' MTC cases found to be RET M+.	RET mutation testing by single strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11, 13-16.	Median age at diagnosis. 31 ± 20.6
(Chang et al. 2009) Taiwan	IV Interventional evidence Moderate quality (3/6)	N=8 probands from 8 unrelated MTC families: 4 MEN2A 2 MEN2B 1 FMTC 1 sporadic MTC (possibly *de novo* MEN2A)	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16	27.4±10.2 years (range 12–40 years) *MEN2A* 32.3±4.5 years (range 27–36 years) *MEN2B* 13.5±2.1 years (range 12–15 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
(Machens et al. 2001) Germany	IV interventional evidence Poor quality (2/6)	N=63 RET M+ patients with MTC 36 were index patients	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14	Medians: Codon 611: 44 years Codon 618: 29 years Codon 620: 36 years Codon 634: 27 years Codon 768: 60 years Codon 790: 39 years Codon 804: 62 years
(Karga et al. 1998) Greece	IV interventional evidence Poor quality (2/6)	N=22 clinically affected RET M+ patients who had a thyroidectomy for MTC before genetic testing	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and 16	22.8±11.1 years (range 12–54 years)
(Neocleous et al. 2011) Cyprus	IV interventional evidence Poor quality (2/6)	N=8 probands from 7 FMTC families and 1 MEN2A family	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index cases, and only exon 10 in family members	27.9±5.8 years (range 19–34 years)

CCH = C-cell hyperplasia; FMTC = familial medullary thyroid cancer; HPT = hyperparathyroidism; MTC = medullary thyroid cancer; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Table 79 Mean age at diagnosis (family members)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
(Machens et al. 2005) Germany	IV interventional evidence High quality (5/6)	N=206 consecutive RET+ patients who underwent surgery for CCH, MTC or PCC: 74 index cases 132 nonindex cases (criteria for diagnosis and/or surgery not reported) Stratified by risk category: 18 highest risk (codon 918) 117 high risk (codons 609-634) 71 less high risk (codons 768-891)	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 Clinical screening Thyroidectomy and/or adrenalectomy	Time to diagnosis: Highest risk 14.3 years (95% CI 10.3, 18.4) High risk 30.1 years (95% CI 26.6, 33.5) Least high risk 51.6 years (95% CI 46.5, 56.6)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
(Nguyen et al. 2001) France	IV interventional evidence High quality (5/6)	N=87 first-degree relatives of index cases in MEN2 families who were RET M+: 84 patients from 52 MEN2A families 3 patients from 3 MEN2B families	MEN2 diagnosed by linkage analysis between 1989 and 1994 RET mutation testing by sequence analysis since 1994 (method not stated)	14.0±7.0 years (range 0.8–29 years)
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	N=30 RET M+ family members of patients with MTC re-classified from sporadic MTC to FMTC or MEN2A due to a RET mutation, who had a thyroidectomy	RET mutation testing, method changed over 15 years Initially used DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy	38.5±17.8 years (range 5–76 years)
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	N=23 RET M+ index cases with MTC: 6 with apparently sporadic MTC 4 with FMTC 9 with MEN2A 4 with MEN2B	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Thyroidectomy Surgical decisions often made before RET mutation testing with no separation of data based on clinical or genetic diagnosis Note: thyroidectomy outcomes data were not available for 1 FMTC index case	Median (range): Exon 10 = 28 years (21–35 years) Exon 11 = 28 years (18–4 years 7) Exon 13 = 44 years (40–48 years) Exon 14 = 49 years (46–52 years) Exon 16 = 20.5 years (14–31 years)
(Frank-Raue et al. 2011) Germany	IV Interventional evidence Moderate quality (4/6)	N=340 patients proven to be carriers of germline mutation in exon 10 of the *RET* gene Identified through: 47% symptomatic 53% screening	RET mutation testing (method not stated) Clinical screening	Median = 35 years (range 4–86 years) Codon 609 median = 37 years (range 4–86 years) Codon 611 median = 42 years (range 14–69 years) Codon 618 median = 35 years (range 5–72 years) Codon 620 median = 31 years (range 6–76 years)
(Chiefari et al. 1998) Italy	IV Interventional evidence Moderate quality (4/6)	N=15 RET M+ members of 8 separate families with hereditary MTC with available data, who had a prophylactic thyroidectomy	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14	24.7±11.4 years (range 11–44 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at diagnosis
			Clinical screening Total thyroidectomy	
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=72 RET M+ index cases and family members from 17 MEN2 families: 49 diagnosed on clinical evidence 23 diagnosed through molecular screening 9/23 had elevated serum basal calcitonin	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15	With clinical disease (n=49): 29.8±11.6 years Without clinical disease (n=23): 21.7±21.6 years (difference p<0.04)
(Shifrin et al. 2009) USA	IV interventional evidence Moderate quality (3/6)	N=40 RET M+ family members from a family with a RET V804M mutation The majority of family members were diagnosed in the same year	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (not clear if relatives tested for specific mutation or all)	Generation I: 71–83 years (median 75 years) Generation II: 41–64 years (median 45 years) Generation III: not stated
(Erdogan et al. 2007) Turkey	IV Interventional evidence Moderate quality (3/6)	N=38 RET M+ patients identified from 12 MEN2A pedigrees 26 were asymptomatic	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–15, and restriction enzyme analysis of exon 16 Total thyroidectomy	Median = 33 years (range 2 months – 58 years)
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=16 RET M+ family members of a MEN2A family with a RET codon 618 mutation, who had a thyroidectomy: 15 identified by biochemical screening 1 identified by genetic testing	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central neck dissection	Mean = 42 years
(Pacini et al. 1995) Italy	IV interventional evidence Moderate quality (3/6)	N=5 clinically unaffected RET M+ family members from 7 MEN2A and 2 MEN2B families	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	Mean = 18.4±6.1 years (range 10–25 years)

CCH = C-cell hyperplasia; FMTC = familial medullary thyroid cancer; MTC = medullary thyroid cancer; RET M+ = RET-mutation-positive

Appendix F Uncontrolled studies reporting age at thyroidectomy in family members

Table 80 Mean age at total thyroidectomy (family members)

Study and location	Level of evidence	Study population	Intervention	Mean age at thyroidectomy
(Skinner et al. 1996) USA	IV interventional evidence High quality (5/6)	N=50 RET M+ patients from MEN2A families, who were <20 years of age at time of thyroidectomy	RET mutation testing by restriction site polymorphism analysis and/or direct DNA sequencing of RET exons 10, 11, 13, 14 and 16 Total thyroidectomy	Mean = 8.6 years (range 3–19 years) Median = 7 years
(Lau et al. 2009) Hong Kong	IV interventional evidence High quality (5/6)	N=22 asymptomatic patients from 8 MEN2A families, who underwent prophylactic total thyroidectomy All had RET codon 634 mutations	RET mutation testing by restriction enzyme analysis and/or direct DNA sequencing (exons not specified) Prophylactic thyroidectomy with or without a unilateral central compartment neck dissection	Mean = 25.2±17.5 years (range 6.1–71.9 years)
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	N=14 RET M+ relatives of MTC cases who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Thyroidectomy Surgical decisions often made prior to RET mutation testing with no separation of data based on clinical or genetic diagnosis Note: thyroidectomy outcomes data were not available for 1 FMTC index case	Mean = 18.8±13.8 years (range 5–51 years)
(Alvares Da Silva et al. 2003) Brazil	IV Interventional evidence Moderate quality (4/6)	N=35 RET M+ members of a large extended FMTC family with a RET G533C mutation, who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exon 8 Prophylactic thyroidectomy	Mean = 42 years (range 5–73 years)
(Frohnauer et al. 2000) USA	IV Interventional evidence Moderate quality (4/6)	N=13 members (degree not stated) from 5 MEN2A kindreds, who had a RET codon 804 mutation and a thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exon 14 Thyroidectomy	Mean = 35.3±15.8 years (range 6–56 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at thyroidectomy
(Decker et al. 1996) USA	IV interventional evidence Moderate quality (4/6)	N=11 RET M+ children from 4 confirmed MEN2A families, who had a prophylactic thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11 Clinical screening Prophylactic thyroidectomy	Mean = 7.5 years (range 2–12 years)
(Learoyd et al. 2005) Australia, New Zealand	IV interventional evidence Moderate quality (4/6)	N=25 RET M+ members of 2 families who had a thyroidectomy: Family 1: 22 family members with a RET V804L mutation Family 2: 3 family members with a RET V804M mutation	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 From 1998, analysis also of exons 13–15 for probands Family members screened for family RET mutation Prophylactic thyroidectomy	Family 1: Mean = 37.1±17.9 years (range = 9-63 years) Family 2: Mean = 34.6±30.5 years (range = 5-68 years)
(Decker et al. 1995) USA	IV Interventional evidence Moderate quality (4/6)	N=17 RET M+ members of MEN2A or FMTC kindreds who had a thyroidectomy based on RET mutational status: 13 MEN2A 4 FMTC	RET mutation testing by denaturing gradient gel analysis of exons 10 and 11, with confirmatory direct DNA sequencing	Mean = 25.1±17.4 years (range = 5-64 years) *MEN2A* Mean = 28.7±18.4 years (range = 5-64 years) *MEN2B* Mean = 15.8±8.5 years (range = 10-28 years)
(Heizmann et al. 2006) Switzerland	IV interventional evidence Moderate quality (4/6)	N=14 RET M+ patients who were presymptomatic, from 2 MEN2A kindreds	RET mutation testing by single-strand conformation polymorphism analysis, denaturing gradient gel electrophoresis and direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central compartment dissection in those older than 6 years of age	Mean = 25±19 years (range = 4-63 years)
(Algun et al. 2002) Turkey	IV Interventional evidence Moderate quality (4/6)	N=12 RET M+ members of a MEN2A family, who had a prophylactic thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exon 11 Clinical screening Total thyroidectomy with central lymph node dissection	Mean = 24.2±16.1 years (range = 1-60 years)
(Rodriguez Gonzalez et al.	IV interventional evidence	N=22 RET M+ patients who had normal basal and pentagastrin-stimulated	RET mutation testing by denaturing gradient gel electrophoresis of exons 10, 11, 13, 14 and 16,	Mean = 15.2±8.7 years (range = 5-36 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at thyroidectomy
2002) Spain	Moderate quality (3/6)	calcitonin levels and received a prophylactic thyroidectomy All had mutations in RET codon 634	confirmed by restriction site polymorphism analysis Clinical screening Prophylactic total thyroidectomy ± central neck dissection	
(Lombardo et al. 2002) France and Italy	IV interventional evidence Moderate quality (3/6)	N=71 patients with RET V804L mutations from 2 families, who underwent thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Clinical screening Total thyroidectomy	Mean = 49.9±16.0 years (range 12–75 years)
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	N=8 RET M+ members of a 3-generation FMTC family, who underwent a total thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of index case Analysis of exon 10 in family members Total thyroidectomy with either central neck dissection or modified radical neck dissection	Mean = 36.8±17.5 years
(Calva et al. 2009) USA	IV Interventional evidence Moderate quality (3/6)	N=16 RET M+ family members who had a thyroidectomy	RET mutation testing (method not stated) Clinical screening Total thyroidectomy for treatment or prophylaxis	Mean = 39.7 years (range 5–59 years)
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	N=8 RET M+ juvenile members from 2 large MEN2A families, who had total thyroidectomy on basis of RET mutation status	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy	Mean = 9.4±4.3 years (range 4–18 years)
(Pacini et al. 1995) Italy	IV interventional evidence Moderate quality (3/6)	N=5 clinically unaffected RET M+ family members from 7 MEN2A and 2 MEN2B families	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	Mean = 18.4±6.1 years (range 10–25 years)
(Gagel et al. 1995) USA	IV Interventional evidence Moderate quality (3/6)	N=4 RET M+ patients (children aged 3–12 years) who had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16 Thyroidectomy based on genetic screening	Mean = 9.1±3.8 years (range 3.5–12 years)

Study and location	Level of evidence	Study population	Intervention	Mean age at thyroidectomy
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=12 family members from 4 families with a RET Y791F mutation, who underwent total thyroidectomy: 1 MEN2B family 1 MEN2A family 1 FMTC family 1 apparently sporadic MTC family	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	Mean = 20.7±14.1 years (range 5–43 years)
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=4 members of an MTC family (including index patient), who had RET L790F mutation and thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–15, and restriction site polymorphism analysis for exon 16 Direct DNA sequencing of exon 13 in family members Clinical screening Prophylactic thyroidectomy	Mean = 52.8±12.0 years (range 45–74 years)

RET M+ = RET-mutation-positive

Appendix G Studies reporting diagnostic yield

Table 81 Diagnostic yield in patients with a hereditary MTC

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Gagel et al. 1995) USA	IV diagnostic evidence	N=71 members from 28 families with clinically confirmed MEN2A	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16	71/71 (100%) MEN2A RET M+
(Shirahama et al. 1998) Japan	IV diagnostic evidence	N=44 patients with MTC: 34 MEN2A 4 MEN2B 6 FMTC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11; if no mutations found, then restriction site polymorphism analysis of codons 768 and 918	42/44 (95.5%) RET M+: 33/34 (97.1%) MEN2A 4/4 (100%) MEN2B 5/6 (83.3%) FMTC
(Elisei et al. 2007) Italy	IV diagnostic evidence	N=37 patients with inherited who underwent genetic screening for RET mutations during 1993–2006	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	36/37 (97.3%) RET M+: 31/32 (96.9%) FMTC 5/5 (100%) MEN2B
(Etit et al. 2008) USA	IV diagnostic evidence	N=32 patients retrospectively identified from hospital records who had undergone a prophylactic thyroidectomy for MTC: 24 MEN2A 8 non-MEN 30 with family history	RET mutation testing by direct DNA sequencing analysis of exons 10, 11 and 13–16	29/32 (90.6%) RET M+ 27/30 (90%) with family history 24/24 (100%) MEN2A 5/8 (62.5%) non-MEN
(Fernandez et al. 2006) Spain	IV diagnostic evidence	N=27 patients clinically diagnosed with familial MTC: 16 MEN2A 3 MEN2B 8 FMTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	23/27 (85.2%) RET M+: 16/16 (100%) MEN2A 3/3 (100%) MEN2B 4/8 (50%) FMTC
(Fink et al. 1996) Austria	IV diagnostic evidence	N=27 patients clinically diagnosed with MTC from 16 families with FMTC, MEN2A, MEN2B, or suspected of inheritable MTC	RET mutation testing by single-strand conformational analysis or restriction site polymorphism analysis, and confirmatory direct DNA sequencing of exons 10, 11, 13 and 16; if no mutation detected, direct DNA sequencing of exon 14 was conducted	20/27 (74.1%) RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Jindrichova et al. 2004) Czech Republic	IV diagnostic evidence	N=24 unrelated index cases with MTC: 10 MEN2A 4 MEN2 10 FMTC	RET mutation testing by single-strand conformation polymorphism analysis, confirmed by direct DNA sequencing of exons 10, 11 and 13–16	17/24 (70.8%) RET M+: 9/10 (90%) MEN2A 4/4 (100%) MEN2B 4/10 (40%) FMTC
(Komminoth et al. 1995) Switzerland	IV diagnostic evidence	N=22 specimens from patients with MTC suspected of having MEN2 or FMTC	RET mutation testing by single-strand conformation polymorphism and heteroduplex gel electrophoresis analysis of exons 10, 11, 13 and/or 16	22/22 (100%) had germline RET mutation 3/22 (13.6%) had exon 10 mutations at codon 618 15/22 (68.2%) had exon 11 mutations at codon 634 3/22 (13.6%) had exon 16 mutations at codon 918
(Punales et al. 2003) Brazil	IV diagnostic evidence	N=17 index cases with MTC from MEN2 families: 11 MEN2A 4 MEN2B 2 FMTC	RET mutation testing by single-strand conformation polymorphism analysis, restriction enzyme analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 15	17/17 (100%) RET M+
(Neumann et al. 1995) Germany	IV diagnostic evidence	N=10 families with MEN2: 9 MEN2A 1 MEN2B	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 (also checked for mutations in SDHB, SDHD and VHL)	8/10 (80%) families were RET M+: 7/9 (77.8%) MEN2A 1/1 (100%) MEN2B 2/10 (20%) had VHL mutations, so were reclassified as VHL families
(Chang et al. 2009) Taiwan	IV diagnostic evidence	N=8 probands from 8 unrelated MTC families: 4 MEN2A 1 suspected MEN2A 2 MEN2B 1 FMTC	RET mutation testing by direct DNA sequencing of exons 1–20	8/8 (100%) RET M+
(Blaugrund et al. 1994) USA	IV diagnostic evidence	N=7 patients with MTC: 3 MEN2A 1 MEN2B	RET mutation testing by DNA sequencing of cloned exons 10, 11 and 16, and Southern blot analysis for genomic rearrangements	7/7 (100%) RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
		3 FMTC		
(Kimura et al. 1995) Japan	IV diagnostic evidence	N=7 specimens from patients: 1 FMTC 2 MEN2A 4 MEN2B	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11	3/7 (42.9%) had germline RET mutations in exons 10 or 11 (test cannot detect MEN2B mutations)
(Gonzalez et al. 2003) Mexico	IV diagnostic evidence	N=6 probands 3 MEN2B 2 MEN2A 4 sporadic MTC	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15	6/9 (66.7%) RET M+
(Kitamura et al. 1997) Japan	IV diagnostic evidence	N=6 unrelated patients with inherited MTC	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11, 13, 14 and 16, followed by direct DNA sequencing of exons 10, 11, 13 and 14, and restriction site polymorphism analysis to detect codon 918 mutation	6/6 (100%) RET M+
(Hedayati et al. 2006) Iran	IV diagnostic evidence	N=4 unrelated index cases with MTC: 1 MEN2A, 1 MEN2B 2 FMTC	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11	1/4 (25%) RET M+: 1/1 MEN2A 0/1 MEN2B 0/2 FMTC
(Abdelhakim et al. 2009) Morocco	IV diagnostic evidence	N=3 index cases: 2 MEN2A 1 hereditary MTC	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16	3/3 (100%) RET M+

RET M+ = RET-mutation-positive

Table 82 Diagnostic yield in patients with an apparently sporadic MTC

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Romei et al. 2011) Italy	IV diagnostic evidence	N=729 patients with apparently sporadic MTC (no familial history of MTC or other endocrine disease)	RET mutation testing method changed over 15 years Initially used DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8	47/729 (6.5%) RET M+: 32/47 in a non-cysteine-encoding codon in exons 5, 11, 13, 14 or 15 15/47 in a cysteine encoding codon in exons 10, or 11 6/47 MEN2A 41/47 FMTC
(Elisei et al. 2007) Italy	IV diagnostic evidence	N=481 apparently sporadic MTC patient samples submitted for genetic screening for RET mutations during 1993–2006	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	35/481 (7.3%) RET M+
(Bugalho et al. 2007) Portugal	IV diagnostic evidence	N=77 apparently sporadic cases of MTC	RET mutation testing by direct DNA sequencing of exons 10–16, or restriction site polymorphism analysis of exons 13–16 Exon 8 was screened for gross insertions/deletions (method not stated)	3/77 (3.9%) RET M+
(Fernandez et al. 2006) Spain	IV diagnostic evidence	N=73 patients identified in a hospital through clinical presentation and classified as sporadic MTCs	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	2/73 (2.7%) RET M+
(Bergant et al. 2006) Slovenia	IV diagnostic evidence	N=69 sporadic MTC patients	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case	13/69 (18.8%) RET M+: 6 x codon 634 4 x codon 790 3 x codon 618
(Eng, Mulligan, et al. 1995) UK	IV diagnostic evidence	N=67 sporadic MTC patients No history of first- or second-degree family MTC or PCC No multiple tumours MTC confirmed histopathologically	RET mutation testing by direct DNA sequencing of exons 10, 11, 13 and 16	1/67 (1.5%) RET M+
(Fink et al. 1996) Austria	IV diagnostic evidence	N=59 sporadic MTC patients	RET mutation testing by single-strand conformational analysis or restriction site polymorphism analysis and confirmatory direct DNA sequencing of exons 10, 11, 13	0/59 (0%) RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
			and 16; if no mutation detected, direct DNA sequencing of exon 14 was conducted	
(Erdogan et al. 2005) Turkey	IV diagnostic evidence	N=56 apparently sporadic MTC, clinically & histopathologically confirmed Family history negative to PCC, HPT or MTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	6/56 (10.7%) RET M+
(Hedayati et al. 2006) Iran	IV diagnostic evidence	N=53 unrelated index cases with apparently sporadic MTC	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11	3/53 (5.7%) RET M+
(Prazeres et al. 2006) Portugal	IV diagnostic evidence	N=53 patients with apparently sporadic MTC	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16, plus restriction site polymorphism analysis when possible	2/53 (3.8%) RET M+: 1 C611Y mutation Other mutation not stated
(Alvandi et al. 2011) Iran	IV diagnostic evidence	N=49 unrelated index patients diagnosed with MTC and classified as apparently sporadic	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16, and restriction site polymorphism analysis to detect C634R mutation	7/49 (14.2%) RET M+
(Lendvai et al. 2012) Hungary	IV diagnostic evidence	N=47 consecutive patients with apparently sporadic MTCs	RET mutation testing by direct DNA sequencing of exons 10, 11 and 14	0/47 (0%) RET M+
(Fitze et al. 2002) Germany	IV diagnostic evidence	N=45 patients clinically identified with sporadic MTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	5/45 (11.1%) RET M+
(Shan et al. 1998) Japan, China	IV diagnostic evidence	N=40 patients with apparently sporadic MTCs	RET mutation testing by restriction site polymorphism analysis of codon 918 mutations	0/40 (0%) had germline mutation on RET codon 918
(Uchino et al. 1998) Japan	IV diagnostic evidence	N=40 patients of apparently sporadic MTCs who had surgery between 1965 and 1996.	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 16	6/40 (15.0%) RET M+: 2 mutations on codon 618 3 mutations on codon 634 1 mutation on codon 804
(Guerrero et al. 2006) Brazil	IV diagnostic evidence	N=24 unrelated patients with apparently sporadic MTC)	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11, and direct DNA sequencing of exons 13–16	8/24 (33.3%) RET M+ 1/24 (4.2%) at exons 10 or 11 7/24 (29.2%) at exons 13 or 15

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Komminoth et al. 1995) Switzerland	IV diagnostic evidence	N=24 specimens from patients with apparently sporadic MTC or PCCs	RET mutation testing by single-strand conformation polymorphism and heteroduplex gel electrophoresis analysis of exons 10, 11, 13 and/or 15	1/24 (4.2%) had germline RET mutation on exon 11
(Decker et al. 1995) USA	IV diagnostic evidence	N=21 patients diagnosed with apparently sporadic MTC	RET mutation testing by denaturing gradient gel electrophoresis mutational analysis of exons 10 and 11, with confirmatory direct DNA sequencing	5/21 (23.8%) RET M+
(Blaugrund et al. 1994) USA	IV diagnostic evidence	N=15 apparently sporadic MTC	RET mutation testing by DNA sequencing of cloned exons 10, 11 and 16, and Southern blot analysis for genomic rearrangements	7/15 (46.7%) RET M+
(Bugalho et al. 1997) Portugal	IV diagnostic evidence	N=13 sporadic MTC No family history of MTC, PCC or parathyroid disease	RET mutation testing by direct DNA sequencing of exons 10, 11, 13, 15 and 16, confirmed using restriction site polymorphism analysis where appropriate	0/13 (0%) RET M+
(Chiefari et al. 1998) Italy	IV diagnostic evidence	N=10 with sporadic MTC	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14	0/10 (0%) RET M+
(Donis-Keller 1995) USA	IV diagnostic evidence	N=8 sporadic MTC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing in exons 1–20	5/9 (55.6%) RET M+
(Abdelhakim et al. 2009) Morocco	IV diagnostic evidence	N=6 sporadic MTC	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16	0/6 (0%) RET M+
(Gonzalez et al. 2003) Mexico	IV diagnostic evidence	N= 4 sporadic MTC	RET mutation testing by single-strand conformational polymorphism analysis, with direct DNA sequencing of exons 10, 11 and 16 and direct DNA sequencing of exons 13–15	1/4 (25%) RET M+
(Kimura et al. 1995) Japan	IV diagnostic evidence	N=3 sporadic MTC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11	1/3 (33.3%) had germline RET mutations in exons 10 or 11

PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Table 83 Diagnostic yield in patients with an unspecified MTC

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Boer et al. 2003) Hungary	IV diagnostic evidence	N=65 consecutive patients during 1992–2000 with MTC undergoing screening	RET mutation testing by direct DNA sequencing (exons not specified)	25/65 (38.5%) RET M+
(Klein et al. 2001) Hungary	IV diagnostic evidence	N=65 unrelated people with MTC (index cases)	RET mutation testing by restriction site polymorphism analysis of exon 11 and/or direct DNA sequencing of exons 10, 13 and/or 14	14/65 (21.5%) RET M+: 12 x codon 634 mutations 1 x codon 609 mutation 1 x codon 804 mutation
(Sharma & Saranath 2011) India	IV diagnostic evidence	N=51 MTC patients	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 for index cases, and specific exons for family members	15/51 (29.4%) RET M+: Mutation at exon 11 codon 634: 9/15 (60%) RET M+ Mutation at exon 10 codon 609/618: 3/15 RET M+ Mutation at exon 16 codon 918: 2/15 RET M+ Mutation at exon 14 codon 814 : 1/15 RET M+
(Ameur et al. 2009) France	IV diagnostic evidence	N=46 tissue samples collected from MTC, CCH, MCC or mixed MTC patients	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 from normal and diseased tissue samples to determine germline and somatic RET status	21/46 (45.7%) had a germline RET mutation
(Chung et al. 2004) Korea	IV diagnostic evidence	N=33 MTC patients who underwent a thyroidectomy (diagnosed clinically and by histopathology)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (postoperative)	9/33 (27.3%) RET M+
(Pinna et al. 2007) Italy	IV diagnostic evidence	N=22 patients with MTC	RET mutation testing by direct DNA sequencing of exons 8–16 in index case, and appropriate exon in family members	7/22 (31.8%) RET M+

CCH = C-cell hyperplasia; RET M+ = RET-mutation-positive

Table 84 Diagnostic yield in patients presenting with a phaeochromocytoma

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
Apparently sporadic				
(Neumann et al. 2002) Germany and Poland	IV diagnostic evidence	N=271 patients with non-syndromic PCC and/or paragangliomas without family history of disease 22 had paragangliomas only 8 had both a paraganglioma and a PCC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 (also checked for mutations in SDHB, SDHD and VHL)	13/271 (4.8%) RET M+: 4 C634R, 1 C634G, 3 C634Y, 1 C634S, 1 C634F, 2 C634T 1 Y791F (exon 13)
(Amar et al. 2005) France	IV diagnostic evidence	N=258 patients with apparently sporadic PCC or paraganglioma	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 All the coding exons of SDHB, SDHD, SDHC and VHL were also sequenced	1/258 (0.4%) RET M+
(Cascon et al. 2009) Spain	IV diagnostic evidence	N=192 consecutively enrolled patients with functioning or non-functioning PCC or paraganglioma with no personal or familial history	Complete genetic characterisation of RET, SDHB, SDHC, SDHD and VHL (method not stated)	1/192 (0.5%) RET M+
(Radien et al. 1997) France	IV diagnostic evidence	N=120 patients with apparently sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10, 11, 13 and 16	1/120 (0.8%) RET codon 790 mutation
(Krawczyk et al. 2010) Poland	IV diagnostic evidence	N=60 patients with diagnosis of apparently sporadic PCC or paraganglioma 53 had PCC 8 had paraganglioma (1 had both) 41 were benign tumours 11 had malignant lesions	RET mutation testing by direct DNA sequencing of exons 10, 11, 14 and 16 (also checked for mutations in SDHB, SDHD and VHL)	11/60 (18.3%) RET M+: 6 had mutations at codon 634 5 had mutations at codon 791
(Iacobone et al. 2011) Italy	IV diagnostic evidence	N=59 patients with apparently sporadic PCC (without evident hereditary disease and/or syndromic appearance)	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 (also checked for mutations in SDHB, SDHC, SDHD and VHL)	0/59 (0%) RET M+
(Lindor et al. 1995) USA	IV diagnostic evidence	N=29 patients who had undergone an operation for a sporadic PCC	RET mutation testing by direct DNA sequencing of exons 10 and 11, and mutation specific PCR for exon 16	0/29 (0%) RET M+
(Bar et al. 1997)	IV diagnostic	N=27 patients diagnosed with sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10, 11 and 16 (also	0/27 (0%) RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
Israel	evidence		checked for mutations in VHL)	
(Beldjord et al. 1995) France	IV diagnostic evidence	N=28 patients diagnosed clinically with sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exons 10, 11 and 16	0/28 (0%) RET M+
(Eng, Crossey, et al. 1995) UK, USA	IV diagnostic evidence	N=48 patients with apparently sporadic PCC	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 9, 10, 11 and 13–16 (also checked for mutations in VHL)	5/48 (10.4%) RET M+
(Lendvai et al. 2012) Hungary	IV diagnostic evidence	N=48 consecutive patients with apparently sporadic PCC Mean age of 36±14 years in men and 42±14 years in women	RET mutation testing by direct DNA sequencing of exons 10, 11 and 14	0/48 (0%) RET M+
(Kimura et al. 1995) Japan	IV diagnostic evidence	N=12 sporadic PCCs	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11	0/12 (0%) RET M+
(Fernandez et al. 2006) Spain	IV diagnostic evidence	N=12 patients identified in a hospital through clinical presentation of PCC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	1/12 (8.3%) RET M+
(Donis-Keller 1995) USA	IV diagnostic evidence	N=8 sporadic PCC patients	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing in exons 1–20	0/8 (0%) RET M+
Hereditary phaeochromoctyoma				
(Eisenhofer et al. 2011) Germany	IV diagnostic evidence	N=173 patients with hereditary PCC and paraganglioma patients (retrospective analysis)	Genetic characterisation of RET, VHL, SDHB, SDHC, and SDHD (method not stated)	38/173 (21.9%) RET M+
(Cascon et al. 2009) Spain	IV diagnostic evidence	N=69 consecutively enrolled patients with functioning or non-functioning PCC or paraganglioma with a personal or familial history of disease: 35 had history of MEN2 34 had other familial syndromes: 10 had history of VHL	Complete genetic characterisation of RET, VHL, SDHB, SDHC and SDHD (method not stated)	54/69 (78.3%) RET M+ 35/35 (100%) with history of MEN2 19/34 (55.9%) with history of other familial syndromes 0/10 (0%) with history of VHL

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Amar et al. 2005) France	IV diagnostic evidence	N=56 patients with a family history of PCC or paraganglioma	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 All the coding exons of SDHB, SDHD, SDHC, and VHL were also sequenced	15/56 (26.8%) RET M+
(Woodward et al. 1997) United Kingdom	IV diagnostic evidence	N=16 kindreds with familial PCC	RET mutation testing of exons 10 and 11 (method not stated), (also checked for mutations in GDNF and VHL)	0/16 (0%) RET M+
Unspecified phaeochromocytoma				
(Erlic et al. 2010) USA, Spain, Germany, Poland, Finland	IV diagnostic evidence	N=1,475 patients identified on the European-American Phaeochromocytoma-Paraganglioma Registry	Genetic characterisation of RET exons 10, 11 and 13–16 (method not stated), (also checked for mutations in SDHB, SDHC, SDHD and VHL)	14/1475 (0.9%) RET M+ 13/1475 (Tyr791Phe) 1/1475 (Ser649Leu)
(Mannelli et al. 2009) Italy	IV diagnostic evidence	N=501 consecutively enrolled patients presenting with PCC or paragangliomas (new or previously identified)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16, and multiplex ligation-dependent probe amplification assay to detect genomic rearrangements (also checked for mutations in SDHB, SDHC, SDHD and VHL)	27/501 (5.3%) RET M+
(Januszewicz et al. 2000) Poland	IV diagnostic evidence	N=77 unselected patients with PCC surgically treated (who responded to invitation, 85 did not respond)	RET mutation testing by single-strand conformation polymorphism analysis confirmed by direct DNA sequencing of exons 10, 11 and 13–16	6/77 (7.8%) RET M+ All 6 had mutations of exon 11, codon 634 (TGC to CGC)
(Patocs et al. 2004) Hungary	IV diagnostic evidence	N=41 patients with PCCs	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 14 (also checked for mutations in VHL)	7/41 (17.1%) RET M+: 2 x C609S 3 x C634F 1 x C634Y 1 x C634R
(De Krijger et al. 2006) Nederlands	IV diagnostic evidence	N=10 PCC tissue samples	RET mutation testing by denaturing gradient gel electrophoresis analysis, confirmed by direct DNA sequencing of exons 10, 11 and 16 (also checked for mutations in SDHB, SDHD and VHL)	4/10 (40%) RET M+

PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Table 85 Diagnostic yield in relatives of someone with a confirmed RET mutation

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
First-degree family members				
(Elisei et al. 2007) Italy	IV diagnostic evidence	N=274 first-degree relatives of patients with confirmed RET mutations screened during 1993–2006	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	91/274 (33.2%) first-degree family members were RET M+
(McMahon et al. 1994) UK	IV diagnostic evidence	N=63 affected or unaffected first-degree relatives from 9 MEN2A families with mutations in RET codon 634: 29 affected 30 unaffected 4 not tested but categorised as non-carriers when parents tested RET M–	RET mutation testing by restriction site polymorphism analysis of codon 634, and confirmatory direct DNA sequencing of exon 11	36/63 (57.1%) first-degree family members were RET M+ 10/30 (33.3%) asymptomatic first-degree family members were RET M+
(Wells Jr & Skinner 1998) USA	IV diagnostic evidence	N=58 first-degree family members from 7 kindreds with MEN2A, showing no clinical signs/symptoms	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 10 and 11	21/58 (36.2%) first-degree family members were RET M+
(Frilling et al. 1995) Germany	IV diagnostic evidence	N=56 clinically unaffected first-degree relatives from 21 MEN2 and FMTC families	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11, and restriction site polymorphism analysis of exon 16	21/56 (37.5%) first-degree family members were RET M+
(Gagel et al. 1995) USA	IV diagnostic evidence	N=54 first-degree relatives (affected parent) from 28 families with MEN2A	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16	19/54 (35.2%) first-degree family members were RET M+
(Pinna et al. 2007) Italy	IV diagnostic evidence	N=43 first-degree relatives of 7 RET M+ index cases with MTC	RET mutation testing by direct DNA sequencing of exons 8–16 in index case, and appropriate exon in family members	22/43 (51.2%) of first-degree family members were RET M+
(Shimotake et al. 1996) Japan	IV diagnostic evidence	N=37 first-degree relatives in a MEN2 family with a RET C634R mutation 6/37 without clinical signs	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exon 11	18/37 (48.6%) first-degree family members were RET M+
(Vestergaard et al. 2007) Denmark	IV diagnostic evidence	N=27 first-degree family members (children of RET M+ patients) from a large kindred with a RET Y791F mutation	RET mutation testing by direct DNA sequencing of exon 13	12/27 (44.4%) first-degree family members were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Karga et al. 1998) Greece	IV diagnostic evidence	N=25 asymptomatic first-degree relatives from 12 unrelated Greek families 9 MEN2A 1 FMTC 3 likely FMTC Aged 3 months to 86 years	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and/or 16	5/25 (20%) asymptomatic first-degree family members were RET M+
(Sharma & Saranath 2011) India	IV diagnostic evidence	N=25 first-degree relatives from 7 RET M+ MTC index patients	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 for index cases and specific exons for family members	13/25 (52%) first-degree family members were RET M+: 11/15 exon 11 codon 634 (C→R) 0/3 exon 11 codon 634 (C→Y) 0/1 exon 10 codon 618 (C→G) 2/2 exon 10 codon 609 (C→R) 0/4 exon 16 codon 918 (M→T)
(Dourisboure et al. 2005) Argentina	IV diagnostic evidence	N=21 first-degree relatives from a MEN2B family with a RET C630R mutation 5 affected with MTC	RET mutation testing by direct DNA sequencing of exon 11	7/21 (33.3%) first-degree family members were RET M+
First- and second-degree, or degree not stated				
(Alvares Da Silva et al. 2003) Brazil	IV diagnostic evidence	N=229 extended family members from 6 generations of an MTC index patient with RET G533C mutation	RET mutation testing by direct DNA sequencing of exon 8	76/229 (33.2%) extended family members were RET M+
(Frank-Raue et al. 1996) Germany	IV diagnostic evidence	N=159 at-risk members (degree not stated) from 35 families with hereditary MTC	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA sequencing or restriction site polymorphism analysis of exons 10 or 11 Direct DNA sequencing of exons 13 or 16	84/159 (52.8%) extended family members were RET M+: 64/111(57.7%) MEN2A 16/31(51.6%) FMTC 4/17(23.5%) MEN2B
(Romei et al. 2011) Italy	IV diagnostic evidence	N=146 relatives (degree not stated) of 47 RET M+ index cases who had MTCs without family history of endocrine disorders	RET mutation testing of relatives by direct DNA sequencing of exon affected in index case	60/146 (41.1%) relatives were RET M+: 35/60 carriers showed clinical or biochemical evidence but were unaware of condition 20/60 showed no signs of disease 5/60 refused clinical/biochemical

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
				investigations
(Punales et al. 2003) Brazil	IV diagnostic evidence	N=133 family members (degree not stated) from 17 MEN2 families 113 were from families with MEN2A 37 had clinical signs of disease	RET mutation testing by single-strand conformation polymorphism analysis, restriction enzyme analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 15	61/133 (45.8%) extended family members were RET M+ 57/113 (50.4%) MEN2A family members were RET M+
(Donis-Keller 1995) USA	IV diagnostic evidence	N=132 relatives (degree not stated) from 7 MEN2A families	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing for family members	21/132 (15.9%) family members were RET M+
(Tsai et al. 1994) USA	IV diagnostic evidence	N=109 members (degree not stated) of 13 kindreds: 9 MEN2A 2 MEN2B 2 FMTC 47 clinically affected 62 non-affected	RET mutation testing by direct DNA sequencing of exons 10 and 11	41/109 (37.6%) relatives were RET M+: 41/85 (48.2%) MEN2A family members 0/16 (0%) MEN2B family members 0/8 (0%) FMTC family members (NB test not appropriate to detect mutations for phenotype MEN2B)
(Shifrin et al. 2009) USA	IV diagnostic evidence	N=107 family members (degree not stated) from a family with RET V804M mutation (exon 14)	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exon 11	40/107 (37.4%) family members were RET M+ Generation I: 7/7 (100%) Generation II: 17/22 (77%) Generation III: 15/22 (68%)
(Sanso et al. 2002) Argentina	IV diagnostic evidence	N=98 relatives (degree not stated) from 17 MEN2A index cases (aged 6 months – 81 years) N=13 relatives (degree not stated) from 5 MEN2B index cases	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, confirmed by restriction site polymorphism analysis	42/98 (42.9%) MEN2A relatives were RET M+ All had the RET C634R mutation 0/13 (0%) MEN2B relatives were RET M+
(Decker et al. 1995) USA	IV diagnostic evidence	N=93 relatives (degree not stated) from 10 MEN2A or FMTC kindreds	RET mutation testing by denaturing gradient gel electrophoresis mutational analysis of exons 10 and 11, with confirmatory direct DNA sequencing	29/93 (31.2%) relatives were RET M+
(Algun et al. 2002) Turkey	IV diagnostic evidence	N=88 relatives (degree not stated) from 4 generations of an extended MEN2A family with a RET C634G mutation	RET mutation testing by restriction site polymorphism analysis of exon 11	18/88 (20.5%) relatives were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Jindrichova et al. 2004) Czech Republic	IV diagnostic evidence	N=77 relatives (degree not stated) of 23 RET M+ index cases with MTC: 6 previously classified as sporadic MTC 4 FMTC families 9 MEN2A families 4 MEN2B families	RET mutation testing by direct DNA sequencing of exons 10, 11, 13, 14 and/or 16	24/77 (42.1%) relatives were RET M+: 3/9 (33.3%) sporadic MTC 9/22 (40.9%) FMTC 10/39 (25.6%) MEN2A 2/7 (28.6%) MEN2B
(Chi et al. 1994) Japan	IV diagnostic evidence	N=74 relatives (degree not stated) from an extended MEN2A pedigree with a RET C634R mutation: 43 clinically affected 31 considered at risk	RET mutation testing by restriction site polymorphism analysis of exon 11	45/74 (60.8%) relatives were RET M+ 43/43 (100%) clinically affected 2/31 (6.5%) at risk relatives
(Halling et al. 1997) USA	IV diagnostic evidence	N=72 family members (degree not stated) from one large FMTC kindred with a RET C609Y mutation	RET mutation testing by direct DNA sequencing of exon 10	34/72 (47%) first- and second-degree family members were RET M+: Generation III: 16/23 (70%) Generation IV: 18/49 (37%)
(Bugalho et al. 2007) Portugal	IV diagnostic evidence	N=65 relatives (degree not stated) of 8 probands of established FMTC/MEN2 kindreds with a RET mutation 53 were asymptomatic	RET mutation testing by direct DNA sequencing of exons 10–16, or restriction site polymorphism analysis of exons 13–16 Exon 8 was screened for gross insertions/deletions (method not stated)	32/65 (49.2%) relatives were RET M+ 20/53 (37.7%) asymptomatic
(Chang et al. 2009) Taiwan	IV diagnostic evidence	N=61 relatives (degree not stated) from 8 unrelated MTC families: 45 from 5 MEN2A families 9 from 2 MEN2B families 7 from 1 FMTC family	RET mutation testing by direct DNA sequencing of exons 1–20	22/61 (36.1%) RET M+: 18/45 (40%) MEN2A 0/9 (0%) MEN2B 4/7 (57.1%)
(Oriola et al. 1996) Spain	IV diagnostic evidence	N=59 family members (degree not stated) from 7 MEN2A families: 20 symptomatic 39 at risk of disease	RET mutation testing by direct DNA sequencing of RET exons 10 and 11, and restriction site polymorphism analysis of exons 10 and exon 11	28/59 (47.5%) family members were RET M+ 8/39 asymptomatic family members
(Karga et al. 1998) Greece	IV diagnostic evidence	N=58 members (degree not stated) of 12 unrelated Greek families	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of	38/58 (65.5%) family members were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
		9 MEN2A 1 FMTC 3 likely FMTC 33 clinically affected (aged 12–65 years)	exons 10, 11, 13, 14 and/or 16	33/33 (100%) were clinically affected
(Pacini et al. 1995) Italy	IV diagnostic evidence	N=58 family members (degree not stated) from 9 MEN2 families: 16 affected 42 at risk of disease	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	21/58 (36.2%) family members were RET M+ 5/42 (11.9%) clinically unaffected family members
(Dos Santos et al. 2007) Brazil	IV diagnostic evidence	N=57 at-risk family members (degree not stated) from 7 index cases: 3 MEN2A 1 MEN2B 3 FMTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index patients, and restricted to specific exon for family members	35/57 (61.4%) relatives were RET M+: 19 MEN2A 15 FMTC 1 MEN2B
(Learoyd et al. 2005) Australia, New Zealand	IV diagnostic evidence	N=54 family members (degree not stated) of 2 probands: 47 family members from 4 generations; proband has RET V804L mutation 7 family members from 3 generations; proband has RET V804M mutation	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 between 1993 and 1998, then exons 13–15 were included	Family 1: 22/47 (46.8%) Family 2: 5/7 (71.4%)
(Fink et al. 1996) Austria	IV diagnostic evidence	N=52 asymptomatic relatives (degree not stated) from 13 families clinically diagnosed with FMTC, MEN2A, MEN2B or suspected of inheritable MTC	RET mutation testing by single-strand conformational analysis or restriction site polymorphism analysis, and confirmatory direct DNA sequencing of exons 10, 11, 13 and 16; if no mutation detected, direct DNA sequencing of exon 14 was conducted	10/52 (19.2%) relatives were RET M+: 5/19 (26.3%) FMTC family members 5/18 (27.8%) MEN2A family members 0/5 (0%) MEN2B family members
(Lecube et al. 2002) Spain	IV diagnostic evidence	N=52 family members (degree not stated) of an FMTC family with a RET V804M mutation	RET mutation testing by restriction site polymorphism analysis of exon 14	25/52 (48.1%) family members were RET M+
(Gonzalez et al. 2003) Mexico	IV diagnostic evidence	N=48 family members (degree not stated) of 6 RET M+ probands: 3 MEN2B 2 MEN2A	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15	15/48 (31.3%) family members were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
		1 sporadic MTC		
(Gosnell et al. 2006) Australia	IV diagnostic evidence	N=48 at-risk family members (degree not stated) from a MEN2A kindred with a RET V804L mutation	RET mutation testing (method not stated)	23/48 (47.9%) family members were RET M+
(Fugazzola et al. 2002) Italy	IV diagnostic evidence	N=44 members (degree not stated) of a large FMTC pedigree with a RET A891S mutation	RET mutation testing by direct DNA sequencing of exon 15	14/44 (31.8%) family members were RET M+
(Klein et al. 2001) Hungary	IV diagnostic evidence	N=43 relatives (degree not stated) of 14 index cases with MTC and RET M+	RET mutation testing by restriction site polymorphism analysis of exon 11, and/or direct DNA sequencing of exons 10, 13 and/or 14	25/43 (58.1%) family members were RET M+
(Marsh et al. 1996) Australia and New Zealand	IV diagnostic evidence	N=39 members (degree not stated) of 16 MEN2A and FMTC families at risk of being a gene carrier	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11	7/39 (17.9%) family members were RET M+ 5/21 (23.8%) from 2 MEN2A families were RET M+ 1/7 (14.3%) with raised stimulated calcitonin levels were RET M+
(Frohnauer et al. 2000) USA	IV diagnostic evidence	N=38 members (degree not stated) from 5 MEN2A kindreds with a RET codon 804 mutation	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exon 14	23/38 (60.5%) family members were RET M+
(Komminoth et al. 1995) Switzerland	IV diagnostic evidence	N=38 members (degree not stated) from 3 MEN2A families, 2 MEN2B families and 4 suspected MEN2 families	RET mutation testing by single-strand conformation polymorphism and heteroduplex gel electrophoresis analysis of exons 10, 11, 13 and/or 15	11/38 (28.9%) family members were RET M+: 5/21 (23.8%) had exon 10 mutations 4/9 (44.4%) had exon 11 mutations 2/8 (25.0%) had exon 16 mutations
(Hernandez et al. 1997) Spain	IV diagnostic evidence	N=36 asymptomatic members (degree not stated) of 3 families with MEN2A 8 had raised pentagastrin-stimulated calcitonin levels	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11	6/36 (16.7%) family members were RET M+ 6/8 (75%) with raised stimulated calcitonin levels were RET M+
(Chiefari et al. 1998)	IV diagnostic evidence	N=34 members (degree not stated) of 9 separate families with hereditary MTC:	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14	22/34 (64.7%) family members were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
Italy		5 MEN2A 2 MEN2B 1 FMTC 1 with <4 MTC cases		
(Siggelkow et al. 2001) Germany	IV diagnostic evidence	N=34 first- and second-degree relatives of an index case with FMTC and a RET C611F mutation	RET mutation testing restriction site polymorphism analysis of exon 10 in family members	19/34 (55.9%) first- and second-degree family members were RET M+: Generation III: 6/8 (75%) Generation IV: 9/17 (52.9%) Generation V:4/9 (44.4%)
(Bergant et al. 2006) Slovenia	IV diagnostic evidence	N=31 relatives (degree not stated) of 13 RET M+ sporadic MTC patients	RET mutation testing by restriction site polymorphism analysis of exon affected in index case	16/31 (17.9%) family members were RET M+: 9 x codon 634 1 x codon 790 6 x codon 618
(Calva et al. 2009) USA	IV diagnostic evidence	N=31 first- and second-degree family members from a MEN2A kindred with a RET C609Y mutation	RET mutation testing (method not stated)	22/31 (70.9%) first- and second-degree family members were RET M+
(Kinlaw et al. 2005) USA	IV diagnostic evidence	N=29 first- and second-degree relatives in a family with MEN2A due to RET C609S mutation 6 with manifestations of MEN2A	RET mutation testing by restriction site polymorphism analysis of exon 10	14/29 (48.3%) family members
(Neocleous et al. 2011) Cyprus	IV diagnostic evidence	N=29 family members (degree not stated) from 7 FMTC families and 1 MEN2A family with a RET C618R mutation	RET mutation testing by direct DNA sequencing of exon 10	15/29 (51.7%) family members 15/15 RET M+ had Cys618Ser mutations
(Jung et al. 2010) Korea	IV diagnostic evidence	N=28 first- and second-degree members (excluding the proband) of a 3-generation FMTC family with a RET C618S mutation 8 had MTC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exon 10	11/28 (39.3%) first- and second-degree family members were RET M+
(Uchino et al. 1999) Japan	IV diagnostic evidence	N=27 members (degree not stated) from 5 MEN2A families whose clinical status was unknown	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 16	6/27 (22.2%) family members were RET M+

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Pasini et al. 2002) Italy	IV diagnostic evidence	N=26 family members (degree not stated) of a patient with Hirschsprung's disease and MEN2 (RET C618R mutation)	RET mutation testing restriction site polymorphism analysis and direct DNA sequencing of exon 10 in family members	12/26 (46.2%) family members were RET M+
(Wu et al. 1998) Taiwan	IV diagnostic evidence	N=26 first- and second-degree relatives of 2 probands from 2 unrelated MEN2A families	RET mutation testing by direct DNA sequencing of exons 10 and 11	11/26 (42.3%) first- and second-degree family members
(Gil et al. 2002) Spain	IV diagnostic evidence	N=23 members of 4 independent MEN2A families (degree not stated): 13 clinically affected: 9 MTC only 4 MTC + PCC 10 unaffected	RET mutation testing by single-strand conformation polymorphism analysis and restriction site polymorphism analysis, with confirmatory direct DNA sequencing of exons 10 and 11	13/23 (56.6%) family members were RET M+: 13/13 (100%) clinically affected 0/10 (0%) unaffected
(Bihan et al. 2012) France	IV diagnostic evidence	N=22 extended family members of an MTC patient confirmed to have a RET L790F mutation on exon 13 3 were symptomatic	RET mutation testing by direct DNA sequencing of exon 13	14/22 (63.6%) extended family members were RET M+
(Mastroianno et al. 2011) Italy	IV diagnostic evidence	N=21 first- and second-degree relatives of a proband with MEN1 (MEN1 IVs4+IG>T mutation) and MEN2 (RET K666M mutation on exon 11)	RET mutation screening of exons 8, 10, 11, 13–16 and 18 (method not stated)	7/21 (33.3%) first- and second-degree relatives were RET M+ 3/21 (14.3%) were both MEN1+ and RET M+
(Chiefari et al. 2001) Italy	IV diagnostic evidence	N=20 first- and second-degree relatives of proband with RET C634F mutation 6 were affected with MTC	RET mutation testing by restriction site polymorphism analysis of exon 11, confirmed by direct DNA sequencing	7/20 (35%) first- and second-degree relatives were RET M+
(Morita et al. 1996) Japan	IV diagnostic evidence	N=20 individuals: 1 proband with MEN2A (RET C618S mutation on exon 10) 6 children of the proband 10 grandchildren 3 great-grandchildren	RET testing by PCR amplification and restriction enzyme analysis of exon 10 C618S	11/19 (55%) family members were RET M+: 4/6 (66.7%) children 4/10 (40%) grandchildren 2/3 (66.7%) great-grandchildren

Study and location	Level of evidence	Study population	Intervention	Diagnostic yield
(Caron et al. 1996) France	IV diagnostic evidence	N=14 extended family members of a confirmed MEN2A patient with a RET C618R mutation	RET mutation testing by direct DNA sequencing of exons 10 and 11	4/14 (28.6%) extended family members were RET M+: All 4 RET M+ family members were symptomatic
(Abdelhakim et al. 2009) Morocco	IV diagnostic evidence	N=13 family members (degree not stated) of 3 RET M+ index cases	RET mutation testing by direct DNA sequencing of exons 8, 10, 11, 13–15 and 16	2/13 (15.4%) family members were RET M+
(Neumann et al. 1995) Germany	IV diagnostic evidence	N=27 family members (degree not stated) from 7 MEN2A families and 1 MEN2B family who had had negative clinical screening (n=19) or unknown phenotype (n=8)	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 (also checked for mutations in SDHB, SDHD and VHL)	4/27 (14.8%) clinically negative family members were RET M+

PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Appendix H Studies reporting rates of treatment

Table 86 Rates of treatment

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	N=60 RET M+ family members of patients with MTC reclassified from spontaneous MTC to FMTC or MEN2A due to RET mutation	RET mutation testing method changed over 15 years Initially used direct DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy	35/60 (58.3%) RET M+ family members were found to be affected clinically or biochemically 25/60 (41.7%) RET M+ were clinically unaffected 5/60 (8.3%) RET M+ refused clinical and/or biochemical examinations 30/35 (85.7%) RET M+-affected patients underwent total thyroidectomy 20/20 (100%) RET M+ clinically unaffected underwent yearly clinical and biochemical assessment.
(Pinna et al. 2007) Italy	IV interventional evidence High quality (5/6)	N=22 RET M+ family members	RET mutation testing by direct DNA sequencing of exons 8–16 Total thyroidectomy	14/22 (63.6%) RET M+ family members underwent prophylactic thyroidectomy
(Alvares Da Silva et al. 2003) Brazil	IV interventional evidence Moderate quality (4/6)	N=229 members spanning 6 generations of a large extended FMTC family with a RET G533C mutation 76 members were RET M+	RET mutation testing by direct DNA sequencing of exon 8 Prophylactic thyroidectomy	35/76 (46.1%) RET M+ family members had a thyroidectomy 37/76 (53.9%) had not yet undergone surgery: 3/37 had scheduled surgery 10/37 presented with low pentagastrin-stimulated calcitonin levels and surgery was delayed 24/37 had not yet completed clinical evaluation because molecular diagnosis was too recent 3/76 refused further clinical investigation 1/76 refused surgery 153/153 (100%) RET M– family members had normal pentagastrin-stimulated calcitonin levels and were excluded from further clinical investigation

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
(Frank-Raue et al. 1996) Germany	IV interventional evidence Moderate quality (4/6)	N=159 members of 35 hereditary MTC families who had RET mutation testing 84 were RET M+	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA sequencing or restriction site polymorphism analysis of exons 10 and 11, and direct DNA sequencing of exons 13 and 16 Clinical screening Prophylactic thyroidectomy	9/17 (52.9%) asymptomatic patients had prophylactic thyroidectomy 4 patients with elevated calcitonin levels who had thyroidectomy prior to genetic testing were RET M– Histopathology results revealed only minor CCH or normal results'
(Learoyd et al. 2005) Australia, New Zealand	IV interventional evidence Moderate quality (4/6)	Family 1: N=48 family members from 4 generations 23 were RET M+ Family 2: N=9 family members from 3 generations 6 were RET M+	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 From 1998, analysis also of exons 13–15 for probands Family members screened for family RET mutation Prophylactic thyroidectomy	Family 1: 22/23 (95.7%) (including proband) RET M+ family members underwent prophylactic thyroidectomy 1/23 (3.7%) was awaiting surgery Family 2: 3/6 (50%) (including proband) RET M+ family members had thyroidectomy 2 had elevated basal calcitonin levels but declined surgery 1 was under further investigation
(Marsh et al. 1996) Australia and New Zealand	IV interventional evidence Moderate quality (4/6)	N=39 members of 16 MEN2A and FMTC families at risk of being a gene carrier 7 were RET M+	Restriction site polymorphism analysis of RET exons 10 and 11 Thyroidectomy	2/32 (6.3%) RET M– members from 2 MEN2A families had undergone thyroidectomy prior to RET mutation testing based on pentagastrin results: 1 had CCH pathology 1 was normal 5 other RET M– members of these 2 families also had elevated calcitonin levels but their management wasn't stated
(Boer et al. 2003) Hungary	IV interventional evidence Moderate quality (4/6)	N=25 RET M+ consecutive unrelated patients with MTC admitted for genetic screening	RET mutation testing by direct DNA sequencing (exons not specified) Thyroidectomy	14/25 (56%) underwent surgery 5/25 (20%) screened postoperatively 6/25 (24%) refused treatment
(Frohnauer et al. 2000) USA	IV interventional evidence Moderate quality (4/6)	N=23 members from 5 MEN2A kindreds who had a RET codon 804 mutation	RET mutation testing by denaturing gradient gel electrophoresis analysis, confirmed by direct DNA sequencing of exon 14 Thyroidectomy	14/23 (60.9%) had a thyroidectomy 2/23 (8.7%) were aged 2 years and awaiting consideration for prophylactic thyroidectomy 2/23 (8.7%) were aged 80 and 85 years with no clinical signs of disease, and refused further testing

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
				1/23 (4.3%) had no further testing (reason not given) 4/23 (17.4%) had calcitonin levels checked 1/4 (aged 84 years) had an elevated basal calcitonin level
(Donis-Keller 1995) USA	IV interventional evidence Moderate quality (4/6)	N=21 RET M+ family members from 7 MEN2A kindreds 7 had elevated calcitonin levels	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of RET exon affected in index case Thyroidectomy	13/21 (61.9%) elected to have prophylactic thyroidectomy based on genetic screening
(Decker et al. 1995) USA	IV interventional evidence Moderate quality (4/6)	N=5 RET M+ patients with apparently sporadic MTC N=10 patients clinically diagnosed as MEN2A from calcitonin levels but dubious results or histopathology	RET mutation testing by denaturing gradient gel analysis of exons 10 and 11, with confirmatory direct DNA sequencing	5 patients with apparently sporadic MTC (lack of family history) were found to have a germline RET mutation, prompting genetic testing of first-degree relatives 9/10 (90%) patients previously classified as MEN2A (but had questionable results) were reclassified as RET M– 7/8 (87.5%) patients who had thyroidectomy prior to genetic testing were RET M– 2 patients were spared surgery based on RET M– status
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	N=69 index cases and family members with a RET codon 634 mutation: 47 were clinically diagnosed 22 had no clinical signs of disease	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15 Total thyroidectomy, with a central cervical lymph node dissection in those with increased calcitonin levels	43/47 (91.5%) clinically affected patients underwent thyroidectomy 7/22 (31.8%) gene carriers without clinical signs underwent thyroidectomy
(Lombardo et al. 2002) France and Italy	IV interventional evidence Moderate quality (3/6)	N=61 patients with RET V804L mutations from 5 families	RET mutation testing by single strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Clinical screening Total thyroidectomy	31/61 (50.8%) underwent thyroidectomy: 3 (index case) due to thyroid tumour 14 based on detectable basal calcitonin levels 13 based on increased pentagastrin-stimulated calcitonin levels 1 based on increase in basal calcitonin levels and the willingness of parents 1 based on follicular tumour

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	N=49 family members of a MEN2A family with a RET codon 618 mutation: 16 were RET M+ 33 were RET M–	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central neck dissection	16/49 patients were RET M+: 15 were initially identified by biochemical screening 1 identified by genetic testing 16/16 (100%) had a thyroidectomy 33/33 RET M– patients previously under biochemical surveillance were informed that they had not inherited the mutation and that they and their descendants would no longer need to be evaluated for disease
(Erdogan et al. 2007) Turkey	IV interventional evidence Moderate quality (3/6)	N=41 RET M+ patients identified from 12 MEN2A, 2 MEN2B and 1 FMTC pedigrees 26 were asymptomatic	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–15, and restriction enzyme analysis of exon 16 Total thyroidectomy	30/41 (73.2%) had a thyroidectomy 29/30 had elevated basal and/or stimulated calcitonin levels preoperatively 10/41 (24.4%) RET mutation carriers refused thyroidectomy despite all efforts of the medical team, including a medical student with a RET codon 634 mutation and elevated calcitonin levels
(Quayle et al. 2004) USA	IV interventional evidence Moderate quality (3/6)	N=39 RET M+ patients with MEN2 or FMTC diagnosed when over 50 years of age: 36 patients from MEN2A families 3 from FMTC families	RET mutation testing (method not stated) Total thyroidectomy	38/39 (97.4%) underwent total thyroidectomy 7/38 also underwent central node dissection Prior to surgery: 28/38 had abnormal basal and/or pentagastrin-stimulated calcitonin levels 8/38 had palpable nodule on physical examination 2/38 were RET M+ with no physical signs of disease
(Halling et al. 1997) USA	IV interventional evidence Moderate quality (3/6)	N=38 family members in 1 large kindred with FMTC who had a thyroidectomy before genetic testing: 28 were RET M+ 10 were RET M–	RET mutation testing by direct DNA sequencing of exon 10 Clinical screening Thyroidectomy	19/38 were RET M+ with elevated pentagastrin-stimulated calcitonin levels (2 were normal, 4 had CCH, 13 had MTC and CCH) 9/38 were RET M+ with normal pentagastrin-stimulated calcitonin levels (3 were normal, 4 had CCH, 2 had MTC and CCH) 7/38 were RET M– with elevated pentagastrin-stimulated calcitonin levels (1 was normal, 5 had CCH, 1 had MTC) 3/38 were RET M– with normal pentagastrin-stimulated calcitonin levels (3 had CCH)
(Bergant et al. 2006) Slovenia	IV interventional evidence Moderate quality (3/6)	N=29 relatives of RET M+ sporadic MTC patients who had the RET mutation and had a total thyroidectomy	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case	27/29 (93.1%) of relatives had a thyroidectomy 2/29 (6.9%) refused surgery

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
			Total thyroidectomy with central neck dissection	
(Lecube et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	N=22 family members of a FMTC family who had a RET V804M mutation	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–16 Biochemical screening on those RET M+	20/22 had normal pentagastrin-stimulated calcitonin levels (test had not yet been performed in 2 children aged 3 and 5 years) Consequently, thyroidectomy was not recommended for these patients at that time
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	N=20 members from 4 large MEN2A families, who had a RET mutation and/or a thyroidectomy 14 were RET M+	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy	14/20 were RET M+ with normal or equivocal pentagastrin-stimulated calcitonin levels: 8/14 (57.1%) had total thyroidectomy on basis of RET mutation status 6/14 (42.9%) were scheduled for surgery 6/20 patients were RET M– but had had a thyroidectomy on the basis of raised pentagastrin-stimulated calcitonin levels: 2/6 (33.3%) had CCH 4/6 (66.7%) had normal thyroid pathology
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	N=11 RET M+ members (including index case) of a 3-generation FMTC family who underwent genetic testing 6 diagnosed with MTC before genetic testing 3 diagnosed with MTC after genetic testing	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of index case Analysis of exon 10 in family members Total thyroidectomy with either central neck dissection or modified radical neck dissection	9/11 (81.8%) RET M+ were clinically affected: 8/9 (88.9%) underwent surgery 1/9 (11.1%) was scheduled for surgery 2/11 (18.2%) were asymptomatic: 1 (a 12-year-old) was being monitored to determine timing of thyroidectomy 1 (a 37-year-old) was recommended prophylactic thyroidectomy and refused
(Gagel et al. 1995) USA	IV Interventional evidence Moderate quality (3/6)	N=4 RET M+ children aged 3–12 years identified from genetic screening of 197 MEN2A patients	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16 Thyroidectomy based on genetic screening	4 RET M+ children underwent thyroidectomies All had positive histopathology for CCH and 1 also had microscopic MTC 1 mother had thyroidectomy based on raised pentagastrin-stimulated calcitonin levels; her son also had raised levels, and both were found to be RET M– It was decided the son would be monitored instead of undergoing a thyroidectomy

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
(Hernandez et al. 1997) Spain	IV interventional evidence Poor quality (2/6)	N=36 asymptomatic members of 3 families with MEN2A 6 identified as RET M+	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11 Clinical screening Total thyroidectomy	6/6 (100%) RET M+ also had raised pentagastrin-stimulated calcitonin levels Treatment decisions the same: total thyroidectomy 2/13 (15.4%) family members tested had false positive pentagastrin-stimulated calcitonin test results 2/30 (6.6%) RET M– had raised pentagastrin-stimulated calcitonin levels RET M– status has changed treatment decisions and prevented unnecessary thyroidectomy
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Poor quality (2/6)	N=31 family members with a RET Y791F mutation 10 index cases	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	20/31 (64.5%) had a thyroidectomy 7/31 (22.6%) have had a thyroidectomy recommended 4/31 (12.9%) have refused the surgery
(Karga et al. 1998) Greece	IV interventional evidence Poor quality (2/6)	N=25 asymptomatic first-degree relatives 5 were RET M+	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and 16 Thyroidectomy	5/25 (20%) family members (all children) were RET M+ 2/5 children (aged 10 and 14 years) underwent thyroidectomy 3/5 children aged <6 years had not yet been operated on 20/20 (100%) RET M– family members were excluded from further screening
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	N=15 patients from an MTC family with a RET L790F mutation (including index patient) 8 had abnormal pentagastrin-stimulated calcitonin levels	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–15, and restriction site polymorphism analysis for exon 16 Direct DNA sequencing of exon 13 in family members Clinical screening Prophylactic thyroidectomy	8/15 (53.3%) had abnormal pentagastrin-stimulated calcitonin levels: 5 had prophylactic thyroidectomy (including index case) 3 refused recommended surgery due to 'fear of future discomfort related to L-thyroxin' 7/15 (46.7%) had normal calcitonin levels, and an annual follow-up to check calcitonin levels was recommended: 4/7 had a mean follow-up of 4.5 years with normal calcitonin levels. 3/7 were lost to follow-up

Study and location	Level of evidence	Study population	Intervention	Rates of treatment/surveillance
(Kinlaw et al. 2005) USA	IV interventional evidence Poor quality (2/6)	N=15 RET M+ family members (including index case) of a MEN2A family with a RET C609S mutation	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index case Restriction site polymorphism analysis to detect C609S mutation in family members Thyroidectomy	11/15 (73.3%) RET M+ underwent further biochemical and clinical screening 3/11 (27.3%) had borderline or slightly elevated pentagastrin-stimulated calcitonin levels 6/15 (40%) had prophylactic thyroidectomy (including 3 with elevated calcitonin levels) 4/15 (26.7%) refused further evaluation
(Neocleous et al. 2011) Cyprus	IV interventional evidence Poor quality (2/6)	N=15 RET M+ family members from 7 FMTC families and 1 MEN2A family	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index cases, and only exon 10 in family members Thyroidectomy	15/15 (100%) RET M+ underwent prophylactic total thyroidectomy
(Uchino et al. 1999) Japan	IV interventional evidence Poor quality (2/6)	N=6 clinically unaffected members from MEN2A families with mutations on RET codon 634 All had raised calcitonin levels	RET mutation testing by single strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11, 13, 14 and 16 Total thyroidectomy	3/6 (50%) patients, aged 15, 32 and 70 years, had total thyroidectomy (the eldest patient also had a left adrenalectomy) 2/6 (33.3%) patients, aged 54 and 67 years, refused a thyroidectomy 1/6 (16.7%) patient, aged 7 years, was being followed; she had no signs of MTC by ultrasound and only slightly elevated tetragastrin-stimulated calcitonin levels (from basal level of 45 pg/mL to peak level of 110 pg/mL)

CCH = C-cell hyperplasia; RET M+ = RET-mutation-positive, RET M– = RET-mutation-negative

Appendix I MBS items associated with investigations and treatment of MEN2

Table 87 summarises the investigations for MEN2 and other hereditary disorders. The use of these tests in those suspected of having MEN2 has likely reduced since the introduction of RET mutation testing as a triage test for further investigations. Table 88 summarises the requirements for liflong surveillance of patients with confirmed or suspectred MEN2.

Table 87 MBS items for investigating clinical features of MEN2

Type of resource and identifier	Description	Fees
MBS item 66695	Quantitation in blood or urine of hormones and hormone binding proteins - ACTH, aldosterone, androstenedione, C-peptide, calcitonin, cortisol, DHEAS, 11-deoxycortisol, dihydrotestosterone, FSH, gastrin, glucagon, growth hormone, hydroxyprogesterone, insulin, LH, oestradiol, oestrone, progesterone, prolactin, parathyroid hormone, renin, sex hormone binding globulin, somatomedin C(IGF-1), free or total testosterone, urine steroid fraction or fractions, vasoactive intestinal peptide - 1 test	Fee: $30.50 Benefit: 75% = $22.90 85% = $25.95
MBS item 66707	5 or more tests described in item 66695 (Item is subject to rule 6)	Fee: $83.35 Benefit: 75% = $62.55 85% = $70.85
MBS item 12527	RENAL FUNCTION TEST (with imaging and at least 2 blood samples)	Fee: $84.95 Benefit: 75% = $63.75 85% = $72.25

Source: March 2013 Medicare Benefits Schedule MBS online
MBS = Medicare Benefits Schedule

Table 88 MBS items for lifelong surveillance regimen for MEN2

Type of resource and identifier	Description	Fees
MBS item 23	LEVEL B CONSULTATION AT CONSULTATION ROOMS Professional attendance at consulting rooms by a general practitioner (not being a service to which any other item in this table applies) lasting less than 20 minutes, including any of the following that are clinically relevant: a) Taking a patient history b) Performing a clinical examination c) Arranging any necessary investigation d) Implementing a management plan e) Providing appropriate preventive health care In relation to 1 or more health-related issues, with appropriate documentation.	Fee: $36.30 Benefit: 100% = $36.30
MBS item 55032	NECK, 1 or more structures of, ultrasound scan of, where: (a) the patient is referred by a medical practitioner for ultrasonic examination not being a service associated with a service to which an item in Subgroups 2 or 3 of this Group applies; and	Fee: $109.10 Benefit: 75% = $81.85 85% = $92.75

Type of resource and identifier	Description	Fees
	(b) the referring medical practitioner is not a member of a group of practitioners of which the providing practitioner is a member (R)	
MBS item 66500	Quantitation in serum, plasma, urine or other body fluid (except amniotic fluid), by any method except reagent tablet or reagent strip (with or without reflectance meter) of: acid phosphatase, alanine aminotransferase, albumin, alkaline phosphatase, ammonia, amylase, aspartate aminotransferase, bicarbonate, bilirubin (total), bilirubin (any fractions), C-reactive protein, calcium (total or corrected for albumin), chloride, creatine kinase, creatinine, gamma glutamyl transferase, globulin, glucose, lactate dehydrogenase, lipase, magnesium, phosphate, potassium, sodium, total protein, total cholesterol, triglycerides, urate or urea - 1 test	Fee: $9.70 Benefit: 75% = $7.30 85% = $8.25
MBS item 66584	Quantitation of ionised calcium (except if performed as part of item 66566) - 1 test	Fee: $9.70 Benefit: 75% = $7.30 85% = $8.25
MBS item 66695	Quantitation in blood or urine of hormones and hormone binding proteins - ACTH, aldosterone, androstenedione, C-peptide, calcitonin, cortisol, DHEAS, 11-deoxycortisol, dihydrotestosterone, FSH, gastrin, glucagon, growth hormone, hydroxyprogesterone, insulin, LH, oestradiol, oestrone, progesterone, prolactin, parathyroid hormone, renin, sex hormone binding globulin, somatomedin C(IGF-1), free or total testosterone, urine steroid fraction or fractions, vasoactive intestinal peptide - 1 test	Fee: $30.50 Benefit: 75% = $22.90 85% = $25.95
MBS item 66650	Alpha-fetoprotein, CA-15.3 antigen (CA15.3), CA-125 antigen (CA125), CA-19.9 antigen (CA19.9), cancer associated serum antigen (CASA), carcinoembryonic antigen (CEA), human chorionic gonadotrophin (HCG), neuron specific enolase (NSE), thyroglobulin in serum or other body fluid, in the monitoring of malignancy or in the detection or monitoring of hepatic tumours, gestational trophoblastic disease or germ cell tumour - quantitation - 1 test (Item is subject to rule 6)	Fee: $24.35 Benefit: 75% = $18.30 85% = $20.70
MBS item 66779	Adrenaline, noradrenaline, dopamine, histamine, hydroxyindoleacetic acid (5HIAA), hydroxymethoxymandelic acid (HMMA), homovanillic acid (HVA), metanephrines, methoxyhydroxyphenylethylene glycol (MHPG), phenylacetic acid (PAA) or serotonin quantitation - 1 or more tests	Fee: $39.95 Benefit: 75% $30.00 80% = $34.00

Source: March 2013 Medicare Benefits Schedule MBS online

NB: A maximum of three pathology costs are claimable under MBS arrangements due to coning.

MBS = Medicare Benefits Schedule

Treatment costs associated with the different clinical features of MEN2 are outlined in Table 89, Table 90 and Table 91.

Table 89: Possible treatment costs associated with treatment of, or prophylaxis for, an MTC

Type of resource and identifier	Description	Fees
MBS item 30296	THYROIDECTOMY, total (Anaes.) (Assist.)	Fee: $1,023.70 Benefit: 75% = $796.80
MBS Item 17615	Pre-anaesthesia consultation - on a patient undergoing advanced surgery or who has complex medical problems, involving a selective history and an extensive examination of multiple systems and the formulation of a written patient management plan documented in the patient notes *AND of more than 15 minutes but not more than 30 minutes duration,* not being	Fee: $85.55 Benefit: 75% = $64.20 85% = $72.75

Type of resource and identifier	Description	Fees
	a service associated with a service to which items 2801–3000 applies	
MBS item 20320	INITIATION OF MANAGEMENT OF ANAESTHESIA - for procedures on oesophagus, thyroid, larynx, trachea, lymphatic system, muscles, nerves or other deep tissues of the neck, not being a service to which another item in this Subgroup applies (6 basic units)	Fee: $118.80 Benefit: 75% = $89.10 85% = $101.00
MBS item 51303	Assistance at any operation identified by the word "Assist." for which the fee exceeds $537.15 or at a series of operations identified by the word "Assist." for which the aggregate fee exceeds $537.15 One fifth of the established fee for the operation or combination of operations	1/5 of $1,023.70 = $204.74
MBS item 25015 *For those with MEN2B or mutations in codons 883, 918, 922*[a]	ANAESTHESIA, PERFUSION OR ASSISTANCE AT ANAESTHESIA - where the patient is less than 12 months of age or 70 years or greater (1 basic unit)	Fee: $19.80 Benefit: 75% = $14.85 85% = $16.85
NHCDC cost weights for K06Z	Accommodation costs for Thyroid procedure, average length of stay 2.04 days	Average total cost: $4,039
PBS item 2173J	Thyroxine sodium, 200 µg	DPMQ: $27.11
PBS item 2175L	Thyroxine sodium, 100 µg	DPMQ: $24.08
PBS item 9287T	Thyroxine sodium, 75 µg	DPMQ: $24.12
PBS item 2174K	Thyroxine sodium, 50 µg	DPMQ: $23.47

Source: March 2013 Medicare Benefits Schedule MBS online, Pharmaceutical Benefits Scheme website update 1 April 2013 Pharmaceutical Benefits Scheme, National Hospital Cost Data Collection private hospital costs, AR-DRG version 5.1 round 13 (2008–2009) Round 13 (2008-09) Cost Report
DPMQ = dispensed price for maximum quantity; MBS = Medicare Benefits Schedule; NHCDC = National Hospital Cost Data Collection; PBS = Pharmaceutical Benefits Schedule
[a] Patients with MEN2 or mutations in codons 883, 918, 922 are recommended to undergo total thyroidectomies in the first month of life;

Table 90: Possible treatment costs associated with hyperparathyroidism

Type of resource and identifier	Description	Fees
MBS item 30315	Parathyroid operation for hyperparathyroidism (Anaes.) (Assist.)	Fee: $1,139.90 Benefit: 75% = $854.95
MBS Item 17615	Pre-anaesthesia consultation - on a patient undergoing advanced surgery or who has complex medical problems, involving a selective history and an extensive examination of multiple systems and the formulation of a written patient management plan documented in the patient notes *AND of more than 15 minutes but not more than 30 minutes duration,* not being a service associated with a service to which items 2801–3000 applies	Fee: $85.55 Benefit: 75% = $64.20 85% = $72.75
MBS item 20320	INITIATION OF MANAGEMENT OF ANAESTHESIA - for procedures on oesophagus, thyroid, larynx, trachea, lymphatic system, muscles, nerves or other deep tissues of the neck, not being a service to which	Fee: $118.80 Benefit: 75% = $89.10

Mutation testing of the *RET* gene

Type of resource and identifier	Description	Fees
	another item in this Subgroup applies (6 basic units)	85% = $101.00
MBS item 51303	Assistance at any operation identified by the word "Assist." for which the fee exceeds $537.15 or at a series of operations identified by the word "Assist." for which the aggregate fee exceeds $537.15 One fifth of the established fee for the operation or combination of operations	1/5 of $1,139.90 = $227.98
NHCDC cost weights for K05Z	Accommodation costs for Parathyroid procedure, average length of stay 1.96 days	Average total cost: $3,481

Source: March 2013 Medicare Benefits Schedule MBS online, National Hospital Cost Data Collection private hospital costs, AR-DRG version 5.1 round 13 (2008–2009) Round 13 (2008-09) Cost Report

MBS = Medicare Benefits Schedule; NHCDC = National Hospital Cost Data Collection; PBS = Pharmaceutical Benefits Schedule; DPMQ = dispensed price for maximum quantity

Table 91: Possible treatment costs associated with adrenal phaeochromocytoma

Type of resource and identifier	Description	Fees
MBS item 30324	ADRENAL GLAND TUMOUR, excision of (Anaes.) (Assist.)	Fee: $1,364.90 Benefit: 75% = $1,023.70
MBS Item 17615	Pre-anaesthesia consultation - on a patient undergoing advanced surgery or who has complex medical problems, involving a selective history and an extensive examination of multiple systems and the formulation of a written patient management plan documented in the patient notes *AND of more than 15 minutes but not more than 30 minutes duration,* not being a service associated with a service to which items 2801–3000 applies	Fee: $85.55 Benefit: 75% = $64.20 85% = $72.75
MBS item 20320	INITIATION OF MANAGEMENT OF ANAESTHESIA - for procedures on oesophagus, thyroid, larynx, trachea, lymphatic system, muscles, nerves or other deep tissues of the neck, not being a service to which another item in this Subgroup applies (6 basic units)	Fee: $118.80 Benefit: 75% = $89.10 85% = $101.00
MBS item 51303	Assistance at any operation identified by the word "Assist." for which the fee exceeds $537.15 or at a series of operations identified by the word "Assist." for which the aggregate fee exceeds $537.15 One fifth of the established fee for the operation or combination of operations	1/5 of $1,364.90 = $272.98
NHCDC cost weights for K03Z	Accommodation costs for Adrenal procedure, average length of stay 5.05 days	Average total cost: $9,454
PBS item 1499X *(for after bilateral adrenalectomy)*	Hydrocortisone, 4 mg	DPMQ: $16.88
PBS item 1500Y *(for after bilateral adrenalectomy)*	Hydrocortisone, 20 mg	DPMQ: $21.31
PBS item 1433K *(for after bilateral adrenalectomy)*	Fludrocortisone acetate, 100 µg	DPMQ: $46.60

Source: March 2013 Medicare Benefits Schedule MBS online, Pharmaceutical Benefits Scheme Website update 1 April 2013 Pharmaceutical Benefits Scheme, National Hospital Cost Data Collection private hospital costs, AR-DRG version 5.1 round 13 (2008–2009) Round 13 (2008-09) Cost Report

MBS = Medicare Benefits Schedule; NHCDC = National Hospital Cost Data Collection; PBS = Pharmaceutical Benefits Schedule; DPMQ = dispensed price for maximum quantity

Appendix J Thyroid cancer incidence, 1982–2009

The total numbers of thyroid cancer cases observed between 1982 and 2009 are presented in Table 92. The average annual increase in thyroid cancer incidence between 1982 and 2009 was 6.73%, with 6.27% observed between 2005 and 2009. Thyroid cancer incidence has increased each year since 1982 with the exception of 1984, 1993 and 1997.

Table 92 Thyroid cancer incidence, 1982–2009

Year	Number of thyroid cancer cases	Annual change (%)
1982	366	
1983	420	14.7541%
1984	406	–3.3333%
1985	411	1.2315%
1986	423	2.9197%
1987	456	7.8014%
1988	472	3.5088%
1989	483	2.3305%
1990	527	9.1097%
1991	571	8.3491%
1992	690	20.8406%
1993	677	–1.8841%
1994	726	7.2378%
1995	814	12.1212%
1996	894	9.8280%
1997	870	–2.6846%
1998	987	13.4483%
1999	1,012	2.5329%
2000	1,062	4.9407%
2001	1,205	13.4652%
2002	1,218	1.0788%
2003	1,414	16.0920%
2004	1,508	6.6478%
2005	1,617	7.2281%
2006	1,664	2.9066%
2007	1,789	7.5120%
2008	1,995	11.5148%
2009	2,039	2.2084%

Source: AIHW thyroid cancer workbook

Appendix K Financial and Budgetary impact, DAP costs

The financial and budgetary impact of the addition of RET gene mutation testing to the MBS using the costs outlined in the Final DAP (*RET* gene screen: $1150, known RET mutation test: $480) are presented in Table 93, Table 94, Table 95 and Table 96.

Total costs to the MBS

Table 93 and Table 94, respectively, present the estimated annual costs of listing diagnostic and predictive RET mutation testing on the MBS between 2007 and 2015, assuming that all services are provided in an outpatient setting, where the MBS is responsible for 85% of the service. Based on an estimated number of 130–260 diagnostic and 150–359 predicitve RET mutation test performed in 2013, the estimated cost to the MBS is $294,705. This increases to $333,008 in 2015, based on 147–294 diagnostic and 169–406 predictive RET mutation tests performed (Table 95). However, an unknown proportion of patients may qualify for the Medicare Safety Net, in which case 100% of the scheduled fee is paid by the MBS. Allowing for application of the Medicare Safety Net, the overall true costs to the Commonwealth health budget would lie between the total costs to the MBS and the total combined costs of RET mutation testing, i.e. up to $346,712 in 2013 and $391,774 in 2015.

Table 93 Estimated cost of diagnostic RET mutation tests 2007–2015, with and without MBS listing

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Number of diagnostic RET mutation tests[b]	89–179	100–200	102–204	108–217	115–230	122–245	130–260	138–277	147–294
Estimated expenditure on diagnostic RET mutation testing[c]	$102,868–$205,735	$114,713–$229,425	$117,246–$234,492	$124,632–$249,265	$132,484–$264,968	$140,831–$281,661	$149,703–$299,406	$159,134–$318,268	$169,160–$338,319
Patient co-payment[d]	$15,430–$30,860	$17,207–$34,414	$17,587–$35,174	$18,695–$37,390	$19,873–$39,745	$21,125–$42,249	$22,455–$44,911	$23,870–$47,740	$25,374–$50,748
Estimated MBS expenditure[e]	$87,437–$174,875	$97,506–$195,011	$99,659–$199,318	$105,937–$211,875	$112,612–$225,223	$119,706–$239,412	$127,248–$254,495	$135,264–$270,528	$143,786–$287,571

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] estimated based on a 5–10% incidence of MTC in all thyroid cancers

[c] assuming that the cost of the diagnostic RET mutation test is $1,150, see final DAP

[d] assuming that most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[e] assuming that all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Table 94 Estimated cost of predictive RET mutation tests 2007–2015, with an MBS listing

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Relatives eligible for screening[b]	257–617	287–688	293–703	312–748	331–795	352–845	374–898	398–955	423–1,015
Number of relatives screened[c]	103–247	115–275	117–281	125–299	132–318	141–338	150–359	159–382	169–406
Estimated expenditure on predictive RET mutation testing[d]	$49,376–$118,503	$55,062–$132,149	$56,278–$135,067	$59,823–$143,576	$63,592–$152,622	$67,599–$162,237	$71,857–$172,458	$76,384–$183,323	$81,197–$194,872
Patient co–payment[e]	$7,406–$17,776	$8,259–$19,822	$8,442–$20,260	$8,974–$21,536	$9,539–$22,893	$10,140–$24,336	$10,779–$25,869	$11,458–$27,498	$12,179–$29,231
Estimated MBS expenditure[f]	$41,970–$100,728	$46,803–$112,326	$47,836–$114,807	$50,850–$122,040	$54,054–$129,728	$57,459–$137,901	$61,079–$146,589	$64,927–$155,824	$69,017–$165,641

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] estimated based on the identification of a positive hereditary mutation in the *RET* gene in 25–30% of tests performed; each patient was assumed to have, on average, 11.5 first- or second-degree relatives eligible for familial screening

[c] assuming an uptake rate of 40% in eligible family members

[d] assuming that the cost of the predictive RET mutation test is $480, see final DAP

[e] assuming that most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[f] assuming that all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Table 95 Total MBS costs associated with RET mutation testing (combined costs of listing for diagnostic purposes and listing for familial screening)

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Total combined cost of RET mutation testing	$238,241	$265,674	$271,541	$288,648	$306,833	$326,164	$346,712	$368,555	$391,774
Lower limit	$152,244	$169,775	$173,524	$184,456	$196,076	$208,429	$221,560	$235,519	$250,356
Upper limit	$324,238	$361,574	$369,559	$392,841	$417,590	$443,898	$471,864	$501,591	$533,191
Total patient co-payment[c]	$35,736	$39,851	$40,731	$43,297	$46,025	$48,925	$52,007	$55,283	$58,766
Lower limit	$22,837	$25,466	$26,029	$27,668	$29,411	$31,264	$33,234	$35,328	$37,553
Upper limit	$48,636	$54,236	$55,434	$58,926	$62,638	$66,585	$70,780	$75,239	$79,979
Total cost to the MBS[a]	$202,505	$225,823	$230,810	$245,351	$260,808	$277,239	$294,705	$313,272	$333,008
Lower limit	$129,407	$144,308	$147,495	$156,787	$166,665	$177,165	$188,326	$200,191	$212,803
Upper limit	$275,603	$307,338	$314,125	$333,915	$354,951	$377,313	$401,084	$426,352	$453,213

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence 2005–09 of 6.3%

[b] assuming all patients qualify for the Medicare Safety Net, then the total cost to the MBS would equate to the total combined cost of RET mutation testing

[c] assuming most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[d] assuming all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Sensitivity analyses assuming upper estimates around disease incidence and a 100% uptake rate of familial screening are also presented in Table 96 to provide an extreme upper limit of predictable financial costs. The cost of RET mutation testing to the MBS under these extreme upper limits increases from $620,968 in 2013 to $701,674 in 2015.

Table 96 Sensitivity analyses

Year	2007	2008	2009	2010[a]	2011[a]	2012[a]	2013[a]	2014[a]	2015[a]
Number of diagnostic RET mutation tests[b]	179	200	204	217	230	245	260	277	294
Total cost of diagnostic RET mutation tests[c]	$205,735	$229,425	$234,492	$249,265	$264,968	$281,661	$299,406	$318,268	$338,319
Total number of relatives screened[d]	617	688	703	748	795	845	898	955	1015
Total cost of predictive RET mutation tests[e]	$296,258	$330,372	$337,668	$358,941	$381,554	$405,592	$431,144	$458,307	$487,180
Combined cost of RET mutation testing[f]	$501,993	$559,797	$572,160	$608,206	$646,522	$687,253	$730,550	$776,575	$825,499
Patient contribution[g]	$75,299	$83,970	$85,824	$91,231	$96,978	$103,088	$109,583	$116,486	$123,825
Total cost to the MBS[h]	$426,694	$475,827	$486,336	$516,975	$549,544	$584,165	$620,968	$660,089	$701,674

MBS = Medicare Benefits Schedule; RET = rearranged during transfection (proto-oncogene)

[a] projected incidence of thyroid cancer based on the average annual incidence during 2005–09 of 6.3%

[b] based on 10% incidence of MTC in all thyroid cancers

[c] assuming that the cost of the diagnostic RET mutation test is $1,150, see final DAP

[d] based on 11.5 relatives per proband and assuming 100% uptake of familial screen

[e] assuming that the cost of the predictive RET mutation test is $480, see final DAP

[f] assuming that all patients qualify for the Medicare Safety Net, then the total cost to the MBS would equate to the combined cost of RET mutation testing

[g] assuming that most patients are outpatients and Medicare pays 85% of the scheduled fees, with no Medicare Safety Net concessions or bulk-billed pathology service

[h] assuming that all services are provided in an outpatient setting such that Medicare pays 85% of the scheduled fees; no allowance for additional MBS if some patients qualify for the Medicare Safety Net

Appendix L Studies included in the review

Table 97 Study profiles of studies showing direct comparative evidence

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Comparator	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Diaz & Wohllk 2012) Chile	III-3 interventional evidence High risk of bias (9/26)	Historical controlled study	N=60 MEN2 patients who underwent total thyroidectomy	N=31 Total thyroidectomy after genetic diagnosis	N=29 Total thyroidectomy after clinical diagnosis	Inclusion MEN2 phenotypes or RET M+ Exclusion Not stated	Incidence of residual/recurrent disease Mortality	Not stated
(Kameyama & Takami 2004) Japan	III-3 interventional evidence High risk of bias (10/26)	Historical controlled study	N=905 MTC patients: 634 patients in 1996 271 patients in 2002 1996: 175 MEN2A 49 FMTC 20 MEN2B 390 sporadic MTC 2002: 83 MEN2A 14 FMTC 11 MEN2B 163 sporadic MTC	N=271 Diagnosis with RET mutation testing (as well as clinical information, neck mass, serum calcitonin, and other findings)	N=634 Diagnosis based on clinical information (mass in the neck, serum calcitonin level, hypertension and other findings)	Inclusion MTC patients from institutional members of the Japanese Society of Thyroid Surgery, surveys in 1996 and 2002 Exclusion Not stated	Age at diagnosis	Not stated
(Lallier et al. 1998) Canada	III-3 interventional evidence High risk of bias (15/26)	Historical controlled study	N=13 MEN2 patients (children) who had a total thyroidectomy between 1981 and 1997 *With RET mutation testing:* 5 codon 620 mutations 1 codon 643 mutation	N=6 Total thyroidectomy when individual identified as gene carrier	N=7 Total thyroidectomy when serum calcitonin was elevated	Inclusion MEN2 and underwent total thyroidectomy between 1981 and 1997 Exclusion Not stated	Incidence and severity of MTC Age at time of thyroidectomy Incidence of residual/recurrent disease	Pre-RET: 2–14 years RET: 1–2 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Comparator	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			Without RET mutation testing: codons unknown					
(Learoyd et al. 1997) Australia	III-3 interventional evidence High risk of bias (16/27)	Historical controlled study	N=164 individuals from families with MEN2 and known RET mutations: 56 were RET M+ 108 were RET M-	N=7 Total thyroidectomy with knowledge of RET	N=45 Total thyroidectomy without knowledge of RET	Inclusion Families who had requested RET mutation testing from the one location in Australia performing the test Exclusion Not stated	Incidence and severity of MTC Age at time of thyroidectomy Rate of surveillance	No long-term outcomes
(Lips et al. 1994) The Netherlands	III-3 interventional evidence High risk of bias (7/26)	Historical controlled study	N=14 members of 4 large MEN2A families, who had a thyroidectomy: 8 on the basis of RET mutation carrier status 6 on the basis of raised pentagastrin-stimulated calcitonin levels, who were later found to be RET M–	N=8 Total thyroidectomy based on RET	N=6 Total thyroidectomy based on raised calcitonin, later found to be RET M–	Included Asymptomatic members of 4 large MEN2A families, who had a thyroidectomy Excluded Not stated	Incidence and severity of MTC	Not stated
(Rohmer et al. 2011) France	III-3 interventional evidence Moderate risk of bias (18/26)	Historical controlled study	N=170 patients with a RET mutation who underwent a total thyroidectomy younger than 21 years of age: 109 MEN2A 24 MEN2B	N=38 Total thyroidectomy after 1992	N=132 Total thyroidectomy before 1993	Inclusion RET mutation from families with MEN or familial MTC and aged 21 years at time of surgery Exclusion Not stated	Incidence and severity of MTC Age at time of thyroidectomy Age-appropriate surgery Incidence of residual/recurrent	Median = 5.8 years (range 0.01–28.7 years)

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Comparator	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			37 FMTC				disease	
(Sanchez Sobrino et al. 2011) Spain	III-3 interventional evidence High risk of bias (10/26)	Historical controlled study	N=8 individuals from a family with MEN2A due to RET C634Y mutation	N=3 Total thyroidectomy after genetic diagnosis	N=5 Total thyroidectomy after clinical diagnosis	Inclusion Family with MEN2A due to C634Y mutation Exclusion Not stated	Incidence and severity of MTC Incidence of PCC Incidence of HPT Age at time of thyroidectomy Progression-free survival	Total over 20 years
(Schreinemakers et al. 2010) Sweden	III-3 interventional evidence High risk of bias (17/26)	Historical controlled study	N=93 patients with a RET mutation, who underwent a total thyroidectomy younger than 20 years of age	N=68 Total thyroidectomy with knowledge of RET	N=25 Total thyroidectomy without knowledge of RET	Inclusion MEN2 syndrome, younger than 20 years of age at the time of surgery, and had undergone a total thyroidectomy Exclusion Not stated	Incidence and severity of MTC Age at time of thyroidectomy Age-appropriate surgery Incidence of residual/recurrent disease	Median duration = 7 years (IQR 3, 11)
(Skinner et al. 1996) USA	III-3 interventional evidence High risk of bias (13/26)	Historical controlled study	N=38 children who underwent thyroidectomy younger than 16 years of age for MEN2A or presence of a RET mutation	N=14 Prophylactic thyroidectomy based on RET mutation status 4/14 with elevated calcitonin	N=24 Thyroidectomy without knowing of RET mutations (elevated calcitonin levels or strong family history of MTC, or characteristics of phenotype)	Inclusion Children who underwent thyroidectomy prior to 16 years of age for MEN2A or MEN2B Exclusion No RET mutation	Incidence and severity of MTC Incidence of residual/recurrent disease	Pre-RET: mean = 9.3 years post thyroidectomy RET mutation testing era: mean = 1.3 years

IQR = interquartile range; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 98 Study profiles of studies showing direct uncomparative evidence

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Abdelhakim et al. 2009) Morocco	IV interventional evidence Moderate Quality (4/6)	Case series	N=9 index patients with diagnosed MTC 3 were RET M+: 2 MEN2A 1 unclassified 0/6 suspected sporadic MTC cases were RET M+	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 Total thyroidectomy	Inclusion Patients with diagnosed MTC Exclusion Not stated	RET mutation status Incidence of MTC Incidence of PCC Incidence of HPT Age at diagnosis	Not stated
(Algun et al. 2002) Turkey	IV interventional evidence Moderate quality (4/6)	Case series	N=88 members from 4 generations of an extended family with MEN2A 18 were RET M+ 12 had a thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exon 11 Clinical screening Total thyroidectomy with central lymph node dissection	Inclusion Members of an extended MEN2A family Exclusion Not stated	RET mutation status Incidence of MMC, CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at time of thyroidectomy	Not stated
(Alvares Da Silva et al. 2003) Brazil	IV interventional evidence Moderate quality (4/6)	Case series	N=229 members spanning 6 generations of a large extended FMTC family with a RET G533C mutation 76 members were RET M+ 35 RET M+ members have undergone a total thyroidectomy	RET mutation testing by direct DNA sequencing of exon 8 Prophylactic thyroidectomy	Inclusion Patients with RET G533C mutation from 1 FMTC family Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Postoperative calcitonin levels Age at time of thyroidectomy Treatment decisions	Not stated
(Ameur et al. 2009) France	IV interventional evidence Moderate quality (3/6)	Case series	N=46 tissue samples collected from MTC, CCH, MCC or mixed MTC patients 21 had a germline RET mutation	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 from normal and diseased tissue samples to determine	Inclusion Thyroid samples from MTC and CCH patients obtained from Institut Gustave-Roussy	RET mutation status Incidence of MMC, CCH, MTC, and lymph node metastases Age at diagnosis	N/A

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
				germline and somatic RET status	Exclusion Not stated		
(Bergant et al. 2006) Slovenia	IV interventional evidence Moderate quality (3/6)	Case series	N=69 sporadic MTC patients 13 found to be RET M+ N=31 relatives of RET M+ sporadic MTC patients 16 were RET M+ 27/29 RET M+ patients had total thyroidectomies	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case Total thyroidectomy with central neck dissection	Inclusion Sporadic MTC patients who underwent RET mutation testing between 1997 and 2003 Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis Treatment decisions	Not stated
(Bihan et al. 2012) France	IV interventional evidence Poor quality (2/6)	Case series	N=22 members of an MTC family (including index patient) 15 had RET L790F mutation 8 had abnormal pentagastrin-stimulated calcitonin levels 5 had a thyroidectomy 3 had clinical signs of disease	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–15, and restriction site polymorphism analysis for exon 16 Direct DNA sequencing of exon 13 in family members Clinical screening Prophylactic thyroidectomy	Inclusion Family members of an index case with MTC Exclusion Not stated	RET mutation status Incidence of MTC and lymph node metastases Age at time of thyroidectomy Safety of thyroidectomy Rate of surveillance	Mean = 4.2 years (range 1–6 years) After surgery: Mean = 6.6 years (range 6–8 years)
(Boer et al. 2003) Hungary	IV interventional evidence Moderate quality (4/6)	Case series	N=65 consecutive unrelated patients with MTC admitted for genetic screening for MEN2A and FMTC 25 were RET M+ 5/25 were screened postoperatively 14/25 underwent thyroidectomy	RET mutation testing by direct DNA sequencing (exons not specified) Thyroidectomy	Inclusion Unrelated probands diagnosed with MTC and no signs of MEN2B between 1992 and 2000 Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of HPT Rates of treatment	Mean = 47 months (range 29–78 months)

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Calva et al. 2009) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=31 family members who underwent genetic testing 22/31 had a RET C609Y mutation 16/22 underwent a thyroidectomy	RET mutation testing (method not stated) Clinical screening Total thyroidectomy for treatment or prophylaxis	Inclusion Members of a 3-generation MEN2 family with a RET C609Y mutation Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at time of thyroidectomy	Not stated
(Chang et al. 2009) Taiwan	IV interventional evidence Moderate quality (3/6)	Case series	N=8 probands from 8 unrelated MTC families: 4 MEN2A 2 MEN2B 1 FMTC 1 sporadic MTC (possibly *de novo* MEN2A)	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16	Inclusion Probands from 8 families affected by MTC Exclusion Not stated	RET mutation status Age at diagnosis	Not stated
(Chiefari et al. 1998) Italy	IV interventional evidence Moderate quality (4/6)	Case series	N=47 patients: 10 with sporadic MTC: 1/10 had a germline RET mutation 37 members of 10 separate families with hereditary MTC 22/37 were RET M+: 3 were asymptomatic 18/22 had available data 16/18 had a thyroidectomy (from 8 families)	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14 Clinical screening Total thyroidectomy	Inclusion Patients either affected by MTC or belonging to families with hereditary MTC Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis Age at time of thyroidectomy Postoperative calcitonin levels	Not stated
(Decker et al. 1996) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=36 children (1 month – 12 years) from confirmed MEN2A patients 18 were RET M+	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11	Inclusion Children from 1 of 4 distinct, well-characterised,	RET mutation status Incidence of CCH and MTC Postoperative calcitonin	36 hours post-surgery

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			11 underwent prophylactic thyroidectomy	Clinical screening Prophylactic thyroidectomy	multigenerational MEN2A kindreds at direct risk for disease <u>Exclusion</u> Not stated	levels Age at time of thyroidectomy Safety of thyroidectomy	
(Decker et al. 1995) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=93 members of 10 MEN2A or FMTC kindreds with known RET mutations 29 were RET M+ 17 had a thyroidectomy 4 are awaiting operation 8 are planned before 5 years of age N=21 with sporadic MTC 5 were RET M+ N=10 patients clinically diagnosed as MEN2A from calcitonin levels but dubious results or histopathology 1 was RET M+	RET mutation testing by denaturing gradient gel analysis of exons 10 and 11 with confirmatory direct DNA sequencing	<u>Inclusion</u> Consecutive patients at risk of MEN2A/FMTC referred for genetic screening during a 3-month period <u>Exclusion</u> Not stated	RET mutation status Incidence of CCH and MTC Age at time of thyroidectomy Treatment decisions	Not stated
(Donis-Keller 1995) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=21 RET M+ family members from 7 MEN2A kindreds 13 had thyroidectomy 7 had elevated calcitonin levels	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of affected exon Thyroidectomy	<u>Inclusion</u> Member of MEN2A family <u>Exclusion</u> Not stated	RET mutation status Incidence of CCH and MTC Treatment decisions	Not stated
(Dralle et al. 1998) Germany	IV interventional evidence Moderate quality (4/6)	Case series	N=75 RET M+ patients <20 years of age who underwent a prophylactic total thyroidectomy Identified retrospectively through a questionnaire 57 underwent additional lymph	RET mutation testing (method not stated) Clinical screening Total thyroidectomy Retrospectively identified	<u>Inclusion</u> Patients who: (1) had a preoperatively proved RET mutation; (2) age at operation	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			node dissections	through questionnaire	was younger than 20 years (3) were clinically asymptomatic with regard to thyroid C-cell disease (4) TNM classification pT0–1/pNX/pN0–1/M0 Exclusion Not stated	Incidence of HPT Postoperative calcitonin levels Safety of thyroidectomy	
(Erdogan et al. 2007) Turkey	IV interventional evidence Moderate quality (3/6)	Case series	N=41 RET M+ patients identified from 15 pedigrees: 12 MEN2A 2 MEN2B 1 FMTC 26 were asymptomatic 30 had a thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–15, and restriction enzyme analysis of exon 16 Total thyroidectomy	Inclusion Patients had to fulfil the clinical and molecular criteria proposed by the International RET Mutation Consortium Exclusion Not stated	RET mutation status Incidence of CCH and MTC Incidence of PCC Incidence of HPT Age at diagnosis Treatment decisions	Not stated
(Etit et al. 2008) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=42 specimens from patients retrospectively identified from hospital records who had undergone a prophylactic thyroidectomy for possible MTC 32 underwent RET mutation testing: 24 MEN2A 8 non-MEN 30 with family history	RET mutation testing by direct DNA sequencing analysis of exons 10, 11 and 13–16	Inclusion Thyroidectomy must have been performed due to a positive family history of MEN2A, MEN2B or FMTC, an elevated serum calcitonin level, or the presence of a RET mutation, between 1977 and 2007 Exclusion Not stated	RET mutation status Incidence of MMC, CCH, MTC and lymph node metastases Incidence of HPT	Mean = 4.7 years (range 1 month – 13 years)

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Feldman et al. 2000) UK, USA, France	IV interventional evidence Moderate quality (4/6)	Case series	N=20 members from 2 FMTC families who have a RET V804M mutation	RET mutation testing by restriction analysis of exon 14 Clinical screening Thyroidectomy	Inclusion A member of 1 of 2 FMTC families who have a RET exon 14 codon 804 V804M mutation Exclusion Not stated	RET mutation status Incidence of CCH and MTC	Not stated
(Frank-Raue et al. 2011) Germany	IV interventional evidence Moderate quality (4/6)	Case series	N=340 patients proven to be carriers of germline mutation in exon 10 of the *RET* gene Identified through: 47% symptomatic 53% screening	RET mutation testing (method not stated) Clinical screening	Inclusion A proven carrier status of a germline mutation in exon 10 of the *RET* gene, or a relative of index registrants if diagnosed with an MTC or PCC (from 14 different countries) Exclusion Not stated	RET mutation status Incidence of MTC Incidence of PCC Incidence of HPT Age at diagnosis	Not stated
(Frank-Raue et al. 1997) Germany	IV interventional evidence Moderate quality (4/6)	Case series	N=11 asymptomatic RET M+ children from 8 MEN2A/FMTC families	RET mutation testing by single-strand conformation polymorphism analysis or restriction site polymorphism analysis, and direct DNA sequencing of exons 10, 11 and 13 Prophylactic thyroidectomy	Inclusion Patients with MEN2A or FMTC from 8 families Exclusion Not stated	RET mutation status Incidence of CCH and MTC Postoperative calcitonin levels	Range = 2–32 months
(Frank-Raue et al. 1996) Germany	IV interventional evidence Moderate quality	Case series	N=178 members of 35 families clinically identified with hereditary MTC	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA	Inclusion A member of 1 of 35 families clinically	RET mutation status Incidence of MMC, CCH and MTC	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
	(4/6)		159 had RET mutation testing 84 were RET M+ 67/84 patients were symptomatic 9/17 presymptomatic patients had prophylactic thyroidectomy	sequencing or restriction site polymorphism analysis of exons 10 and 11 Direct DNA sequencing of exons 13 and 16 Clinical screening Prophylactic thyroidectomy	identified as having hereditary MTC since 1993 Exclusion Not stated	Postoperative calcitonin levels Safety of thyroidectomy Treatment decisions	
(Franz & Wells Jr 1997) USA, Germany	IV interventional evidence Moderate quality (4/6)	Case series	N=20 RET M+ patients: 19 MEN2A patients 1 FMTC patient	RET mutation testing by restriction site polymorphism analysis and/or DNA sequencing (exons not specified) Clinical screening Prophylactic thyroidectomy based on RET status	Inclusion Patients with MEN2A or FMTC from 12 distinct kindreds Exclusion Not stated	RET mutation status Incidence of CCH and lymph node metastases Postoperative calcitonin levels	Not stated
(Frohnauer et al. 2000) USA	IV interventional evidence Moderate quality (4/6)	Case series	N=38 members from 5 MEN2A kindreds with a RET codon 804 mutation 23 were RET M+ 14 had a thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis analysis, confirmed by direct DNA sequencing of exon 14 Thyroidectomy	Inclusion At-risk family members of a MEN2A kindred Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Age at time of thyroidectomy Treatment decisions Mortality	Not stated
(Gagel et al. 1995) USA	IV interventional evidence Moderate quality (3/6)	Case series	N=178 members from 28 families with MEN2A: 71 were clinically confirmed: all were found to be RET M+ 53 were clinically negative: all were found to be RET M– 54 were unknown status but at 50% risk of RET mutation: 19	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16 Thyroidectomy based on genetic screening	Inclusion Member of a MEN2A kindred Exclusion Not stated	RET mutation status Incidence of CCH and MTC Age at time of thyroidectomy Treatment decisions	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			were found to be RET M+ 4 RET M+ patients (children aged 3–12 years) had a thyroidectomy				
(Gimm et al. 2002). Germany, Austria	IV interventional evidence Moderate quality (4/6)	Case series	N = 40 patients with a RET codon 790/791 mutation who underwent thyroid operations: 13 were index patients 27 were identified during RET mutation screening 10 had a thyroidectomy 30 had a thyroidectomy and lymph node dissection	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14 Thyroidectomy	Inclusion Patients diagnosed with a codon 790/791 mutation who underwent thyroid operations Exclusion Not stated	RET mutation status Incidence of CCH, MTC, and lymph node metastases Incidence of PCC Incidence of HPT Postoperative calcitonin levels Safety of thyroidectomy	Not stated
(Gonzalez et al. 2003) Mexico	IV interventional evidence High quality (5/6)	Case series	N=57 patients 9 Probands: 3 MEN2B 2 MEN2A 4 sporadic MTC 48 Family members 21 had MTC or CCH 17 were RET M+ (5 probands and 12 relatives) 11 had thyroidectomy 4 sporadic MTC patients were RET M–	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15 Clinical screening Thyroidectomy	Inclusion Proband had MTC present, elevated calcitonin and thyroid resection for histopathological confirmation Exclusion Patients found to be RET M– were excluded from further study	RET mutation status Progression to disease over time Mortality	Mean = 6.7 years (range 1–24 years)
(Gosnell et al. 2006) Australia	IV interventional evidence Moderate quality (4/6)	Case series	N=48 at-risk individuals in a single MEN2A kindred with a RET V804L mutation 23 were RET M+ 22 RET M+ family members (including proband) underwent thyroidectomy	RET mutation testing (method not stated) Clinical screening Prophylactic thyroidectomy	Inclusion At-risk individuals in 1 MEN2A kindred Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Guyetant et al. 2003) France	IV interventional evidence High quality (5/6)	Case series	N=66 patients who had been operated on for CCH or MTC: 43 with diffuse goiter or nodular thyroid disease 18 were potential MEN2 carriers due to prior familial MTC 5 had isolated hypercalcitoninemia N=46 sporadic cases 27 sporadic MTC 19 sporadic CCH	RET mutation testing of exons 8, 10, 11 and 13–16 (method not stated)	Inclusion Consecutive patients with an MTC or CCH diagnosis in pathology department files from 1993 to 2000), representing 2.9% of 3,342 thyroid pathological specimens examined Exclusion Consultation cases and patients with non-total thyroidectomy, or with incomplete biological or clinical data	RET mutation status Incidence of CCH, MTC and lymph node metastases	Not stated
(Halling et al. 1997) USA	IV interventional evidence Moderate quality (3/6)	Case series	N=72 family members from 1 large FMTC kindred with a RET C609Y mutation 34 were RET M+ 41 had thyroidectomy before genetic testing 28 were RET M+ (19 with elevated pentagastrin-stimulated calcitonin levels) 10 were RET M– (6 with elevated pentagastrin-stimulated calcitonin levels) I had an unknown RET mutation status with normal pathology 2 had no available pathology	RET mutation testing by direct DNA sequencing of exon 10 Clinical screening Thyroidectomy	Inclusion Member of large FMTC kindred Exclusion Not stated	RET mutation status Incidence of CCH and MTC Treatment decisions	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Heizmann et al. 2006) Switzerland	IV interventional evidence Moderate quality (4/6)	Case series	N=14 RET M+ patients who were presymptomatic, from 2 MEN2A kindreds	RET mutation testing by single-strand conformation polymorphism analysis, denaturing gradient gel electrophoresis and direct DNA sequencing of exons 10 and 11 Total thyroidectomy with central compartment dissection in those older than 6 years of age	Inclusion Presymptomatic kindreds with MEN2A operated on between 1997 and 2004 by a senior surgeon Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Age at time of thyroidectomy Safety of thyroidectomy	6 weeks postoperative
(Hernandez et al. 1997) Spain	IV interventional evidence Poor quality (2/6)	Before and after case series	N=53 members of 3 MEN2A families: 17 members affected 36 members asymptomatic 6 identified as RET M+: all 6 had raised pentagastrin-stimulated calcitonin levels and a thyroidectomy 2/13 who tested as RET M– had raised pentagastrin-stimulated calcitonin levels	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11 Clinical screening Total thyroidectomy	Inclusion Family members from 3 families with MEN2A, where both clinical and genetic information was available Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Treatment decisions	Not stated
(Jindrichova et al. 2004) Czech Republic	IV interventional evidence High quality (5/6)	Case series	N=106 unrelated index cases with MTC 23 index cases were RET M+ N=76 relatives of RET M+ cases: 6 previously sporadic MTC (9 family members tested) 4 FMTC families (21 family members tested) 9 MEN2A families (39 family	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Thyroidectomy Surgical decisions often made prior to genetic testing with no separation of data based on clinical or genetic diagnosis Note: thyroidectomy outcomes data were not	Inclusion Unrelated index cases with MTC, and family members of those with RET mutations Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Age at diagnosis Age at time of thyroidectomy	1–20 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			members tested) 4 MEN2B families (7 family members tested) 24 relatives were RET M+ 10 had normal levels with no signs of disease 14 had high calcitonin levels and had thyroidectomy	available for 1 FMTC index case			
(Jung et al. 2010) Korea	IV interventional evidence Moderate quality (3/6)	Case series	N=30 members of a 3-generation FMTC family 29 (index case plus 28 relatives) underwent genetic testing: 11 were RET M+ (including index case) 6 relatives (including index case) diagnosed with MTC before genetic testing 3 relatives diagnosed with MTC after genetic testing 8 relatives (including index case) underwent total thyroidectomy	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 of index case Analysis of exon 10 in family members Total thyroidectomy with either central neck dissection or modified radical neck dissection	Inclusion Members of a FMTC family Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Age at time of thyroidectomy Postoperative calcitonin levels Treatment decisions Mortality	Median = 10 years
(Kameyama, Okinaga & Takami 2004) Japan	IV interventional evidence Moderate quality (4/6)	Case series	N=271 patients with histologically confirmed MTC 108 had hereditary MTC: 83 MEN2A 11 MEN2B 14 FMTC 53 were symptomatic: 39 had a neck mass 14 had other (e.g. adrenal) tumour	RET mutation testing (method not stated).	Inclusion Histologically proved MTC between 1995 and 2002 Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT Age at diagnosis	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			55 were asymptomatic: 45 identified as RET M+ 10 had elevated calcitonin levels 163 had sporadic MTC				
(Karga et al. 1998) Greece	IV interventional evidence Poor quality (2/6)	Case series	N=58 individuals from 12 unrelated Greek families 9 MEN2A 1 FMTC 3 probable FMTC (only 3 members diagnosed with MTC) 33 clinically affected patients had a thyroidectomy for MTC prior to genetic testing 25 asymptomatic first-degree relatives of patients 5 children were RET M+	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and 16	Inclusion Individuals from families with MEN2A or FMTC Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT Age at diagnosis Rate of surveillance Treatment decisions	Not stated
(Kinlaw et al. 2005) USA	IV interventional evidence Poor quality (2/6)	Case series	N=30 family members (including index case) of a MEN2A family with a RET C609S mutation 15 were RET M+ 6 had thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in the index case Restriction site polymorphism analysis to detect C609S mutation in family members Thyroidectomy	Inclusion Family members in a family with MEN2A due to Cys609Ser mutation Exclusion Refusal to be tested	RET mutation status Incidence of CCH and MTC Incidence of PCC Treatment decisions	Not stated
(Lau et al. 2009) Hong Kong	IV interventional evidence High quality (5/6)	Case series	N=22 asymptomatic patients from 8 MEN2A families who underwent prophylactic total thyroidectomy based on RET mutation status All had RET codon 634 mutations:	RET mutation testing by restriction enzyme analysis and/or direct DNA sequencing (exons not specified) Prophylactic thyroidectomy with or without a unilateral	Inclusion Genetic carriers who were completely asymptomatic and had no clinical evidence of MTC	RET mutation status Incidence of CCH, MTC and lymph node metastases Age at time of thyroidectomy	Median = 49 months (range 13–128 months)

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			12 C634Y 4 C634R 3 C634W 3 C634G	central compartment neck dissection	Exclusion Not stated	Incidence of residual/recurrent disease Safety of thyroidectomy	
(Learoyd et al. 2005) Australia, New Zealand	IV interventional evidence Moderate quality (4/6)	Case series	N=57 members of 2 families Family 1: 1 proband diagnosed with PCC and MTC had RET V804L mutation 47 family members from 4 generations: 22 were RET M+ 22/23 (including proband) had thyroidectomy Family 2: 1 proband diagnosed with MTC had RET V804M mutation 8 family members from 3 generations: 5 were RET M+ 3/6 (including proband) had thyroidectomy	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 From 1998, analysis also of exons 13–15 for probands Family members screened for family RET mutation Prophylactic thyroidectomy	Included Families referred to their institute for genetic testing from the early 1990s Excluded Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at time of thyroidectomy Treatment decisions	Not stated
(Lecube et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	Case series	N=52 family members of an FMTC family with RET V804M mutation 25 were RET M+	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11 and 13–16 Biochemical screening on those RET M+	Included Family members of an FMTC family with V804M mutations Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT Treatment decisions	Up to 2 years
(Lindskog et al. 2004) Sweden	IV interventional evidence Moderate quality (3/6)	Before and after case series	N=16 RET M+ family members of a MEN2A family with a RET codon 618 mutation, who had a thyroidectomy: 15 identified by biochemical	RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy with	Included Family members from 1 large family	RET mutation status Incidence of MTC and lymph node metastases Incidence of PCC	Mean = 19±9 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			screening 1 identified by genetic testing N=33 RET M– family members	central neck dissection		Incidence of HPT Age at diagnosis Age at time of thyroidectomy Postoperative calcitonin levels Treatment decisions Rate of surveillance	
(Lips et al. 1994) The Netherlands	IV interventional evidence Moderate quality (3/6)	Before and after case series	N=148 members from 4 large MEN2A families: 80 MEN2A gene carriers (61 diagnosed by DNA sequence analysis) 14 were symptomatic 14 had normal pentagastrin-stimulated calcitonin results 8 had total thyroidectomy on basis of RET mutation status 6 were scheduled for surgery 68 non-carriers	MEN2 diagnosed by linkage analysis until June 1993 RET mutation testing by direct DNA sequencing of exons 10 and 11 Total thyroidectomy	Included Members of 4 large families with MEN2A Exclusion Not stated	RET mutations status Incidence of CCH and MTC Incidence of PCC Incidence of HPT Age at time of thyroidectomy Treatment decisions Mortality	Not stated
(Lombardo et al. 2002) France and Italy	IV interventional evidence Moderate quality (3/6)	Case series	N=61 patients with RET V804L mutations, from 5 families 31/61 underwent thyroidectomy: 3 index cases with MTC 1 patient with follicular tumour 14 patients with detectable basal calcitonin levels 13 patients with significant increase in pentagastrin-stimulated calcitonin levels	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15, and restriction site polymorphism analysis of exon 16 Clinical screening Total thyroidectomy	Included Patients with V80L RET mutations Excluded Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Age at time of thyroidectomy Incidence of residual/recurrent disease Postoperative calcitonin levels	Median = 8.5 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
						Treatment decisions	
(Machens et al. 2006) Germany	IV interventional evidence Poor quality (2/6)	Case series	N=219 patients with RET mutations divided into three categories RET codons 918 mutations (highest risk) RET codons 609–634 (high risk) RET codons 768–891 (least high risk 206 patients previously described (Machens et al. 2005)	RET mutation testing (method not stated).	Included RET mutation carriers recruited between November 1994 and April 2005 Excluded Not stated	RET mutation status Incidence of PCC	Not stated
(Machens et al. 2005) Germany	IV interventional evidence High quality (5/6)	Case series	N=206 consecutive RET M+ patients who underwent surgery for CCH, MTC or PCC: 74 index cases 132 non-index cases (criteria for diagnosis and/or surgery not reported) Stratified by risk category: 18 highest risk (codon 918) 117 high risk (codons 609–634) 71 less high risk (codons 768–891)	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 Clinical screening Thyroidectomy and/or adrenalectomy	Inclusion Patients submitted to surgery for MTC, CCH or PCC and/or adrenalectomy Exclusion Not stated	RET mutation status Incidence of MTC Time to progression stratified by RET risk category	Not stated Mean time to progression is stated
(Machens et al. 2001) Germany	IV interventional evidence Poor quality (2/6)	Case series	N=63 RET M+ patients with MTC who had a thyroidectomy 36 were index patients	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11, 13 and 14 Thyroidectomy	Inclusion Patients operated on for MTC between November 1994 and October 1999 and had RET mutations in exons 10, 11, 13 or 14 Exclusion	RET mutation status Incidence of MTC and lymph node metastases Age at diagnosis	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
					Carriers of codon 918 mutation (7/198)		
(Marsh et al. 1996) Australia, New Zealand	IV interventional evidence Moderate quality (4/6)	Case series	N=39 asymptomatic members of 16 MEN2A and FMTC families at risk of being a gene carrier 7 were RET M+ 21 members were from 2 MEN2A families: 5 were RET M+	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11 Thyroidectomy	Inclusion Family member at risk of carrying RET mutation Exclusion Not stated	RET mutation status Incidence of CCH and MTC Incidence of PCC Treatment decisions	Not stated
(Milos et al. 2008) Worldwide (Romania, Germany, Chile, Brazil, Agentina, Hungary, Spain, The Netherlands, Czech Republic, Poland, USA)	IV interventional evidence Moderate quality (4/6)	Case series	N=92 carriers of RET C634W mutation from 20 unrelated MEN2A families 81 underwent thyroid operations 49 had available histological data 34 had available postoperative data	RET mutation testing (method not stated)	Inclusion Patients with C643W mutation Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Postoperative calcitonin levels Mortality	Mean = 12 years (range 1–29 years)
(Neocleous et al. 2011) Cyprus	IV interventional evidence Poor quality (2/6)	Case series	N=8 probands from 7 FMTC families and 1 MEN2A family N=29 family members from 7 of the probands: 15 were RET M+	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index cases, and only exon 10 in family members Thyroidectomy	Inclusion FMTC or MEN2A patients and their family members Exclusion Not stated	RET mutations status Age at diagnosis Treatment decisions	Range = 1–10 years
(Neumann et al. 2002) Germany and Poland	IV interventional evidence High quality (5/6)	Case series	N=271 patients with non-syndromic PCC without family history of disease 13 were RET M+	RET mutation testing by single-strand conformation polymorphisms and direct DNA sequencing of exons 13–16 Also checked for mutations in SDHB, SDHD and VHL	Inclusion Patients with PCCs consecutively registered in Freiburg, Germany, and Warsaw, Poland Exclusion Cases discovered by	RET mutation status Incidence of MTC Age at diagnosis	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
					clinical or genetic screening without symptoms of illness Excluded 11 with neurofibromatosis type 1 due to clear clinical diagnosis, 14 with family history of VHL or MEN1		
(Nguyen et al. 2001) France	IV interventional evidence High quality (5/6)	Case series	N=87 first-degree relatives of index cases in MEN2 families who were diagnosed with MTC and found to be RET M+: 84 patients from 52 MEN2A families 3 patients from 3 MEN2B families	MEN2 diagnosed by linkage analysis between 1989 and 1994 RET mutation testing by sequence analysis since 1994 (method not stated)	Inclusion Non-index patients, descendants of index cases MEN2A or 2B diagnosed by sequence analysis Exclusion Not stated	RET mutation status Incidence of CCH and MTC Incidence of PCC Incidence of HPT Age at diagnosis	Mean = 7.6±2.8 years (range 1.5–10 years)
(Pacini et al. 1995) Italy	IV interventional evidence Moderate quality (3/6)	Case series	N=58 family members from 7 MEN2A and 2 MEN2B families 16 clinically affected patients were RET M+ 5/42 clinically unaffected but at risk of disease were RET M+ 4/5 had thyroidectomy	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16	Inclusion Non-index patients, descendants of index cases MEN2A or 2B diagnosed by sequence analysis Exclusion Not stated	RET mutation status Incidence of CCH and MTC Incidence of PCC Incidence of HPT Age at diagnosis Age at time of thryoidectomy	Not stated
(Paszko et al. 2007) Poland	IV interventional evidence Moderate quality (3/6)	Case series	N=46 patients with MTC who were RET M+ N=19 RET M+ asymptomatic relatives	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16	Inclusion Patients with MTC Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT	2 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Patocs et al. 2006) Hungary	IV interventional evidence Moderate quality (3/6)	Case series	N=40 patients from 18 families who had had a thyroidectomy due to hereditary MTC or CCH: 33 MEN2A 1 MEN2B 6 from MTC families without PCC or HPT	RET mutation testing by single-strand conformation polymorphism analysis, restriction site polymorphism analysis, and direct DNA sequencing of exons 10, 11, 13, 14 and, in MEN2B phenotype, exons 15 and 16	Inclusion Patients operated on for hereditary MTC or CCH Exclusion Patients with unidentified mutations of the *RET* gene	RET mutations status Incidence of MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis Mortality	Not stated
(Patocs et al. 2004) Hungary	IV interventional evidence Moderate quality (4/6)	Case series	N=41 patients with PCCs 7 were RET M+	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 14	Inclusion Adrenal tumours (only results for PCC presented) Exclusion Not stated	RET mutation status Age at diagnosis	Not stated (only short-term outcomes)
(Pinna et al. 2007) Italy	IV interventional evidence High quality (5/6)	Case series	N=22 patients with MTC who had a total thyroidectomy 7 were RET M+ N=43 relatives of the 7 index cases 22 RET M+ family members 14 have undergone prophylactic total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 8–16 Total thyroidectomy	Inclusion Family members who underwent prophylactic total thyroidectomy Exclusion Not stated	RET mutation status Incidence of CCH and MTC Treatment decisions	Not stated
(Punales et al. 2003) Brazil	IV interventional evidence Moderate quality (3/6)	Case series	N=160 individuals: 150 family members in 17 MEN2 families (54 had clinical signs of thyroid neoplasia or endocrine-related neoplasia) 10 patients with apparently sporadic MTCs 88 patients were RET M+:	RET mutation testing by single-strand conformational polymorphism analysis, restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–15 Total thyroidectomy, with a central cervical lymph node	Inclusion Diagnosis of MTC, or family member Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis Treatment decisions	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			17/88 index cases 61/88 family members 10/88 sporadic MTC cases 24/78 patients from MEN2 families had no clinical evidence of disease 69/88 had RET codon 634 mutation 50/69 underwent thyroid surgery: 43/50 had clinical disease 7/50 were clinically asymptomatic gene carriers	dissection in those with increased calcitonin levels		Mortality	
(Quayle et al. 2007) USA	IV interventional evidence Poor quality (2/6)	Case series	N=323 patients from 65 MEN2A families who were RET M+	RET mutation testing (method not stated)	Inclusion Patients with MEN2A and data on PCC status, with a RET mutation Exclusion Patients with mutations in codons 611 and 804	RET mutation status Incidence of PCC	Median = 9 years
(Quayle et al. 2004) USA	IV interventional evidence Moderate quality (3/6)	Case series	N=39 RET M+ patients with MEN2 or FMTC diagnosed when over the age of 50 years: 36 patients with MEN2A 3 patients with FMTC 5 RET codon 609 mutations 15 RET codon 618 mutations 6 RET codon 620 mutations 12 RET codon 634 mutations 1 unknown mutation	RET mutation testing (method not stated) Total thyroidectomy	Inclusion Patients with MEN2A, MEN2B or FMTC who were diagnosed after the age of 50 years Exclusion Not stated	RET mutations status Incidence of CCH, MTC and lymph node metastases Incidence of residual/recurrent disease Postoperative calcitonin levels Treatment decisions Mortality	Median = 6.4 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			38 had thyroidectomy				
(Rodriguez Gonzalez et al. 2002) Spain	IV interventional evidence Moderate quality (3/6)	Case series	N=203 MTC patients 82 patients were RET M+ (RET codon 634 mutation) and diagnosed as MEN2A 60 patients had high calcitonin levels and were not discussed further in this study 22 had normal basal and pentagastrin-stimulated calcitonin levels and received prophylactic thyroidectomy	RET mutation testing by denaturing gradient gel electrophoresis of exons 10, 11, 13, 14 and 16, and confirmed by restriction site polymorphism analysis Clinical screening Prophylactic total thyroidectomy ± central neck dissection	Inclusion Patients at risk (family) or with confirmed MTC or CCH Only clinically negative but RET M+ were used for further data analysis Exclusion Patients RET M– or clinically confirmed MTC and CCH (not considered to be prophylactic surgery)	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at time of thyroidectomy Incidence of residual/recurrent disease Postoperative calcitonin levels Safety of thyroidectomy	Mean = 23 months (range 6–57 months)
(Romei et al. 2011) Italy	IV interventional evidence High quality (5/6)	Case series	N=60 RET M+ family members of patients with MTC reclassified from sporadic MTC to FMTC or MEN2A due to a RET mutation: 5 refused treatment 20 had no clinical or biochemical signs of disease and were monitored 35 showed clinical and/or biochemical signs of disease on screening 30 (29 FMTC, 1 MEN2A) underwent total thyroidectomy	RET mutation testing method changed over 15 years Initially used direct DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 Total thyroidectomy	Inclusion Family members who underwent a total thyroidectomy after RET mutation testing Exclusion Did not undergo surgery (n=5)	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis Incidence of residual/recurrent disease Progression-free survival Treatment decisions	Mean = 6.0 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Sanso et al. 2002) Argentina	IV interventional evidence Moderate quality (4/6)	Case series	N=133 patients: 17 index cases with MEN2A 5 index cases with MEN2B 98 relatives of MEN2A patients 13 relatives from MEN2B families 42 (26 juveniles and 16 adults) had RET mutation 18 carriers (aged 17 months – 21 years) had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, and confirmed by restriction site polymorphism analysis Prophylactic thyroidectomy	Inclusion Cases with clinical signs of MEN2 and their relatives Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Postoperative calcitonin levels	Not stated
(Schellhaas et al. 2009) Germany	IV interventional evidence High quality (5/6)	Case series	N=17 patients with a RET codon 634 mutation 14 with MEN2A 3 with apparent FMTC	RET mutation testing (method not stated) Prophylactic total thyroidectomy with bilateral cervicocentral lymphadenectomy	Inclusion Total thyroidectomy between 1992 and 1999 with mutation in codon 634 in exon 11 Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of residual/recurrent disease Postoperative calcitonin levels Safety of thyroidectomy	Median = 147 months (range 90–181 months)
(Schuffenecker et al. 1998) France	IV interventional evidence Moderate quality (4/6)	Case series	N=188 patients from 30 families with RET 634 mutations: 10 with C634R mutations 11 with C634Y mutations 9 with other 634 mutations	Exhaustive RET mutation testing (method not stated, data from registry)	Inclusion Family with 634 mutation and genotyping data from at least 2 generations with comprehensive follow-up of MTC, PCC and HPT Exclusion Not stated	RET mutation status Incidence of HPT	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Schuffenecker et al. 1994) France	IV interventional evidence Moderate quality (4/6)	Case series	N=86 unrelated MTC patients with RET codon 618, 620 or 634 mutations 54/58 MEN2A were RET M+ 6/9 FMTC were RET M+ 10/19 with other hereditary MTC were RET M+ N=259 affected members from 53 families with RET codon 634 mutations N=60 affected members from 13 families with RET codon 618 or 620 mutations	RET mutation testing by direct DNA sequencing of exons 10 and 11	Inclusion Individuals from French families with hereditary MTC Exclusion MEN2B	RET mutation status Incidence of PCC	Not stated
(Shifrin et al. 2009) USA	IV interventional evidence Moderate quality (3/6)	Case series	N=107 members of a family with a RET V804M mutation 81 underwent genetic testing 40/81 had RET V804M mutation 15/40 had total thyroidectomy	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (not clear if relatives tested for specific mutation or all) Total thyroidectomy with central and ipsilateral lateral neck dissection	Inclusion Family members from family with RET V804M mutation Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Age at diagnosis	Not stated
(Shimotake et al. 1996) Japan	IV interventional evidence Moderate quality (3/6)	Case series	N=6 children without clinical signs of disease who underwent RET mutation testing 3 were RET M+	RET mutation testing by direct DNA sequencing of exons 10 and 11	Inclusion First-degree family members in 1 Japanese pedigree Exclusion Did not undergo genetic testing	RET mutation status Rate of surveillance	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Skinner et al. 2005) USA	IV interventional evidence High quality (5/6)	Case series	N=50 RET M+ patients from MEN2A families who were <20 years of age at time of thyroidectomy	RET mutation testing by restriction site polymorphism analysis and/or direct DNA sequencing of RET exons 10, 11, 13, 14 and 16 Total thyroidectomy	Inclusion RET M+ patients, <20 years of age at time of thyroidectomy and were followed up at least 5 years postoperatively Exclusion Older than 19 years of age, or not followed up at 5 years	RET mutation status Incidence of CCH, MTC and lymph node metastases Incidence of residual/recurrent disease Age at time of thyroidectomy Postoperative calcitonin levels	Range = 5–10 years
(Spinelli et al. 2010) Italy	IV interventional evidence High quality (4/6)	Case series	N=13 patients (8–17 years of age) with MEN2 who underwent surgery for MTC: 7 (54%) MEN2A 4 (31%) FMTC 2 (15%) MEN2B	RET mutation testing by direct DNA sequencing (exons not specified) Curative or prophylactic total thyroidectomy	Inclusion Patients with MEN2 who underwent surgery for MTC, ≤17 years of age Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Safety of thyroidectomy	N/A
(Uchino et al. 1999) Japan	IV interventional evidence Low quality (2/6)	Case series	N=36 members from 5 MEN2A families with mutations on codon 634 15 were RET M+: 9 clinically affected 6 clinically non-affected	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11, 13, 14 and 16 Total thyroidectomy	Inclusion Members of 5 MEN2 kindreds Exclusion Not stated	RET mutation status Incidence of MTC and lymph node metastases Incidence of PCC Treatment decisions	Not stated
(Vaclavikova et al. 2009) Czech Republic	IV interventional evidence Low quality (2/6)	Case series	N=10 index cases with a RET Y791F mutation: 3 with apparently sporadic MTC 3 with FMTC/MEN2A/MEN2B 1 with PCC 3 with HSCR N=21 RET M+ family members	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 Total thyroidectomy	Inclusion Families with germline Y791F mutations Exclusion Not stated	RET mutation status Incidence of MMC, CCH, MTC and lymph node metastases Incidence of PCC Incidence of HPT Incidence of	Up to 15 years

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
			18 patients (12 relatives from 4 families and 6 index cases) had total thyroidectomy			residual/recurrent disease Postoperative calcitonin levels Age at time of thyroidectomy Treatment decisions Mortality	
(Vestergaard et al. 2007) Denmark	IV interventional evidence Moderate Quality (4/6)	Case series	N=27 first-degree family members of index case with a RET Y791F mutation 12 had the RET Y791F mutation	RET mutation testing by direct DNA sequencing of exon 13 No thyroidectomy	Inclusion First-degree family members of an index case with FMTC and a RET Y791F mutation Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT Incidence of abnormal calcitonin tests	Not stated
(Wells Jr & Skinner 1998) USA	IV interventional evidence High quality (5/6)	Case series	N=58 first-degree relatives, aged 21 years or younger, from 7 MEN2A kindreds with no clinical symptoms 21 were RET M+ 18 underwent a thyroidectomy 8 had elevated pentagastrin-stimulated calcitonin levels	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 10 and 11 Prophylactic thyroidectomy with bilateral cervicocentral lymphadenectomy	Inclusion First-degree family members from 7 kindreds at risk of MEN2A Exclusion Not stated	RET mutation status Incidence of CCH, MTC and lymph node metastases Postoperative calcitonin levels Safety of thyroidectomy	Range = 0–3 years
(Wu et al. 1998) Taiwan	IV interventional evidence Moderate quality (3/6)	Case series	N=28 first- and second-degree relatives from 2 unrelated MEN2A families: 15/17 members of family 1: 10 were RET M+ 13/15 members of family 2: 3 were RET M+	RET mutation testing by direct DNA sequencing of exons 10 and 11	Inclusion Members of 2 unrelated Taiwanese MEN2A kindreds Exclusion Not stated	RET mutation status Incidence of MTC Incidence of PCC Incidence of HPT	Not stated

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Yoshida et al. 2009) Japan	IV interventional evidence Moderate quality (3/6)	Case series	N=12 adults who underwent total thyroidectomy for MTC and had MEN2 5 were symptomatic All had raised pentagastrin-stimulated calcitonin levels	RET mutation testing (method not stated) Total thyroidectomy, the parathyroid gland was also removed and autotransplanted (unclear whether treatment decisions influenced by RET mutation)	Inclusion MEN2A patients aged over 25 years who underwent surgery for MTC between 1994 and 2006 Exclusion Not stated	RET mutation status Incidence of PCC Incidence of HPT Incidence of residual/recurrent disease Safety of thyroidectomy Mortality	1–14 years

CCH = C-cell hyperplasia; HPT = hyperparathyroidism; MCC = medullary microcarcinoma; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive; RET M– = RET-mutation-negative

Table 99 Study profiles of studies showing diagnostic yield

Study and location	Level of evidence	Study design	Study population	Intervention
(Abdelhakim et al. 2009) Morocco	IV diagnostic evidence	Diagnostic yield	N=9 index cases with MTC: 2 MEN2A, 1 hereditary MTC, 6 sporadic MTC N=13 family members (degree not stated) of 3 RET M+ index cases	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16
(Algun et al. 2002) Turkey	IV diagnostic evidence	Diagnostic yield	N=88 relatives (degree not stated) from 4 generations of an extended MEN2A family with a RET C634G mutation	RET mutation testing by restriction site polymorphism analysis of exon 11
(Alvandi et al. 2011) Iran	IV diagnostic evidence	Diagnostic yield	N=49 unrelated index patients diagnosed with MTC and classified as apparently sporadic	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16, and restriction site polymorphism analysis to detect C634R mutation
(Alvares Da Silva et al. 2003) Brazil	IV diagnostic evidence	Diagnostic yield	N=229 extended family members from 6 generations of an MTC index patient with a RET G533C mutation	RET mutation testing by direct DNA sequencing of exon 8

Study and location	Level of evidence	Study design	Study population	Intervention
(Amar et al. 2005) France	IV diagnostic evidence	Diagnostic yield	N=258 patients with apparently sporadic phaeochromocytoma or paraganglioma N=56 patients with a family history	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–6 All the coding exons of SDHB, SDHD, SDHC, and VHL were also sequenced
(Ameur et al. 2009) France	IV diagnostic evidence	Diagnostic yield	N=46 tissue samples collected from MTC, CCH or mixed MTC patients	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 from normal and diseased tissue samples to determine germline and somatic RET status
(Bar et al. 1997) Israel	IV diagnostic evidence	Diagnostic yield	N=27 patients diagnosed with sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10, 11 and 16 (also checked for mutations in VHL)
(Beldjord et al. 1995) France	IV diagnostic evidence	Diagnostic yield	N=28 patients diagnosed clinically with sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exons 10, 11 and 16
(Bergant et al. 2006) Slovenia	IV diagnostic evidence	Diagnostic yield	N=69 sporadic MTC patients N=31 relatives (degree not stated) of RET M+ sporadic MTC patients	RET mutation testing by single-strand conformational analysis and confirmatory direct DNA sequencing of exons 10, 11 and 13–16, or restriction site polymorphism analysis of exon affected in index case
(Bihan et al. 2012) France	IV diagnostic evidence	Diagnostic yield	N=22 extended family members of an MTC patient confirmed to have a RET L790F mutation on exon 13	RET mutation testing by direct DNA sequencing of exon 13
(Blaugrund et al. 1994) USA	IV diagnostic evidence	Diagnostic yield	N=22 specimens from patients with MTC: 15 apparently sporadic MTC; 3 MEN2A; 1 MEN2B; 3 FMTC	RET mutation testing by DNA sequencing of cloned exons 10, 11 and 16, and Southern blot analysis for genomic rearrangements
(Boer et al. 2003) Hungary	IV diagnostic evidence	Diagnostic yield	N=65 consecutive patients from 1992–2000 with MTC and no signs of MEN2B who were undergoing genetic screening	RET mutation testing by direct DNA sequencing (exons not specified)
(Bugalho et al. 2007) Portugal	IV diagnostic evidence	Diagnostic yield	N=77 apparently sporadic cases of MTC N=65 relatives (degree not stated) of 8 probands of established FMTC/MEN2 kindreds with a RET mutation 53 were asymptomatic	RET mutation testing by direct DNA sequencing of exons 10–16 or restriction site polymorphism analysis of exons 13–16 Exon 8 was screened for gross insertions/deletions (method not stated)
(Bugalho et al. 1997) Portugal	IV diagnostic evidence	Diagnostic yield	N=13 sporadic MTC No family history of MTC, PCC or parathyroid disease	RET mutation testing by direct DNA sequencing of exons 10, 11, 13, 15 and 16, confirmed using restriction site polymorphism analysis where appropriate

Study and location	Level of evidence	Study design	Study population	Intervention
(Calva et al. 2009) USA	IV diagnostic evidence	Diagnostic yield	N=31 first- and second-degree family members from a MEN2A kindred with a RET C609Y mutation	RET mutation testing (method not stated)
(Caron et al. 1996) France	IV diagnostic evidence	Diagnostic yield	N=14 extended family members of a confirmed MEN2A patient with a RET C618R mutation 4 were symptomatic	RET mutation testing by direct DNA sequencing of exons 10 and 11
(Cascon et al. 2009) Spain	IV diagnostic evidence	Diagnostic yield	N=237 consecutively enrolled patients identified in Spanish hospital with functioning or non-functioning PCCs and/or paragangliomas 35 had personal or familial history of MEN2 10 had personal or familial history of VHL 24 had other familial syndrome	Complete genetic characterisation of RET, SDHB, SDHC, SDHD and VHL (method not stated)
(Chang et al. 2009) Taiwan	IV diagnostic evidence	Diagnostic yield	N=69 members from 8 unrelated MTC families: 8 probands and 61 relatives (degree not stated) N=7 sporadic MTC patients	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 For sporadic cases RET mutation testing by direct DNA sequencing was extended to includes all exons 1–20
(Chi et al. 1994) Japan	IV diagnostic evidence	Diagnostic yield	N=74 relatives (degree not stated) from an extended MEN2A pedigree with a RET C634R mutation: 43 clinically affected; 31 considered at risk	RET mutation testing by restriction site polymorphism analysis of exon 11
(Chiefari et al. 2001) Italy	IV diagnostic evidence	Diagnostic yield	N=20 first- and second-degree relatives of proband with RET C634F mutation 6 affected with MTC	RET mutation testing by restriction site polymorphism analysis of exon 11, confirmed by direct DNA sequencing
(Chiefari et al. 1998) Italy	IV diagnostic evidence	Diagnostic yield	N=10 with sporadic MTC N=37 members (degree not stated) of 10 separate families with hereditary MTC: 6 MEN2A; 2 MEN2B; 1 FMTC; 1 with <4 MTC cases	RET mutation testing by restriction analysis of exons 11, 13, 15 and 16, and DNA sequencing of exons 10 and 14
(Chung et al. 2004) Korea	IV diagnostic evidence	Diagnostic yield	N=33 MTC patients who underwent a thyroidectomy (diagnosed clinically and by histopathology)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (postoperative)
(De Krijger et al. 2006) Nederlands	IV diagnostic evidence	Diagnostic yield	N=10 PCC tissue samples	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exons 10, 11 and 16 (also checked for mutations in SDHB, SDHD and VHL)

Study and location	Level of evidence	Study design	Study population	Intervention
(Decker et al. 1995) USA	IV diagnostic evidence	Diagnostic yield	N=103 consecutive patients at risk for MEN2A/FMTC 93 relatives (degree not stated) from 10 MEN2A or FMTC kindreds 21 patients diagnosed with apparently sporadic MTC	RET mutation testing by denaturing gradient gel electrophoresis mutational analysis of exons 10 and 11, with confirmatory direct DNA sequencing
(Donis-Keller 1995) USA	IV diagnostic evidence	Diagnostic yield	N=9 sporadic MTC patients N=8 sporadic PCC patients N=132 relatives (degree not stated) from 7 MEN2A families	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing in exons 1–20 for index cases Restriction site polymorphism analysis or direct DNA sequencing for family members
(Dos Santos et al. 2007) Brazil	IV diagnostic evidence	Diagnostic yield	N=57 at-risk family members (degree not stated) from 7 index cases: 3 MEN2A; 1 MEN2B; 3 FMTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 in index patients, and restricted to specific exon for family members
(Dourisboure et al. 2005) Argentina	IV diagnostic evidence	Diagnostic yield	N=22 members from a MEN2B family with a RET C630R mutation: 21 first-degree relatives 1 second-degree relative (grandchild with deceased parent)	RET mutation testing by direct DNA sequencing of exon 11
(Eisenhofer et al. 2011) Germany and USA	IV diagnostic evidence	Diagnostic yield	N=173 hereditary PCC and paraganglioma patients: 22 from Europe; 151 from USA	Genetic characterisation of RET, VHL, SDHB, SDHC, and SDHD (method not stated)
(Elisei et al. 2007) Italy	IV diagnostic evidence	Diagnostic yield	N=37 patients with familial MTC N=481 apparently sporadic MTC patients submitted for RET screening between 1993 and 2006 N=274 first-degree relatives of patients with confirmed RET mutations	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16
(Eng, Crossey, et al. 1995) UK and USA	IV diagnostic evidence	Diagnostic yield	N=48 patients with apparently sporadic PCC	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 9, 10, 11 and 13–16 (also checked for mutations in VHL)
(Eng, Mulligan, et al. 1995) UK	IV diagnostic evidence	Diagnostic yield	N=67 apparently sporadic MTC patients No history of first- or second-degree family MTC or PCC, no multiple tumours, and MTC confirmed histopathologically	RET mutation testing by direct DNA sequencing of exons 10, 11, 13 and 16

Study and location	Level of evidence	Study design	Study population	Intervention
(Erdogan et al. 2005) Turkey	IV diagnostic evidence	Diagnostic yield	N=56 apparently sporadic MTC patients Histopathologically and clinically confirmed, with a negative family history of MTC, PCC or HPT	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16
(Erlic et al. 2010) USA, Spain, Germany, Poland, Finland	IV diagnostic evidence	Diagnostic yield	N=1,475 patients identified on the European-American Phaeochromocytoma-Paraganglioma Registry	Genetic characterisation of RET exons 10, 11 and 13–16 (method not stated), (also checked for mutations in SDHB, SDHC, SDHD and VHL)
(Etit et al. 2008) USA	IV diagnostic evidence	Diagnostic yield	N=32 patients retrospectively identified from hospital records who had undergone a prophylactic thyroidectomy for MTC 30 with family history: 24 MEN2A; 8 non-MEN	RET mutation testing by direct DNA sequencing analysis of exons 10, 11 and 13 –16
(Fernandez et al. 2006) Spain	IV diagnostic evidence	Diagnostic yield	N=27 clinically diagnosed patients with hereditary MTC: 16 MEN2A; 3 MEN2B; 8 FMTC N=73 sporadic MTC N=14 clinically diagnosed patients who presented with PCC (N=12) and/or HPT (N=4) N = 238 family members	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16
(Fink et al. 1996) Austria	IV diagnostic evidence	Diagnostic yield	N=33 patients clinically diagnosed with FMTC, MEN2A, MEN2B or suspected of inheritable MTC from 16 families: 27 had MTC; 6 had CCH N=52 asymptomatic relatives from the 13 families N=59 sporadic MTC patients	RET mutation testing by single-strand conformational analysis or restriction site polymorphism analysis, and confirmatory direct DNA sequencing of exons 10, 11, 13 and 16; if no mutation detected, direct DNA sequencing of exon 14 was conducted
(Fitze et al. 2002) Germany	IV diagnostic evidence	Diagnostic yield	N=45 patients clinically identified with sporadic MTC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16
(Frank-Raue et al. 1996) Germany	IV diagnostic evidence	Diagnostic yield	N=159 at-risk family members (degree not stated) from 35 families with hereditary MTC: 111 MEN2A; 31 FMTC; 17 MEN2B	RET mutation testing by single-strand conformation polymorphism analysis, and then either direct DNA sequencing or restriction site polymorphism analysis of exons 10 or 11 Direct DNA sequencing of exons 13 or 16
(Frilling et al. 1995) Germany	IV diagnostic evidence	Diagnostic yield	N=56 clinically unaffected first-degree relatives (at 50% risk) from 21 hereditary MTC families: 15 MEN2A; 2 MEN2B; 4 FMTC	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11, and restriction site polymorphism analysis of exon 16

Study and location	Level of evidence	Study design	Study population	Intervention
(Frohnauer et al. 2000) USA	IV diagnostic evidence	Diagnostic yield	N=38 members (degree not stated) from 5 MEN2A kindreds with a RET codon 804 mutation	RET mutation testing by denaturing gradient gel electrophoresis analysis confirmed by direct DNA sequencing of exon 14
(Fugazzola et al. 2002) Italy	IV diagnostic evidence	Diagnostic yield	N=44 members (degree not stated) of a large FMTC pedigree with a RET A891S mutation	RET mutation testing by direct DNA sequencing of exon 15
(Gagel et al. 1995) USA	IV diagnostic evidence	Diagnostic yield	N=178 members (degree not stated) from 28 families with MEN2A: 54 first-degree relatives (affected parent); 71 clinically affected with MTC, PCC or HPT; 53 clinically negative	RET mutation testing by direct DNA sequencing of exons 10, 11 or 16
(Gil et al. 2002) Spain	IV diagnostic evidence	Diagnostic yield	N=23 members of 4 independent MEN2A families (degree not stated): 13 clinically affected (9 MTC only, 4 MTC + PCC); 10 unaffected	RET mutation testing by single-strand conformation polymorphism analysis and restriction site polymorphism analysis, with confirmatory direct DNA sequencing of exons 10 and 11
(Gonzalez et al. 2003) Mexico	IV diagnostic evidence	Diagnostic yield	N=9 proband: 3 MEN2B; 2 MEN2A; 4 sporadic MTC N=48 family members (degree not stated) of 6 RET M+ probands	RET mutation testing by single-strand conformational polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16, and direct DNA sequencing of exons 13–15
(Gosnell et al. 2006) Australia	IV diagnostic evidence	Diagnostic yield	N=48 at-risk family members (degree not stated) from a MEN2A kindred.	RET mutation testing (method not stated).
(Guerrero et al. 2006) Brazil	IV diagnostic evidence	Diagnostic yield	N=24 unrelated patients with apparently sporadic MTC	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10 and 11, and direct DNA sequencing of exons 13–16
(Halling et al. 1997) USA	IV diagnostic evidence	Diagnostic yield	N=72 family members (degree not stated) from 1 large FMTC kindred with a RET C609Y mutation	RET mutation testing by direct DNA sequencing of exon 10
(Hedayati et al. 2006) Iran	IV diagnostic evidence	Diagnostic yield	N=57 unrelated index cases with MTC: 1 MEN2A; 1 MEN2B; 2 FMTC; 53 apparently sporadic	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11
(Hernandez et al. 1997) Spain	IV diagnostic evidence	Diagnostic yield	N=36 asymptomatic members of 3 families with MEN2A	RET mutation testing by direct DNA sequencing and/or restriction site polymorphism analysis of exons 10 and 11
(Iacobone et al. 2011) Italy	IV diagnostic evidence	Diagnostic yield	N=59 with apparently sporadic PCC (without evident hereditary disease and/or syndromic appearance)	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16 (also checked for mutations in SDHB, SDHC, SDHD and VHL)

Study and location	Level of evidence	Study design	Study population	Intervention
(Januszewicz et al. 2000) Poland	IV diagnostic evidence	Diagnostic yield	N=77 unselected patients with PCC surgically treated (who responded to invitation; 85 did not respond)	RET mutation testing by single-strand conformation polymorphism analysis confirmed by direct DNA sequencing of exons 10, 11 and 13–16
(Jindrichova et al. 2004) Czech Republic	IV diagnostic evidence	Diagnostic yield	N=106 unrelated index cases with MTC: 10 MEN2A; 4 MEN2B; 10 FMTC; 82 sporadic MTC N=77 relatives(degree not stated) of 23 RET M+ index cases with MTC	RET mutation testing by direct DNA sequencing of exons 10, 11, 13, 14 and/or 16
(Jung et al. 2010) Korea	IV diagnostic evidence	Diagnostic yield	N=28 first- and second-degree members (excluding the proband) of a 3-generation FMTC family with a RET C618S mutation	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exon 10
(Karga et al. 1998) Greece	IV diagnostic evidence	Diagnostic yield	N=58 members (degree not stated) of 12 unrelated Greek families 9 MEN2A families; 1 FMTC families; 3 likely FMTC families 25 asymptomatic first-degree relatives of patients 33 clinically affected family members	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 and/or 16
(Kimura et al. 1995) Japan	IV diagnostic evidence	Diagnostic yield	N=25 specimens from patients: 1 with FMTC; 2 with MEN2A; 4 with MEN2B; 3 with neurofibromatosis type 1; 3 with apparently sporadic MTCs; 12 with sporadic PCCs	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11 (NB test not appropriate to detect mutations for phenotype MEN2B)
(Kinlaw et al. 2005) USA	IV diagnostic evidence	Diagnostic yield	N=29 first- and second-degree relatives in a family with MEN2A due to RET C609S mutation 6 with manifestations of MEN2A	RET mutation testing by restriction site polymorphism analysis of exon 10
(Kitamura et al. 1997) Japan	IV diagnostic evidence	Diagnostic yield	N=33 unrelated MTC patients: 27 clinically sporadic; 6 classified as inherited	RET mutation testing by single-strand conformation polymorphism analysis of exons 10, 11, 13, 14 and 16, followed by direct DNA sequencing of exons 10, 11, 13 and 14, and restriction site polymorphism analysis to detect codon 918 mutation
(Klein et al. 2001) Hungary	IV diagnostic evidence	Diagnostic yield	N=108 individuals: 65 unrelated index cases with MTC; 43 relatives (degree not stated) of RET M+ index cases	RET mutation testing by restriction site polymorphism analysis of exon 11 and/or direct DNA sequencing of exons 10, 13 and/or 14

Study and location	Level of evidence	Study design	Study population	Intervention
(Komminoth et al. 1995) Switzerland	IV diagnostic evidence	Diagnostic yield	N=46 specimens from patients with MTC: 22 suspected of being MEN2 or FMTC; 24 apparently sporadic MTC or PCCs N=38 members (degree not stated) from 3 MEN2A families, 2 MEN2B families, and 4 suspected MEN2 families	RET mutation testing by single-strand conformation polymorphism and heteroduplex gel electrophoresis analysis of exons 10, 11, 13 and/or 15
(Krawczyk et al. 2010) Poland	IV diagnostic evidence	Diagnostic yield	N=60 patients with diagnosis of apparently sporadic PCC or paraganglioma 53 had PCC; 8 had paraganglioma; 1 had both a PCC and a paraganglioma 41 were benign tumours; and 11 had malignant lesions	RET mutation testing by direct DNA sequencing of exons 10, 11, 14 and 16 (also checked for mutations in SDHB, SDHD and VHL)
(Learoyd et al. 2005) Australia and New Zealand	IV diagnostic evidence	Diagnostic yield	N=54 family members (degree not stated) of 2 probands: 47 family members from 4 generations, proband had RET V804L mutation 7 family members from 3 generations, proband had RET V804M mutation	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exons 10, 11 and 16 between 1993 and 1998; then exons 13–15 were included
(Lecube et al. 2002) Spain	IV diagnostic evidence	Diagnostic yield	N=52 family members (degree not stated) of an FMTC family with a RET V804M mutation	RET mutation testing by restriction site polymorphism analysis of exon 14
(Lendvai et al. 2012) Hungary	IV diagnostic evidence	Diagnostic yield	N=95 patients: 47 consecutive patients with apparently sporadic MTCs 48 consecutive patients with apparently sporadic PCC	RET mutation testing by direct DNA sequencing of exons 10, 11 and 14
(Lindor et al. 1995) USA	IV diagnostic evidence	Diagnostic yield	N=29 patients who had undergone an operation for a sporadic PCC	RET mutation testing by direct DNA sequencing of exons 10 and 11, and mutation specific PCR for exon 16
(Mannelli et al. 2009) Italy	IV diagnostic evidence	Diagnostic yield	N=501 consecutively enrolled patients presenting with PCC or paragangliomas (new or previously identified)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16, and multiplex ligation-dependent probe amplification assay to detect genomic rearrangements (also checked for mutations in SDHB, SDHC, SDHD and VHL)
(Marsh et al. 1996) Australia and New Zealand	IV diagnostic evidence	Diagnostic yield	N=39 members (degree not stated) of 16 MEN2A and FMTC families at risk of being a gene carrier	RET mutation testing by restriction site polymorphism analysis of exons 10 and 11

Study and location	Level of evidence	Study design	Study population	Intervention
(Mastroianno et al. 2011) Italy	IV diagnostic evidence	Diagnostic yield	N=21 first- and second-degree relatives of a proband with MEN1 (MEN1 IVs4+IG>T mutation) and MEN2 (RET K666M mutation on exon 11)	RET mutation testing by of exons 8, 10, 11, 13–16 and 18 (method not stated)
(McMahon et al. 1994) UK	IV diagnostic evidence	Diagnostic yield	N=63 affected or unaffected first- degree relatives from 9 MEN2A families with mutations in RET codon 634: 29 affected; 30 unaffected; 4 not tested but categorised as non-carriers when parents tested RET M–	RET mutation testing by restriction site polymorphism analysis of codon 634, and confirmatory direct DNA sequencing of exon 11
(Morita et al. 1996) Japan	IV diagnostic evidence	Diagnostic yield	N=20 members of one MEN2A family: 6 children of the proband; 10 grandchildren of the proband; 3 great-grandchildren of the proband	RET mutation testing by PCR amplification and restriction enzyme analysis of exon 10 C618S
(Neocleous et al. 2011) Cyprus	IV diagnostic evidence	Diagnostic yield	N=29 family members from 7 FMTC families and 1 MEN2A family	RET mutation testing by direct DNA sequencing of exon 10
(Neumann et al. 2002) Germany and Poland	IV diagnostic evidence	Diagnostic yield	N=271 patients with non-syndromic PCC and/or paragangliomas without family history of disease: 241 had PCCs only; 22 had paragangliomas only; 8 had both a PCC and a paraganglioma	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 13–16 (also checked for mutations in SDHB, SDHD and VHL)
(Neumann et al. 1995) Germany	IV diagnostic evidence	Diagnostic yield	N=10 families with MEN2A or MEN2B N=27 family members (degree not stated) from 7 MEN2A families and 1 MEN2B family who had had negative clinical screening (N=19) or unknown phenotype (N=8)	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16; if no RET mutation identified, screened for VHL mutation
(Oriola et al. 1996) Spain	IV diagnostic evidence	Diagnostic yield	N=59 family members (degree not stated) from 7 MEN2A families: 20 symptomatic; 39 'at risk'	RET mutation testing by direct DNA sequencing of RET exons 10 and 11, and restriction site polymorphism analysis of exons 10 and exon 11
(Pacini et al. 1995) Italy	IV diagnostic evidence	Diagnostic yield	N=58 family members (degree not stated) from 9 MEN2 families: 16 affected; 42 at risk of disease	RET mutation testing by restriction site polymorphism analysis of exons 10, 11 or 16
(Pasini et al. 2002) Italy	IV diagnostic evidence	Diagnostic yield	N=26 family members (degree not stated) of a patient with Hirschsprung's disease and MEN2 (RET C618R mutation).	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10, 11, 13, 14 and 16, and restriction site polymorphism analysis of exon 15, with confirmatory direct DNA sequencing of exon 10 in the proband

Study and location	Level of evidence	Study design	Study population	Intervention
				Restriction site polymorphism analysis and direct DNA sequencing of exon 10 was used to identify RET mutation in family members
(Patocs et al. 2004) Hungary	IV diagnostic evidence	Diagnostic yield	N=41 patients with PCCs	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11 and 14 (also checked for mutations in VHL)
(Pinna et al. 2007) Italy	IV diagnostic evidence	Diagnostic yield	N=22 patients with MTC N=43 first-degree relatives of 7 index cases with RET	RET mutation testing by direct DNA sequencing of exons 8–16 in index case and appropriate exon in family members
(Prazeres et al. 2006) Portugal	IV diagnostic evidence	Diagnostic yield	N=53 patients with apparently sporadic MTC	RET mutation testing by direct DNA sequencing of exons 8, 10, 11 and 13–16, plus restriction site polymorphism analysis when possible
(Punales et al. 2003) Brazil	IV diagnostic evidence	Diagnostic yield	N=160 individuals 133 family members plus 17 index cases from 17 MEN2 families 113 family members were from families with MEN2A 54 (37 + 17 index cases) had clinical signs of disease 10 patients with apparently sporadic MTCs	RET mutation testing by single-strand conformation polymorphism analysis, restriction enzyme analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 15
(Radien et al. 1997) France	IV diagnostic evidence	Diagnostic yield	N=120 patients with apparently sporadic PCC	RET mutation testing by denaturing gradient gel electrophoresis analysis of exons 10, 11, 13 and 16
(Romei et al. 2011) Italy	IV diagnostic evidence	Diagnostic yield	N=729 patients with apparently sporadic MTC (no familial history of MTC or other endocrine disease) N=146 relatives (degree not stated) of 47 RET M+ index cases who had MTCs without family history of endocrine disorders	RET mutation testing method changed over 15 years Initially used DNA sequencing of exons 10, 11 and 16; later added exons 13–15; and recently added exons 5 and 8 RET mutation testing of relatives by direct DNA sequencing of exon affected in index case
(Sanso et al. 2002) Argentina	IV diagnostic evidence	Diagnostic yield	N=98 relatives from 17 MEN2A index patients N=13 relatives from 5 MEN2B index patients	RET mutation testing by direct DNA sequencing of exons 10, 11 and 16, and confirmed by restriction site polymorphism analysis
(Shan et al. 1998) Japan – China	IV diagnostic evidence	Diagnostic yield	N=40 patients with apparently sporadic MTCs	RET mutation testing by restriction site polymorphism analysis of codon 918 mutations
(Sharma & Saranath 2011) India	IV diagnostic evidence	Diagnostic yield	N=51 MTC patients N=25 first-degree relatives from 7 index cases with family history and a RET mutation	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 for index cases, and specific exons for family members

Study and location	Level of evidence	Study design	Study population	Intervention
(Shifrin et al. 2009) USA	IV diagnostic evidence	Diagnostic yield	N=107 family members from family with V804M mutation	RET mutation testing by direct DNA sequencing of exons 10, 11 and 13–16 (not clear if relatives tested for specific mutation or all)
(Shimotake et al. 1996) Japan	IV diagnostic evidence	Diagnostic yield	N=37 first-degree relatives in a MEN2 family with a RET C634R mutation 6 without clinical signs	RET mutation testing by restriction site polymorphism analysis and direct DNA sequencing of exon 11
(Shirahama et al. 1998) Japan	IV diagnostic evidence	Diagnostic yield	N=71 patients with MTC: 44 from MEN2 or FMTC families; 22 apparently sporadic MTCs; 5 patients without familial information	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10 and 11; if no mutations found, then restriction site polymorphism analysis of codons 768 and 918
(Siggelkow et al. 2001) Germany	IV diagnostic evidence	Diagnostic yield	N=34 first- and second-degree relatives of an index case with FMTC and a RET C611F mutation: 8/14 at risk members of the 3rd generation 17/17 at risk members of the 4th generation 9/15 at risk members of the 5th generation	RET mutation testing by direct DNA sequencing of exon 10 to identify mutation in proband, and restriction site polymorphism analysis of exon 10 in family members
(Tsai et al. 1994) USA	IV diagnostic evidence	Diagnostic yield	N=109 members (degree not stated) of 13 kindreds: 9 MEN2A; 2 MEN2B; 2 FMTC 47 clinically affected, 62 non-affected	RET mutation testing by direct DNA sequencing of exons 10 and 11
(Uchino et al. 1999) Japan	IV diagnostic evidence	Diagnostic yield	N=27 members (degree not stated) from 5 MEN2A families whose clinical status was unknown	RET mutation testing by single-strand conformation polymorphism analysis and direct DNA sequencing of exons 10, 11, 13, 14 or 16
(Uchino et al. 1998) Japan	IV diagnostic evidence	Diagnostic yield	N=40 patients of apparently sporadic MTCs who had surgery between 1965 and 1996	RET mutation testing by direct DNA sequencing of exons 10, 11, 13, 14 and 16, and confirmed with restriction site polymorphism analysis
(Vestergaard et al. 2007) Denmark	IV diagnostic evidence	Diagnostic yield	N=27 first-degree family members (children of RET M+ patients) from a large kindred with a RET Y791F mutation	RET mutation testing by direct DNA sequencing of exon 13
(Wells Jr & Skinner 1998) USA	IV diagnostic evidence	Diagnostic yield	N=58 first-degree family members from 7 kindreds with MEN2A, showing no clinical signs/symptoms	RET mutation testing by restriction site polymorphism analysis or direct DNA sequencing of exons 10 and 11
(Woodward et al. 1997) UK	IV diagnostic evidence	Diagnostic yield	N=16 kindreds with familial PCC	RET mutation testing of exons 10 and 11 (method not stated) (also checked for mutations in GDNF and VHL)

Study and location	Level of evidence	Study design	Study population	Intervention
(Wu et al. 1998) Taiwan	IV diagnostic evidence	Diagnostic yield	N=26 first- and second-degree relatives of 2 probands from 2 unrelated MEN2A families	RET mutation testing by direct DNA sequencing of exons 10 and 11

CCH = C-cell hyperplasia; HPT = hyperparathyroidism; MTC = medullary thyroid cancer; PCC = phaeochromocytoma; RET M+ = RET-mutation-positive

Table 100 Study profiles of studies showing change in management

Study and location	Level of evidence and quality assessment	Study design	Study population	Intervention	Comparator	Inclusion/exclusion criteria	Outcomes assessed	Duration of follow-up
(Spinelli et al. 2010) Italy	III-2 interventional evidence High risk of bias (16/26)	Cohort study	N=13 patients (8–17 years of age) with MEN2 who underwent surgery for MTC: 7 (54%) MEN2A 4 (31%) FMTC 2 (15%) MEN2B	Prophylactic thyroidectomy performed on clinically asymptomatic patients, basal calcitonin <100 pg/mL with pentagastrin-stimulated calcitonin test lower than 250 pg/mL	Total thyroidectomy based on clinical signs (thyroid nodulation, altered basal calcitonin >100 pg/mL, pentagastrin-stimulated calcitonin test >250 pg/mL, characteristic phenotype (in MEN2B))	Inclusion Patients with MEN2 who underwent surgery for MTC, aged ≤17 years	Incidence and severity of MTC Safety of thyroidectomy	Mean = 8.6 years (range 1.5–15 years)

MTC = medullary thyroid cancer

Appendix M Excluded studies

Data could not be extracted:

Eisenhofer, G, Lenders, JWM et al. 1999, 'Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel-Lindau disease and multiple endocrine neoplasia type 2', *New England Journal of Medicine,* vol. 340, no. 24, pp. 1872–1879.

Heshmati, HM, Gharib, H et al. 1997, 'Genetic testing in medullary thyroid carcinoma syndromes: mutation types and clinical significance', *Mayo Clinic Proceedings,* vol. 72, no. 5, pp. 430–436.

International *RET* mutation consortium 2006, 'The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2', *JAMA: Journal of the American Medical Association,* vol. 276, no. 19, pp. 1575–1579.

Kaldrymides, P, Mytakidis, N et al. 2006, 'A rare RET gene exon 8 mutation is found in two Greek kindreds with familial medullary thyroid carcinoma: implications for screening', *Clinical Endocrinology,* vol. 64, no. 5, pp. 561–566.

Lips, CJ, Landsvater, RM et al. 1995, 'From medical history and biochemical tests to presymptomatic treatment in a large MEN2A family', *Journal of Internal Medicine,* vol. 238, no. 4, pp. 347–356.

Machens, A & Dralle, H 2008, 'Familial prevalence and age of RET germline mutations: implications for screening', *Clinical Endocrinology,* vol. 69, no. 1, pp. 81–87.

Moore, SW & Zaahl, MG 2010, 'Chasing the ubiquitous RET proto-oncogene in South African MEN2 families: implications for the surgeon', *South African Journal of Surgery,* vol. 48, no. 4, pp. 127–131.

Rodriguez, JM, Balsalobre, M et al. 2008, 'Pheochromocytoma in MEN2A syndrome: study of 54 patients', *World Journal of Surgery,* vol. 32, no. 11, pp. 2520–2526.

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