Genetic test for fragile X syndrome

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Assessment report

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Hard copies of the report can be obtained from:

The Secretary
Medical Services Advisory Committee
Department of Health and Ageing
Mail Drop 61
GPO Box 9848
Canberra ACT 2601

Inquiries about the content of the report should be directed to the above address.

The Medical Services Advisory Committee is an independent committee which has been established to provide advice to the Commonwealth 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.

This report was prepared by the Medical Services Advisory Committee with the assistance of Ms Ornella Clavisi, Dr Renea Johnston, Mr Jason Wasiak, Ms Alexandra Raulli and Ms Emily Petherick (Monash Institute of Health Services Research) and Dr Bruce Hollingsworth (Health Economics Unit) from Monash University. The report was endorsed by the Commonwealth Minister for Health and Ageing on 20 August 2002.

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MSAC recommendations do not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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The procedure

There are two molecular genetic techniques used in the diagnosis of fragile X; polymerase chain reaction (PCR) (a form of nucleic acid amplification) and Southern blot. Fragile X testing is most accurate if a combination of both techniques are employed, depending on the exact fragile X genotype. The fragile X mutation involves an expansion of a section of DNA on a gene specific to fragile X mental retardation. The expanded section of DNA consists of repeated trinucleotides with the sequence cytosine-guanine-guanine (CGG). There are two principle recognised mutations for fragile X based on the number of CGG repeats: the full mutation, in which there are more than 200–230 repeats; and the premutation, which consists of between 55 and 230 repeats. PCR is most useful for accurate determination of CGG repeat numbers for normal, premutation and grey zone genotypes, while Southern blot analysis is best suited to detecting full mutations or large premutations. PCR amplifies deoxyribonucleic acid (DNA) samples containing the CGG repeats obtained from blood or mouthwash into millions of copies to calculate the number of repeats. Southern blot analysis involves isolating a portion of DNA from a blood sample and cutting it into fragments before 'blotting' them onto a charged surface to estimate the size of the repeats.

Medical Services Advisory Committee – role and approach

The Medical Services Advisory Committee (MSAC) is a key element of a measure taken by the Commonwealth Government to strengthen the role of evidence in health financing decisions in Australia. MSAC advises the Commonwealth Minister for Health and Ageing on the evidence relating to the safety, effectiveness and cost-effectiveness of new and existing medical technologies and procedures, and under what circumstances public funding should be supported.

A rigorous assessment of the available evidence is thus the basis of decision making when funding is sought under Medicare. A team from the Monash Institute of Health Services Research was engaged to conduct a systematic review of literature on genetic test for fragile X syndrome. A supporting committee with expertise in this area then evaluated the evidence and provided advice to MSAC.

MSAC's assessment of genetic test for fragile X syndrome

Clinical need

The prevalence of fragile X syndrome reported in the published literature varies markedly and exceeds the expected variation due to population differences. Reported prevalence of the full mutation ranges from 2.3/10,000 to 222/10,000, due in part to the selective sampling of individuals more likely to have the disorder. However, the prevalence reported by a community survey in Australia of 2.3 per 10,000 is more representative of the true population prevalence than the rates reported in other studies.

Although there are Australian data on the prevalence of full mutations, there is no corresponding data on the prevalence of premutations. The only available data are from overseas, with the published literature reporting premutation prevalence for males and females ranging from 18.9/10,000 to 233/10,000.

Safety

An extensive literature search did not identify any reports of adverse events associated with testing individuals suspected of having fragile X syndrome or cascade testing of relatives of affected individuals. Similarly, no reports of adverse events specific to prenatal diagnosis of fragile X were identified in the literature, however, potential adverse events associated with prenatal diagnosis due to the invasive nature of amniocentesis or chorionic villus sampling required to obtain foetal DNA are well documented. Minor medical events such as transient vaginal spotting or amniotic fluid leakage following amniocentesis have been reported in two to three per cent of women. The exact rate of foetal loss following amniocentesis is difficult to quantify due to the relatively high background rate of spontaneous abortion of three to four per cent in mid-trimester pregnancy; the excess rate of foetal loss is usually stated to be between 0.5 and one per cent above the background rate. The additional rate of spontaneous abortion associated with chorionic villus sampling is similarly difficult to quantify precisely but is believed to be comparable to amniocentesis.

Effectiveness

Item 1 Diagnostic characteristics

Two factors were considered in determining the effectiveness of genetic tests for fragile X syndrome: accuracy; and usefulness in improving outcomes for people undergoing the test. Accuracy is measured by diagnostic characteristics such as sensitivity and specificity. The ideal method for assessing the usefulness of the test in improving patient outcomes is a randomised controlled trial comparing outcomes of people undergoing the test to people not exposed to the test. No such trials were identified. Evidence of the accuracy of the tests from the published literature indicates that cytogenetic testing is not as accurate as molecular techniques in detecting the fragile X full mutation and cytogenetic testing is unable to accurately detect a premutation at all. Sensitivity of cytogenetic testing varied across studies, but specificity was consistently high with few false positive cytogenetic results reported. Thus, a positive cytogenetic test result is likely to rule in a diagnosis of fragile X, but a negative cytogenetic result is not indicative of the true fragile X status, particularly in prenatal testing, and thus testing with molecular techniques is required.

Item 2 Family cascade testing

Cascade testing for fragile X identifies individuals within families at high risk of having an affected child. This type of testing is aimed at providing reproductive choice, with a number of studies demonstrating that women at risk of having children with fragile X carefully consider their options when faced with the prospect of having an affected child.

Due to the complex nature of the disease and the emotional impact of having a positive diagnosis, fragile X testing is seldom administered without genetic counselling. A number of studies have shown that genetic counselling can help those affected by fragile X syndrome understand the nature of the disease and its heritability, in order to cope with the emotional burden and make informed reproductive decisions. It is desirable that genetic counselling and informed consent be included in the process of cascade testing.

One of the issues surrounding cascade testing is whether it is appropriate to test children at the request of their parents. There is no consensus on this issue in Australia or internationally, and no evidence to direct practice. In general, a cautious approach is advised, with postponement of testing until the child is old enough to give informed consent.

Cost-effectiveness

A cascade testing program is estimated to cost up to $4 million annually, and would result in a cost per initial case detected of between $14,000 and $28,000, depending on assumptions made, especially the detection rates in the population.

This does not account for the costs of anxiety surrounding testing programs, although there may be certain benefits associated with reassurance to be balanced against these costs. Nor does it take into account the social costs and consequences of providing the information to individuals such as the decision to abort a pregnancy or the additional costs to society of caring for a disabled person.

Costs may be greater downstream in terms of the costs of choices individuals make. These may include further diagnosis, abortion, or lifetime costs of having a child with fragile X.

Recommendation

MSAC recommended that on the strength of the evidence pertaining to Genetic Test for Fragile X Syndrome public funding should be supported for the use of:

Nucleic Acid Amplification (NAA) in those with specific clinical features of Fragile X (A) syndrome, including intellectual disabilities, and in first and second degree relatives of individuals with the Fragile X (A) mutation and Southern Blot where the results of NAA testing are inconclusive.

- The Minister for Health and Ageing accepted this recommendation on 20 August 2002 -

Introduction

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

MSAC's terms of reference and membership are at Appendix A. MSAC is a multidisciplinary expert body, comprising members drawn from such disciplines as diagnostic imaging, pathology, surgery, internal medicine and general practice, clinical epidemiology, health economics, consumer health and health administration.

This report summarises the assessment of current evidence relating to genetic tests for fragile X syndrome.

Background

Fragile X (A) syndrome

Fragile X (A) syndrome is the second most frequent cause of intellectual disability after Down syndrome (Kaufmann & Reiss 1999). Individuals affected by the disorder generally have a number of phenotypic features in addition to intellectual disability such as facial characteristics (eg large ears, prominent jawline, broad forehead and high arched palate), speech and language problems and behavioural abnormalities. Macro-orchidism in post-pubertal males is another feature (Pimentel 1999).

The disorder is caused by a mutation in the fragile site mental retardation 1 (FMR1) gene situated on the X chromosome at position Xq27.3. The mutation is characterised by expansion in the number of copies of a repeated sequence (cytosine-guanine-guanine; CGG). Expansion of the sequence can potentially result in silencing of the gene, resulting in failure of production of the FMR protein (FMRP). Although the precise function of FMRP is unclear, it is believed to be needed for neuron formation and synaptic connections since absence of the protein hinders the development of the neuronal network which is important for intelligence (Kallinen et al 2000).

The number of CGG repeats in the FMR1 gene varies in the normal population from five to 55 repeats. Copy numbers between 55 and 200-230 are described as premutations (PMs) and copy numbers exceeding 200–230 are described as full mutations (FMs). There is some uncertainty regarding the repeat size cut-offs between normal and PM, and between PM and FM forms of the gene. In addition to PM and FM, some individuals are mosaics, with some cells in their body showing PM and some showing FM when tested (Murray et al 1997; Pimentel 1999).

Genotype-phenotype correlation

The relationship between genotype and phenotype is complex, depending on the gender of the individual concerned and whether the mutation is PM or FM. In males, an FM or mosaic mutation will usually result in the characteristic features of fragile X syndrome, although some FM and mosaic males will be clinically normal (DeVries et al 1994; Murray et al 1997; Pembrey et al 2001). Females with an FM will have a varying phenotype, with approximately 50% having intellectual disability. Of the remaining 50% of FM women, up to 70% have low or borderline cognitive function, with neuropsychiatric problems present in most regardless of Intelligence Quotient (IQ) level. For both genders, phenotype is influenced by the level of FMRP produced. Individuals with a PM are usually normal with a frequency of intellectual disability no different to that in the general population (Hagerman & Cronister 1996). Women with a PM are at increased risk of premature ovarian failure (Murray et al 1997) and may have subtle emotional and neurocognitive deficiencies (Loesch et al 1993; Sobesky et al 1994).

Risk to offspring

Inheritance of PM, FM or mosaic phenotypes is dependent on whether the gene is inherited from a mother or father and the size of the CGG repeat. There is a tendency for further expansion of the CGG repeat sequence as the mutated gene is passed from parent to child. For this reason, the mutation that causes fragile X syndrome is known as a dynamic mutation (Richards & Sutherland 1992). The likelihood of further expansion

of the CGG repeat sequence is different for males and females. Each of the children of a mother with a PM or FM has a 50% chance of inheriting a mutated gene. When the mother has an FM, each of her sons and daughters will be at 50% risk of inheriting an FM. For mothers with a PM, the risk to offspring is dependent on the number of CGG repeats. As the number increases, the chance that a child will inherit an FM increases. Fathers with a premutation can only transmit their X chromosome to their daughters and there is no potential for expansion to a full mutation (Murray et al 1997).

The procedure

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a rapid and effective method of nucleic acid amplification (NAA) for diagnosing different fragile X genotypes by assessing the repeat size of the FMR1 gene. This technique uses a small quantity of DNA that can be obtained from many tissues, but is usually obtained from a sample of blood or mouthwash. The material need not be as highly purified as that required for a Southern Blot. This technique can amplify a specific DNA sequence into many millions of copies using a heat stable DNA polymerase in a cyclical reaction (Darnell et al 1990). The size, which indicates the number of repeats of the PCR product (amplified DNA) is then determined using radioactive or fluorescence techniques. The diagnosis of specific fragile X genotypes is dependent on the number of CGG repeats in the amplified allele and therefore the size of the PCR product. Those with a full mutation have in excess of ~200–230 CGG repeats and those with a premutation have between 55 and ~200–230 CGG repeats in the FMR1 region of the gene (Murray et al 1997; Pembrey et al 2001).

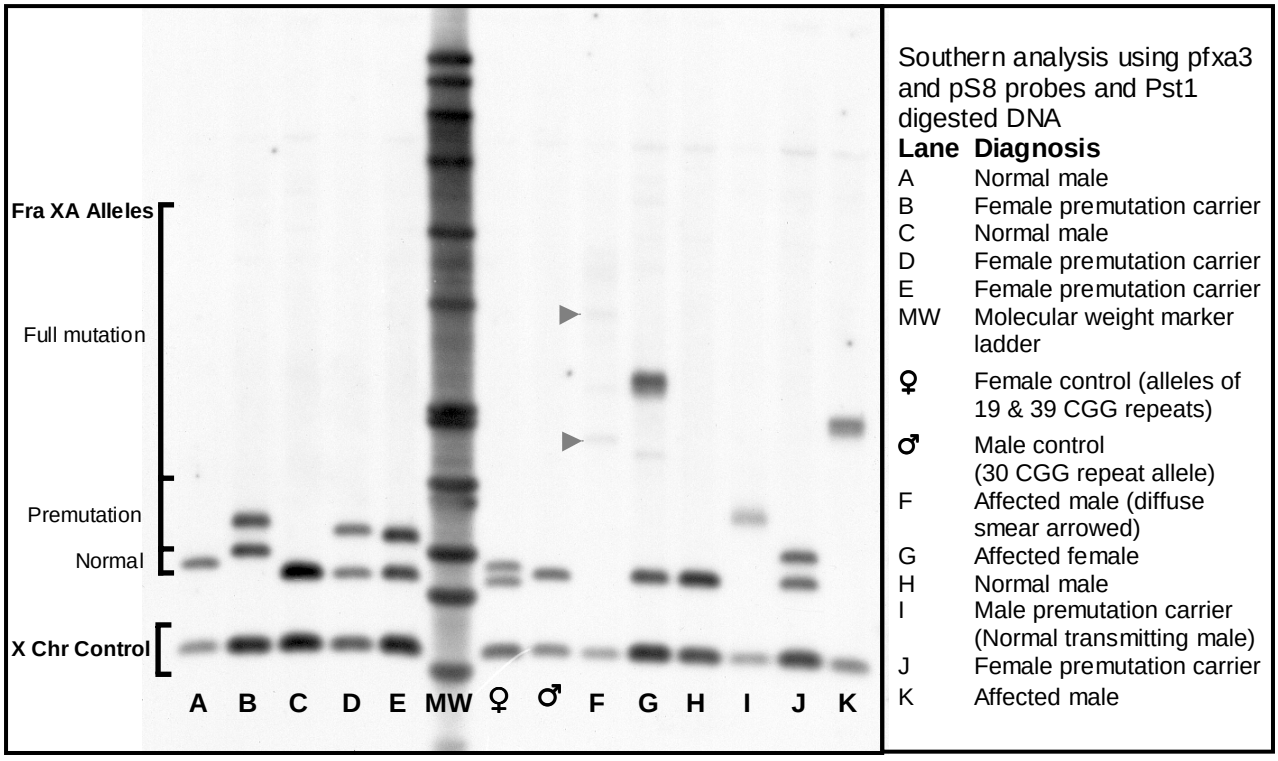
In the case of fragile X diagnosis, PCR is most suitable for detecting normal range and premutation alleles, however, its use in detecting full mutations is limited. There is also the potential for large premutations in females to be missed, especially if there is strong preferential amplification of the normal allele. Where there is a lack of amplification by PCR, or for those who do not have distinguishable alleles on PCR, Southern blot should be used (Murray et al 1997; Pembrey et al 2001).

Southern blot

Southern blot is the method of choice for the detection of full mutations and mosaicism. This method requires a high quality DNA sample derived from whole blood or chorionic villus sampling (CVS), which is digested using one or more restriction enzymes. The DNA is cut at specific sequences, generating fragments of different sizes, which are then separated by electrophoresis through an agarose gel. The distribution of the DNA fragments is preserved as they are denatured and transferred by blotting to a solid substrate with a charged surface (usually a charged nylon filter). The trinucleotide repeat size of the FMR1 fragments is then determined by the hybridisation of a radioactive probe. Diagnosis of the different fragile X genotypes, premutations, full mutations and mosaics is determined from the specific banding patterns seen after autoradiography of the nylon filter (Figure 1). Males and females who do not have a fragile X(A) mutation will show one or more lower molecular weight bands depending on the probe and enzyme(s) used, and on the presence of active and inactive X chromosomes. Premutations appear as a small increase in the molecular weight of the germline band. For full mutations, males show absence of the normal band and have a high molecular weight band or a diffuse smear. Females will also produce a high molecular weight band

or smear in addition to their normal allele. Mosaics will show a combination of premutation, full mutation and normal bands.

Figure 1 Example of fragile X(A) Southern blot results



Intended purpose

It is intended that PCR be used as a first line diagnostic test for individuals suspected of having fragile X syndrome and for cascade testing of first degree blood relatives of affected individuals. Where PCR fails to amplify or cannot identify distinguishable alleles, Southern blot may be used as a second line diagnostic test. Although PCR may be limited in identifying full mutations, the advantages are that it is a more rapid method than Southern blot (approximately two day turnaround time for PCR versus one week for Southern blot), it requires less stringent sample preparation, and it is cheaper (the cost of PCR is approximately $100 compared to $200 for Southern blot).

For individuals who are intellectually disabled, cytogenetic testing may be performed in conjunction with molecular tests for the detection of other abnormalities besides fragile X syndrome.

Clinical need / burden of disease

Burden of disease estimates for fragile X syndrome over the past decades have been quite varied as the definition of fragile X has changed with the introduction of new diagnostic tests. Initially, using cytogenetic testing, the definition of fragile X was based on the presence of a fragile site at a particular location on the X chromosome (Xq27.3) in males who were intellectually handicapped. However, the proportion of cells that expressed the site considered to indicate a positive result differed, as did the methods of cell culture. It was later discovered that this cytogenetic definition included FRAXE,

FRAXD and FRAXF (other nearby fragile sites unrelated to FRAXA). A prevalence of approximately 1/1000 was widely quoted and accepted before the advent of molecular tests (Morton et al 1997). This figure was suspected to be an overestimate. With the advent of Southern blotting and PCR tests the definition was refined as an intellectual disability associated with an absolute or relative deficiency of the protein FMR1 and currently the measurement of the size of the CGG trinucleotide repeats is the method used to diagnose fragile X (Pieretti et al 1991).

A population study by Webb et al (1986) of school children in the city of Coventry, England gave an overall prevalence of 1/952 (10.5/10,000) using cytogenetic tests. The follow-up study (Morton et al 1997) in which the original children were retested using molecular diagnostic techniques gave a revised overall prevalence of 1/4090 (2.4/10,000). A similar study by Turner et al (1996) testing children in Sydney, Australia using cytogenetic tests, gave a prevalence of 1/2610 (3.8/10,000). A follow-up survey (Turner et al 1996) where the children were re-examined using DNA molecular tests gave a revised prevalence of 1/4350 (2.3/10,000). These studies indicated that previous measures of prevalence using cytogenetic testing may have overestimated the true prevalence of fragile X.

As DNA molecular tests are able to measure the number of CGG trinucleotide repeats, distinguishing between types of affected individuals is possible. Affected individuals can either have a full mutation or a premutation. Table 1 lists the prevalence of fragile X (full mutation and premutation) as determined by DNA molecular tests in several countries.

Even though the advent of DNA molecular tests brought us closer to the true prevalence of fragile X, the prevalence reported in the published literature varies markedly. The variation exceeds the expected variation due to population differences with the prevalence (full mutation) ranging from 2.1/10,000 to 222/10,000. However, the papers reporting the highest prevalence, Jacobs et al (1993) and Murray et al (1996), selected their subjects from intellectually disabled populations where it would be expected that there would be a higher prevalence of fragile X. Therefore, their calculated prevalence cannot be considered to be representative of the general population prevalence. The prevalence that Turner et al (1996) reported is likely to be more representative of the true population prevalence in males as the authors carried out community surveys in the general population. The prevalence reported by Israeli papers (Pesso et al 2000; Toledano-Alhadef et al 2001), although using large sample sizes, only screened women with no family history of mental retardation which may mean that their reported prevalence could be underestimated.

Although there is Australian data on the prevalence of full mutations there is no corresponding data on the prevalence of premutations. The only available data is that from overseas with the published literature reporting premutation prevalence ranging from 18.9/10,000 to 233/10,000. Three studies (Jacobs et al 1993; Mazzocco et al 1998; Beresford et al 2000) examined the prevalence of premutations in males. The largest study, Mazzocco et al (1998), selected their subjects from developmental paediatric or speech and language clinics where it would be expected that there would be a higher prevalence of fragile X.

The three largest studies that examined the prevalence of premutations in females were a Canadian study by Rousseau et al (1995) testing female outpatients of a general hospital and two Israeli studies (Pesso et al 2000; Toledano-Alhadef et al 2001) testing preconceptional or pregnant women with no family history of mental retardation.

Although these sample populations are preferable to selectively testing affected individuals, the prevalence may be less precise than estimates of prevalence derived from community surveys.

Table 1 Prevalence of Fragile X as determined by DNA molecular tests

Country	Reference	Source of subjects	No. studied	No. affected	Males	Females
Prevalance of full mutation						
Australia	(Turner et al 1996)	Community survey	4350 (males)	1	2.3/10,000	-
Canada	(Beresford et al 2000)	Screening samples from newborns	470 (males) 378 (females)	0 (males) 0 (females)	0/10,000	0/10,000
Finland	(Ryynanen et al 1994)	Pregnant women accepting a carrier test	1447 (females)	0	-	0/10,000
Israel	(Pesso et al 2000)	Preconceptional or pregnant women with no family history of mental retardation	9459 (females)	4	-	4.2/10,000
Israel	(Toledano-Alhadef et al 2001)	Preconceptional or pregnant women with no family history of mental retardation	14,334 (females)	3	-	2.1/10,000
UK	Cited from (Turner et al 1996)	Community survey	4090 (males)	1	2.4/10,000	-
UK	(Jacobs et al 1993)	Children with an intellectual disability in mainstream schools	180 (males) 74 (females)	4 (males) 0 (females)	222/10,000	0/10,000
UK	(Murray A et al 1996)	Children with an intellectual disability in mainstream schools	1013 (males)	5	49.3/10,000	-
Prevalence of premutation						
Canada	(Rousseau et al 1995)	Outpatients from a general hospital	10,624 (females)	41	-	38.6/10,000
Canada	(Beresford et al 2000)	Screening samples from newborns	470 (males) 378 (females)	0 (males) 0 (females)	0/10,000	0/10,000
Finland	(Ryynanen et al 1994)	Pregnant women accepting a carrier test	1447 (females)	6	-	41.4/10,000
Israel	(Pesso et al 2000)	Preconceptional or pregnant women with no family history of mental retardation	9459 (females)	130	-	137/10,000
Israel	(Toledano-Alhadef et al 2001)	Preconceptional or pregnant women with no family history of mental retardation	14,334 (females)	334	-	233/10,000
USA	(Mazzocco et al 1998)	Children referred to speech disorders clinic with language delay	529 (males)	1	18.9/10,000	-
UK	(Jacobs et al 1993)	Children with an intellectual disability in mainstream schools	180 (males) 74 (females)	0 (males) 0 (females)	0/10,000	0/10,000

Although the estimated prevalence of fragile X based on cytogenetic tests has been shown to be spuriously high and there is great variability in the published literature, the

prevalence measured more accurately by DNA diagnostic tests still leaves the fragile X syndrome as one of the most commonly inherited causes of intellectual disability (Rousseau et al 1995).

Existing procedures

Traditionally, identification of fragile sites for the diagnosis of fragile X has relied on cytogenetic techniques. This technique identifies specific abnormalities such as constrictions, gaps or breaks within the chromosomes of cultured cells. Using this method, fragile sites are best identified by culturing cells under specific conditions such as folic acid and thymidine deprivation (Murray et al 1997). The fragile site unique to fragile X syndrome is FRAXA, which is identified as a non-staining gap located on the long arm of the X chromosome at the Xq27.3 location.

Diagnosis of fragile X using cytogenetic techniques is dependent on the proportion of cells expressing the FRAXA site. There is no consensus as to what proportion of affected cells is regarded as diagnostic. For males, specific guidelines have been published suggesting 4% as the lower limit for diagnosis (Jacky et al 1991). For females, where frequencies of expression are lower than that of males, a lower limit of 3% has been suggested (Hagerman & Cronister 1996). However, expert opinion in Australia suggests that the detection of just one affected cell may indicate the presence of a full mutation and would require further investigation. Further limitations of cytogenetic testing include:

- the inability to distinguish FRAXA from the other neighbouring fragile sites;
- variable results between laboratories as well as variability in tissue culture media used;
- uncertainty surrounding the proportion of cells that constitute a positive diagnosis;
- the sensitivity to other factors such as dietary folic acid which may affect FRAXA expression; and
- the inability to identify a full mutation.

Therefore, cytogenetic testing is limited in its diagnosis of fragile X syndrome (Murray et al 1997).

Comparator

In order to assess the effectiveness of molecular techniques in diagnosing different fragile X genotypes, cytogenetic testing was chosen to be the most appropriate comparator. Both PCR and Southern blot were compared individually and also in combination with cytogenetic testing. In addition, comparisons between PCR and Southern blot were also considered.

Marketing status of the device / technology

This test, which was developed in public hospital laboratories, is not commercially available in Australia, nor is it listed with the Therapeutic Goods Administration.

Current reimbursement arrangement

Cytogenetic testing is listed under two Medicare Benefits Schedule items for chromosome studies including preparation, count, karyotyping and identification by banding techniques or fragile X-site: Item number 73289 for blood tests; and Item number 73287 for non-blood testing. PCR and Southern blot do not have a schedule number for the diagnosis of fragile X syndrome or cascade testing.

Approach to assessment

Review of literature

The Monash Institute of Health Services Research, as part of its contract to the Medical Services Advisory Committee, have undertaken an investigation of the quality of evidence to support the use of genetic test for fragile X syndrome.

Specifically, the evaluation seeks to answer the following questions:

Item 1 Diagnostic characteristics

1.1 What are the diagnostic characteristics of PCR compared to cytogenetic testing?

1.2 What are the diagnostic characteristics of Southern blot compared to cytogenetic testing?

1.3 What are the diagnostic characteristics of combined PCR and Southern blot compared to cytogenetic testing?

1.4 What are the diagnostic characteristics of Southern blot compared to PCR?

Item 2 Family cascade testing

2.1 Does family cascade testing for fragile X affect informed reproductive decision making for people diagnosed with fragile X syndrome pre-mutation or full mutation?

2.2 Does family cascade testing for fragile X improve health and psychosocial outcomes for people diagnosed with fragile X syndrome pre-mutation or full mutation?

2.3 Does family cascade testing for fragile X improve health and psychosocial outcomes for people whose diagnosis is negative?

2.4 For cascade testing, when is the most appropriate age to screen for fragile X in terms of improved health and psychosocial outcomes?

2.5 What is the effectiveness of pre- or post-genetic counselling for patients undergoing fragile X testing?

The search strategy included a search of electronic databases and secondary citations of original references.

Electronic databases

The following electronic databases (Table 2) were accessed to provide a list of citations.

Table 2 Electronic databases (including edition) accessed for the literature review

Database	Issue/Period Covered
Cochrane Library including: Cochrane Databases of Systematic Reviews Database of Abstracts of Reviews of Effectiveness Cochrane Controlled Trials Register NHS Economic Evaluation Database Health Technology Database	Issue 2, 2001
EBM Reviews-ACP Journal Club	1991 to January/February 2001
Medline (OVID) PreMedline (OVID)	1966 to June Week 3 2001 June 27 2001
Current Contents (OVID)	1993 Week 26 to 2001 Week 27
Biological Abstracts (OVID)	1980 to March 2001
PsycINFO*	1887 to June week 3 2001

* Only accessed for Item 2: Family cascade testing

Search terms

Table 3 lists the search terms used to identify papers.

Table 3 Search terms used for the literature review

Fragile -X terms	DNA test terms	Cytogenetic test terms	Cascade testing terms	Genetic counselling terms	Cost-effectiveness
exp Fragile X Syndrome/ exp Trinucleotide Repeats/ fragile x.mp. fmr$.mp. fraxa.mp. frax-a.mp. martin bell.mp. premutation.mp. pre-mutation.mp. trinucleotide repeat$.mp. triplet repeat$.mp	exp Blotting, Southern/ exp DNA Probes/ exp DNA/an [Analysis] exp DNA/du [Diagnostic Use] exp Polymerase Chain Reaction/ polymerase chain reaction.mp. pcr.mp. southern blot$.mp.	exp cytogenetics/ cytogenetic$.mp. chromosome test$.mp.	exp Genetic Predisposition to Disease/ Genetic screening/ Mass screening/ screening.mp testing.tw cascade.tw predisposition.tw susceptibility.tw	Genetic Counseling/ counseling.mp counselling.mp counsel$.mp	**Cost terms:** economic$ cost costs costly costing price prices pricing pharmacoeconomic (expenditure$ not energy) (value adj1 money) budget$ preference? qaly? quality adjusted **Excluded terms:** editorial.pt. letter.pt. note.pt. table of contents.pt. (energy adj cost). (energy adj expenditure). (oxygen adj expenditure).(oxygen adj cost).(utility or utilities)

* Only accessed for Item 2: Family cascade testing

Inclusion and Exclusion criteria

The following criteria were developed *a priori* to determine eligibility of relevant studies.

Item 1 Diagnostic characteristics

Patient population

Inclusion: clinical or experimental studies involving humans or human tissues

Exclusion: animal studies

Characteristics of the diagnostic test

Inclusion: use of Southern blot, polymerase chain reaction (PCR) techniques or cytogenetic tests

Outcomes

Inclusion: all outcomes that address the diagnostic characteristics of fragile X

Methodology

Inclusion: studies that evaluate diagnostic characteristics of at least two tests (PCR, Southern blot or cytogenetic test) in a cross-sectional study

Exclusion: cross-sectional studies that examine a genetic test in a group of affected individuals known to have the disorder and a group of normal controls, case series, case reports, narrative reviews, editorials, letters, foreign language studies

Item 2 Family cascade testing

Characteristics of the intervention

Inclusion: articles that specified screening or cascade testing for fragile X with or without genetic counselling

Outcomes

Inclusion: reproductive outcomes, psychosocial or health outcomes

Search Results

An initial assessment of the abstracts allowed for the exclusion of articles that did not meet the selection criteria. Ambiguous or unclear citations proceeded to the next assessment stage for examination of the full text . Three independent reviewers examined each citation for inclusion. Discrepancies in selection were discussed and resolved through consensus. A final decision to reject or accept articles was based on a thorough reading of the complete article. Only studies that successfully passed this process were included in this report.

Item 1 Diagnostic characteristics

An initial search for diagnostic characteristics of genetic tests for fragile X identified 591 articles of which 495 were rejected, leaving 96 articles to be assessed further in full text. Of these, 17 met inclusion criteria and were eligible for critical appraisal. Table 4 lists the number of included articles by question (citations are listed in Appendix C).

Table 4 Item 1 - Selected articles listed by question

Question	Number of articles selected*
What are the diagnostic characteristics of PCR compared to cytogenetic testing?	1
What are the diagnostic characteristics of Southern blot compared to cytogenetic testing?	9
What are the diagnostic characteristics of Southern blot compared to PCR?	7
What are the diagnostic characteristics of combined PCR and Southern blot compared to cytogenetic testing?	3

*some articles were selected for more than one question

Item 2 Family cascade testing

An initial search for cascade testing in fragile X retrieved 547 articles of which 496 were rejected, leaving 51 articles to be assessed in full text. Of these, 10 met inclusion criteria and are cited in Appendix C.

Assessment of validity

Item 1 Diagnostic characteristics

Diagnostic evidence for articles included in each of Items 1.1 to 1.4 was evaluated separately. Articles were critically appraised to assess the validity of the methodology based on criteria focusing on important aspects of study design for diagnostic studies (Jaeschke et al 1994; Cochrane Methods Working Group on Systematic Review of Screening and Diagnostic Tests 1996; Sackett et al 2000). The most rigorous study design for assessing the validity of diagnostic tests is considered to be a prospective blind comparison of the test and a reference, or 'gold' standard, in a consecutive series of patients from a relevant clinical population (Jaeschke et al 1994; Sackett et al 2000). The Cochrane Methods Working Group on Systematic Review of Screening and Diagnostic Tests (1996) expand on this definition and recommend the following six criteria for assessment of validity of evidence pertaining to diagnostic tests.

1. Test being evaluated (study test) is compared with a reference standard (gold standard).

2. Study test and reference test are measured independently (blind) of each other.

3. Choice of patients who were assessed by the reference standard was made independent of the study test's results.

4. Study test was measured independently of all other clinical information.

5. Reference standard was measured before any interventions were started with knowledge of test results.

6. Tests were compared in a valid study design: tests done independently on each person (most valid), different tests done on randomly allocated individuals, all tests done on each person but not assessed independently, different tests on different individuals, not randomly allocated (least valid).

Some of these criteria require some modification in relation to assessment of genetic tests. For example, the fifth criterion is not particularly relevant to a genetic disorder such as fragile X as the disorder cannot be cured and available interventions would not be expected to alter the results of the genotype. Similarly, as the tests are objective, and clinical suspicion of fragile X syndrome or a premutation or full mutation is usually a pre-requisite for performing the test, prior knowledge of the clinical signs would not be expected to alter the results of the test. Based on these criteria, the validity of the methodology of included articles was assessed against the following checklist (Table 5).

Table 5 Criteria and definitions for assessing validity of included articles

Validity criteria	Definition
Test is compared with a reference standard (gold standard)	Subjects in the study should have undergone both the diagnostic test in question and a reference test that would provide confirmatory proof that they do or do not have the target disorder
Appropriate spectrum of subjects	Study included subjects that the test would normally be used on in clinical practice, ie. in the case of fragile X, those with clinical signs of the disorder and cascade testing of family members, and prenatal testing of those at risk. An inappropriate spectrum compares patients already known to have the disorder with a group of normal non-diseased subjects (case-control) or with patients already diagnosed with another condition
Masked assessment of study and reference tests results	The study test and the reference test should be interpreted separately by persons unaware of the results of the other (avoidance of review bias)
All study subjects tested with both study and reference tests	The reference test should be applied regardless of a positive or negative result from the study test (avoidance of work-up / verification bias)
Study test measured independently of clinical information	The person interpreting the test should be masked to clinical history and results of any other tests performed previously

Item 2 Family cascade testing

All accepted articles were assessed for study validity (Table 6) based on criteria that focused on important aspects of study design for intervention studies (Schulz et al 1995; Jadad et al 1996).

Table 6 Criteria and definitions for assessing validity of intervention studies

Validity criterion	Definition
Randomisation	
Adequate	Adequate measures to conceal allocations such as central randomisation; serially numbered, opaque, sealed envelopes; or other descriptions that contain convincing elements of concealment
Unclear	Trials in which the author failed to describe the method of concealment with enough detail to determine its validity
Inadequate	Method of allocation is not concealed, such as alternation methods or the use of case numbers
None	No randomisation method was employed
Masking	Masking strategy applied (triple, double, etc.)
Losses to Follow-up	Losses specified

Critical appraisal was conducted by three reviewers with expertise in basic science, clinical research, epidemiology and biostatistics. Articles that presented difficulties in interpretation were discussed among reviewers and consensus reached.

Expert advice

A supporting committee with expertise in diagnosis of genetic diseases and health economics was established to evaluate the evidence and provide advice to MSAC from a clinical perspective. In selecting members for supporting committees, MSAC's practice is to approach the appropriate medical colleges, specialist societies and associations, and consumer bodies for nominees. Membership of the supporting committee is provided at Appendix B.

Results of assessment

Is it safe?

An extensive literature search failed to identify any reports of adverse events associated with either the testing of individuals suspected of having a clinical risk of fragile X or in cascade testing of relatives of affected individuals. Sample requirements for fragile X testing are approximately 10ml of fresh blood from adults and at least 3ml from infants (Taylor 2001). Presumably, potential risks of collecting material for fragile X testing, such as infection, are the same for any procedure that involves blood sampling, but no reports of such adverse events associated with fragile X testing were identified in the published literature. The psychological burden of testing and screening for fragile X is discussed below in the section titled, 'Is it effective?' under 'Item 2: Family cascade testing'.

Prenatal diagnosis

Females who have a premutation or full mutation may undertake prenatal diagnosis. Foetal DNA is usually obtained by amniocentesis or CVS, which are sampling procedures potentially associated with adverse events. However, data specifically reporting adverse events associated with prenatal diagnosis of fragile X were not identified in the literature search.

Amniocentesis

In general, minor medical events such as transient vaginal spotting or amniotic fluid leakage following amniocentesis were reported in two to three per cent of women on a United States National Registry (US NICHHD National Registry for Amniocentesis Study Group 1976). As it has been estimated that the rate of spontaneous abortion in mid-trimester pregnancy is three to four per cent (Murray et al 1997), it is difficult to quantify the exact increase above this background rate. One randomised trial of amniocentesis indicated that the rate increased by 0.8 per cent above the background rate following amniocentesis (Tabor et al 1986). Thus, the excess rate of foetal loss is usually stated to be between 0.5 and one per cent.

Chorionic villus sampling

Similar to amniocentesis, it is difficult to quantify the additional rate of spontaneous abortion associated with CVS over the background rate. A Canadian randomised controlled trial found a similar rate of spontaneous abortion in women assigned to CVS and amniocentesis, but a European multicentre trial reported a statistically significant increase in the rate of spontaneous abortion in women assigned to CVS (Medical Research Council Working Party on the Evaluation of Chorion Villus Sampling 1991). A systematic review including data from three large trials (Alfirevic et al 2001) reported an increase in non-life threatening adverse events in women undergoing CVS compared to those undergoing amniocentesis, but no difference in the rate of neonatal complications, including stillbirths and neonatal deaths, between the two procedures.

Is it effective?

Item 1 Diagnostic characteristics

Two factors are considered necessary to determine the effectiveness of a diagnostic test:

- Accuracy of the test; and
- Usefulness of the test in improving outcomes for affected individuals.

Accuracy of the tests

The accuracy of a diagnostic test is primarily determined by its ability to identify the target disorder. Accuracy is measured by diagnostic characteristics such as sensitivity and specificity. The diagnostic characteristics of each test were reviewed, subject to the availability of studies in which subjects are tested with at least two of the diagnostic tests under investigation and the reporting of sufficient data. The minimum requirements for computing sensitivity are sufficient data to compute the proportion of subjects with the disorder whose tests were correctly identified as positive. For specificity, data are required to compute the proportion of patients without the disorder whose tests were correctly identified as negative.

Diagnostic test results may be summarised in two-by-two tables (Table 7). Individuals who test positive for the disease in both the study test under investigation and the reference test are represented in cell 'a' and are called true positives (TP). Individuals without the disease who test negative in both tests (the 'd' cell) are called true negatives (TN). A diagnostic test may produce discordance between the test result and the true disease status of the subject. When this occurs a false result is reported. These situations are illustrated by cells 'b' and 'c'. In the case of the former, the test is positive in individuals without the disease; in the latter's case, the test is negative in diseased individuals. These two sets of false results are called false positives (FP) and false negatives (FN), respectively.

Table 7 The generic relationship between results of the diagnostic test and disease status.*

Study Test Results	True Disease Status (Reference test)		
	Diseased	Not Diseased	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	a+b+c+d

*Abbreviations: a=number of diseased individuals detected by the test; b=number of individuals without disease detected by the test; c=number of diseased individuals not detected by the test; d=number of individuals without disease not detected by the test; a+b=total number of individuals testing positive; c+d=total number of individuals testing negative; a+c=total number of diseased individuals; b+d=total number of individuals without disease; a+b+c+d=total number of individuals studied.

Sensitivity (Sen) is the proportion of diseased individuals who test positive. It is a measure of the probability of correctly diagnosing a case, or the probability that any given case will be identified by the test. Referring to Table 7;

$$Sen = \frac{a}{a+c} = \frac{TP}{TP+FN}$$

Specificity (Spe) is the proportion of individuals without disease who test negative. It is the probability of correctly identifying a non-diseased person with the study test.

$$Spe = \frac{d}{b+d} = \frac{TN}{TN+FP}$$

The complement of specificity is called the false positive rate (FPR).

$$FPR = 1 - Spe$$

Likelihood ratios (LR), which indicate by how much a given diagnostic test result will raise or lower the pre-test probability of the target disorder, were also computed if appropriate data could be extracted from individual articles. Thus, likelihood ratios express the odds that a given level of a test result would be expected in a patient with the condition compared to one without the condition. The likelihood ratio for a positive test result is related to sensitivity and the false positive rate by:

$$LR+ = \frac{Sen}{FPR}$$

The likelihood ratio for a negative test result is calculated by:

$$LR- = \frac{1-Sen}{Spe}$$

Note that large positive likelihood ratios of 10 or more, and small negative likelihood ratios (<0.1), indicate large changes in disease likelihood. If the likelihood ratio for a positive test result lies below two and the likelihood ratio for a negative test result lies above 0.5, then there is little or no change in disease likelihood after taking the test.

The choice of a cut-off value in determining the presence of disease or positivity of test results will affect computed test characteristics. In cytogenetic tests, there is inconsistency between studies as to what proportion of affected cells is regarded as diagnostic in fragile X syndrome (where an affected cell is one in which the break in Xq27.3 can be observed cytologically). Studies varied in their cut-off levels from 1% to 4%, and some specified different proportions for males and females such as Ramos et al (1993). Affected males generally have reasonable levels of positive cells and guidelines have been published suggesting 4% as the lower limit (Jacky et al 1991). However, current expert opinion in Australia suggests that just one affected cell may indicate the presence of a full mutation and would require further investigation with molecular techniques. Therefore, this evaluation assessed the accuracy of cytogenetic tests using cut-offs of both less than 4%, and 4% or more, where such data could be extracted from included studies.

Patient outcomes

Even if the diagnostic test under consideration is able to detect pathology, this is not a good indicator of the usefulness of the test. Application of the test should improve patient management options, otherwise its usefulness is limited. The ideal method for assessing patient outcomes after using the diagnostic test is a randomised controlled trial that compares outcomes of patients who have had the test to those who have not had the test. No trials of this type were identified. Critical appraisal of the diagnostic test

articles included an assessment of whether patient management options were discussed as a result of subjecting patients to the diagnostic test.

Findings

Item 1.1 Cytogenetic testing vs PCR

One study met inclusion criteria for critical appraisal. Brown et al (1993) carried out prenatal testing on 28 amniotic fluid or chorionic villus samples from pregnant women known to be carriers (Table 8). It was not stated how it was determined that the pregnant women were 'carriers'.

Table 8 Study characteristics

Study	Setting, dates of enrolment	Spectrum of patients			Selection criteria
		Sample size	Age (yrs) Mean (range)	Sex Ratio (M:F)	
(Brown et al. 1993)	USA, dates not stated	28 (prenatal samples)	prenatal	4:3 (foetal)	Pregnant 'carrier women'

Validity

Critical appraisal of Brown et al (1993) against the validity criteria is shown in Table 9. Prenatal diagnosis in women who are at risk of carrying the genetic mutation would be an appropriate spectrum of patients in which to perform the diagnostic test, and the study test was measured independently of clinical information. However, it was not clear if the results of the study (cytogenetic test) and reference (PCR) tests were assessed masked to the results of the other test, and two cytogenetic results were not obtained from the prenatal samples, leaving 26 samples that underwent both tests.

Table 9 Assessment of validity

Study	Validity of study methods			
	Appropriate spectrum of study subjects	Masked assessment of study and reference test results	All study subjects tested with both study and reference test	Study test measured independently of clinical information
(Brown et al 1993)	Yes	Not stated	No, 26/28 were	Yes

Diagnostic characteristics

The diagnostic characteristics of the cytogenetic test compared to PCR are shown in Tables 10 and 11. The sample size was small (n=26), and no false positives were measured. The number of true positives decreased and the number of false negatives increased with the higher cut-off (Table 10). Thus, sensitivity of the cytogenetic test decreased with the higher cut-off as expected, but as there were no false positive tests, the specificity was 100% using both <4% and ≥4% affected cells as cut-offs for the cytogenetic test. The sensitivity of the cytogenetic test was 44% using <4% as the cut-off, dropping to 22% with ≥4% as the cut-off, as the number of false negative results increased. Due to the absence of false positives, only negative likelihood ratios could be calculated (Table 11). These were 0.56 for the cytogenetic test cut-off of <4% and 0.78

using the cytogenetic test cut-off of ≥4%. Negative likelihood ratios lying above 0.5 generally indicate that a negative test result does not provide a clinically important change in pre- to post-test probability of not having the target disorder.

Table 10 Test results (PCR as reference)

Study	Sample	n	True positives		False positives		True negatives		False negatives	
			<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Brown et al 1993)	Prenatal tests	26	4	2	0	0	17	17	5	7

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation

Table 11 Diagnostic characteristics

Study	Sensitivity		Specificity		LR+		LR-	
	<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Brown et al 1993)	44%	22%	100%	100%	n/a	n/a	0.56	0.78

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation
n/a = could not be calculated
LR+ = positive likelihood ratio; LR- = negative likelihood ratio

Interpretation

The test results were followed up after the birth or termination of the foetus. As expected, it appears that the cytogenetic test was less accurate than PCR, particularly if the proportion of cells with the mutation was low. The number of false negative tests increased when the cytogenetic test cut-off of ≥4% of cells positive for the mutation was used to indicate the presence of fragile X. From follow-up, a low proportion of cells positive for the mutation was not always indicative of an absence of fragile X syndrome, and in one sample there were no cells with the mutation detected with the cytogenetic test even though PCR and follow-up after delivery indicated the presence of a premutation. Similarly, in three cases a full mutation was present even though cytogenetic results indicated less than one or two per cent of cells tested positive.

Item 1.2 Cytogenetic testing vs Southern blot (Southern blot as reference test)

Nine studies comparing cytogenetic tests and Southern blot for the detection of fragile X were included. Descriptive characteristics of these studies are in Table 12. Two studies (Brown et al 1993; Maddalena et al 1994) provided diagnostic characteristics for prenatal diagnosis only. The remaining papers examined individuals with clinical characteristics of fragile X or family members considered at risk, two included prenatal samples (Rousseau et al 1991, 1994), as well as unrelated controls.

Table 12 Study characteristics

Study	Setting, dates of enrolment	Spectrum of patients			Selection criteria
		Sample size	Age (yrs) Mean (range)	Sex Ratio (M:F)	
(Brown et al 1993)	USA, dates not stated	28 (prenatal samples)	prenatal	4:3 (foetal)	Pregnant 'carrier women'
(Snow et al 1992)	USA, dates not stated	246 + 37 controls	Not stated	95:151	Fragile X pedigree members and unrelated controls.
(Diaz-Gallardo et al 1995)	Mexico, dates not stated	58 + 76 controls	Not stated	1:1	All individuals tested were members of a family that had at least one fragile X case.
(Rousseau et al 1991)	France, dates not stated	511 + 19 controls	Not stated (incl. 28 prenatal samples)	231:271 (excl. prenatal)	Fragile X families and 19 unrelated normal subjects.
(Ramos et al 1993)	USA, dates not stated	396 + 35 controls	Not stated	7:169 excludes controls	Fragile X families and mentally retarded individuals
(Rousseau et al 1994)	Multicentre trial (14 settings), dates not stated	2253	Not stated	1049:1204	Selected from 318 fragile X families, including prenatal diagnosis.
(Malmgren et al 1992)	Sweden, dates not stated	127	Not stated	55:72	21 fragile X families
Yu 1992 (Yu et al 1992)	Australia, dates not stated	420	Not stated	41:43	All consenting members of fragile X families.
(Maddalena et al 1994)	USA, dates not stated	34 (18 male; prenatal)	n/a	9:8	Women with full mutations or premutations

Validity

Critical appraisal of the nine included studies against the validity criteria is summarised in Table 13. No studies met all of the validity criteria. All studies met the criterion of including an appropriate spectrum of patients to be tested for fragile X. No study explicitly stated that the assessors of the study test were masked to the results of the reference test and vice-versa, although with the objective nature of these tests, bias would be expected to be minimal. A shortcoming which may have biased the results was that all studies except Diaz-Gallardo et al (1995), did not appear to test all their included subjects with both cytogenetic testing and Southern blot, or if they did, presented data in such a way that diagnostic characteristics could only be extracted for a proportion of tested subjects. Authors did not state the reasons for presenting results from selected subjects, but omitting some test results may compromise the calculation of test accuracy.

Table 13 Assessment of validity

Study	Validity of study methods			
	Appropriate spectrum of study subjects	Masked assessment of study and reference test results	All study subjects tested with both study and reference test	Study test measured independently of clinical information
(Brown et al 1993)	Yes	Not stated	No, 23/26*	Yes
(Snow et al 1992)	Yes	Not stated	No, 236/246	No
(Diaz-Gallardo et al 1995)	Yes	Not stated	Yes, 56/56	No
(Rousseau et al 1991)	Yes	Not stated	No, 388/511	No
(Ramos et al 1993)	Yes	Not stated	No, 93/396	No
(Rousseau et al 1994)	Yes	Not stated	No, 1476/2253	No
(Malmgren et al 1992)	Yes	Not stated	No, 116/127	No
(Yu et al 1992)	Yes	Not stated	No, 21/420	No
(Maddalena et al 1994)	Yes	Not stated	No, 20/34	Yes

* proportion of included subjects with results reported for both cytogenetic and Southern blot testing

Diagnostic characteristics

The diagnostic characteristics extracted from the included studies are presented in Tables 14 and 15. Calculated sensitivities of the test ranged from 15% to 77% and specificity was high, lying between 93% and 100% (Table 15).

Two cytogenetic cut-offs (<4% cells with the mutation and ≥4% cells with the mutation) for positive diagnosis of fragile X could be extracted from four studies (Rousseau et al 1991; Snow et al 1992; Brown et al 1993; Maddalena et al 1994). Of these, when the higher cut-off was used the number of true positives decreased and the number of false negatives increased, resulting in decreased sensitivity and increased specificity.

Likelihood ratios for positive tests could be calculated from the four larger studies that reported false positives (Rousseau et al 1991; Snow et al 1992; Maddalena et al 1994; Rousseau et al 1994). These were high, ranging from 11.8 to 47, indicating that a subject with a positive cytogenetic test is likely to have a full mutation. Negative likelihood ratios fell between 0.23 and 0.85. These values are unlikely alter to the post-test likelihood of having the disorder.

Table 14 Test results (Southern blot as reference)

Study	Sample	n	True positives		False positives		True negatives		False negatives	
			<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Brown et al 1993)	Prenatal tests	23	4	2	0	0	16	16	3	5
(Maddalena et al 1994)	Prenatal tests	20	5	2	8	11	7	7	8	11
(Rousseau et al 1991)	Fragile X families	388	185	149	10	0	130	140	63	99
(Snow et al 1992)	Fragile X families	236	106	95	2	1	76	77	52	63
(Diaz-Gallardo et al 1995)*	Fragile X families	56	7		0		28		21	
(Malmgren et al 1992)*	Fragile X families	116	54		1		27		34	
(Ramos et al 1993)†	Fragile X families	93	45		0		29		19	
(Rousseau et al 1994)*	Fragile X families	388	185		149		10		0	
(Yu et al 1992)‡	Fragile X families	21	10		0		8		3	

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation
* cytogenetic cut-off not reported
† = positive diagnosis of fragile X if 3% of cells are affected in females and 4% in males
‡ = cytogenetic results reported as 0% for negative diagnosis and ≥4% for positive diagnosis

Table 15 Diagnostic characteristics (Southern blot as reference)

Study	Sensitivity		Specificity		LR+		LR-	
	<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Brown et al 1993)	57%	29%	100%	100%	n/a	n/a	0.43	0.71
(Maddalena et al 1994)	38%	15%	100%	100%	n/a	n/a	0.62	0.85
(Rousseau et al 1991)	75%	60%	93%	100*	10.4	n/a	0.27	0.4
(Snow et al 1992)	67%	60%	97%	99%	26	47	0.34	0.4
(Diaz-Gallardo et al 1995) *	25%		100%		n/a		0.75	
(Malmgren et al 1992)*	61%		96%		17		0.34	
(Ramos et al 1993)†	70%		100%		n/a		0.3	
(Rousseau et al 1994)*	68%		94%		11.8		0.34	
(Yu et al 1992)‡	77%		100%		n/a		0.23	

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation
n/a = could not be calculated
LR+ = positive likelihood ratio; LR- = negative likelihood ratio
* cytogenetic cut-off not reported
† = positive diagnosis of fragile X if 3% of cells are affected in females and 4% in males
‡ = cytogenetic results reported as 0% for negative diagnosis and ≥4% for positive diagnosis

Item 1.3 Cytogenetic vs PCR/Southern blot (PCR/Southern as reference test)

Three studies met inclusion criteria that assessed cytogenetic testing and combined PCR/Southern blotting procedures; their characteristics are presented in Table 16. Seki et al (1994) included only six subjects, while Hagerman et al (1994) and Wang et al (1993) were much larger studies. Hagerman et al (1994) restricted subjects to a select group of males already known to have fragile X. Seki et al (1994) examined a family with a fragile X member and Wang et al (1993) selected a range of patients referred consecutively for testing on clinical suspicion of fragile X.

Table 16 Study characteristics

Study	Setting, dates of enrolment	Spectrum of patients			Selection criteria
		Sample size	Age (yrs) Mean (range)	Sex Ratio (M:F)	
(Hagerman R et al 1994)	USA, 1981-1993	250	13 (0.25-60)	1:0	High-functioning fragile X males
(Seki et al 1994)	Japan, dates not stated	6	Not stated	5:1	A pedigree of a fragile X family
(Wang et al 1993)	UK, dates not stated	525	Not stated	17:4	Individuals referred consecutively with suspected fragile X

Validity

Results of critical appraisal of included studies against validity criteria are presented in Table 17. Hagerman et al (1994) appeared to select a narrow spectrum of subjects whose fragile X status was already established. Seki et al (1994) tested a small pedigree while Wang et al (1993) tested subjects referred for testing due to clinical signs - both studies selecting subjects likely to be tested in practice. These two studies also presented data from which diagnostic characteristics could be extracted for all included subjects, while Hagerman et al (1994) only presented sufficient results for 22 of 250 subjects. Wang et al (1993) was the most rigorously designed of the three studies, meeting all validity criteria, except that it was unclear if subjects were tested without knowledge of clinical information. As stated earlier, this particular criterion may not be as critical for objective molecular tests.

Table 17 Assessment of validity

Study	Validity of study methods			
	Appropriate spectrum of study subjects	Masked assessment of study and reference test results	All study subjects tested with both study and reference test	Study test measured independently of clinical information
(Hagerman et al 1994)	No	Not stated	No, 22/250*	No
(Seki et al 1994)	Yes, but small	Not stated	Yes	No
(Wang et al 1993)	Yes	Yes	Yes	No

* proportion of included subjects with results reported for both study and reference tests

Diagnostic characteristics

Test results revealed no or very low false positive and false negative tests (Table 18). No true negatives were reported in Hagerman et al (1994), perhaps reflecting the

inappropriateness of the narrow selection of subjects. Sensitivities were calculated between 87% and 100% and specificity of the test was 100% (Table 19). Likelihood ratios could only be calculated for Wang et al (1993). The positive likelihood ratio was high, indicating a large change in the post-test probability if a positive cytogenetic test is measured. The low likelihood ratio for a negative test indicated a low probability of disease if a negative cytogenetic result was obtained.

Table 18 Test results (PCR/Southern as reference)

Study	Sample	n	True positives		False positives		True negatives		False negatives	
			<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Hagerman et al 1994)	Fragile X males	22	21	22	0	0	0	0	1	0
(Seki et al 1994)*	Fragile X pedigree	6	4		0		2		0	
(Wang et al 1993)*	Suspected fragile X	525	13		1		510		2	

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation
* cytogenetic cut-off not reported

Table 19 Diagnostic characteristics (PCR/Southern as reference)

Study	Sensitivity		Specificity		LR+		LR-	
	<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
(Hagerman et al 1994)	95%	100%	n/a	n/a	n/a	n/a	n/a	n/a
(Seki et al 1994) *	100%		100%		-		-	
(Wang et al 1993) *	87%		99.8		443		0.13	

<4% = positive diagnosis of fragile X if 1, 2 or 3% of cells are positive for the mutation
≥4%= positive diagnosis of fragile X if 4% or more cells are positive for the mutation
LR+ = positive likelihood ratio; LR- = negative likelihood ratio
* cytogenetic cut-off not reported
n/a = could not be calculated

Item 1.4 PCR vs Southern blot (Southern blot as reference test)

Descriptive characteristics of nine studies that examined PCR compared to Southern blot are presented in Table 20. One study (Brown et al 1993) examined the tests in prenatal diagnosis. Hofstee et al (1994) assessed the tests in institutionalised children and adults. Das et al (1998) tested males previously diagnosed by Southern blotting as normal, premutation, mosaic or affected. Similarly, Haddad et al (1994) assessed the test in male subjects known previously by unstated methods to be affected or normal. Details of the selection criteria of subjects in the remaining two studies were scarce.

Table 20 Study characteristics

Study	Setting, dates of enrolment	Spectrum of patients			Selection criteria
		Sample size	Age (yrs) Mean (range)	Sex Ratio (M:F)	
(Brown et al 1993)	USA, dates not stated	28 (prenatal)	prenatal	4:3 (foetal)	Pregnant 'carrier women'
(Das et al 1997)	USA, dates not stated	100 (52 normal)	Not stated	1:0	Sample previously analysed by Southern blot as normal, premutation, mosaic or affected
(Haddad et al 1996)	Brazil, dates not stated	115	Not stated	1:0	Males, previously known to be normal or affected
(Hofstee et al 1994)	Japan, dates not stated	434	Males: 38(9-66); Females: 38(13-70)	129:305	Institutionalised intellectually disabled individuals
(Levinson et al 1994)	USA, dates not stated	28	Not stated	2:5	Not stated
(Strelnikov et al 1999)	Russia, dates not stated	178	Not stated	143:35	Not stated

Validity

Assessment of validity of each study is presented in Table 21. No study met all validity criteria. Four of the studies chose an appropriate spectrum of patients in which to perform the test, while two did not provide enough details to determine if appropriate subjects were selected. Das et al (1998) and Haddad et al (1996) assessed the test in subjects whose status was previously known but stated that this information was masked in assessment of PCR results. Four studies (Hofstee et al 1994; Levinson et al 1994; Das et al 1997; Strelnikov et al 1999) reported results for all of their included subjects. Brown et al (1993) were missing results for one subject, while Haddad et al (1996) reported results from which diagnostic characteristics could be calculated in only 65 of their 115 subjects. These omissions may substantially affect the validity of their results.

Table 21 Assessment of validity

Study	Validity of study methods			
	Appropriate spectrum of study subjects	Masked assessment of study and reference test results	All study subjects tested with both study and reference test	Study test measured independently of clinical information
(Brown et al 1993)	Yes	Not stated	No, 25/26*	Yes
(Das et al 1997)	Yes	Yes	Yes	Yes
(Haddad et al 1996)	Yes	Yes	No, 65/115	Yes
(Hofstee et al 1994)	Yes	No	Yes	No
(Levinson et al 1994)	Unclear	Not stated	Yes	No
(Strelnikov et al 1999)	Unclear	Not stated	Yes	No

* proportion of included subjects with results reported for both PCR and Southern blot testing

Diagnostic characteristics

No study reported any false positive results using PCR as a test, and only three studies found a small number of false negatives (Table 22). A very high sensitivity of PCR was calculated for all studies, ranging from 85% to 100% (Table 23). Similarly, as no study recorded a false positive result with PCR, specificity was 100% for all included studies. Likelihood ratios for a positive test could not be calculated due to the lack of false positive results. Likelihood ratios for a negative test were calculated for three studies (Hofstee et al 1994; Levinson et al 1994; Haddad et al 1996). These values were low, and unlikely to alter post-test probability. Hofstee et al (1994) was the largest study, but results were consistent across studies.

Table 22 Test results (Southern blot as reference)

Study	Sample	n	True positives	False positives	True negatives	False negatives
(Brown et al 1993)	Prenatal tests	25	8	0	17	0
(Das et al 1997)	Normal and affected males	100	48	0	52	0
(Haddad et al 1996)	Normal and affected males	65	40	0	24	1
(Hofstee et al 1994)	Institutionalised subjects	434	11	0	421	2
(Levinson et al 1994)	Broad range of allele sizes	28	16	0	10	2
(Strelnikov et al 1999)	Males	178	18	0	160	0

Table 23 Diagnostic characteristics (Southern blot as reference)

Study	Sensitivity	Specificity	LR+	LR-
(Brown et al 1993)	100%	100%	n/a	n/a
(Das et al 1997)	100%	100%	n/a	n/a
(Haddad et al 1996)	98%	100%	n/a	0.02
(Hofstee et al 1994)	85%	100%	n/a	0.15
(Levinson et al 1994)	89%	100%	n/a	0.11
(Strelnikov et al 1999)	100%	100%	n/a	n/a

LR+ = positive likelihood ratio; LR- = negative likelihood ratio
n/a = could not be calculated

Summary of findings

Evidence from published studies suggests that cytogenetic testing is not as sensitive as molecular techniques (PCR or Southern blot) in detecting a full mutation and may not detect a premutation at all. Studies tended to report premutations and full mutations together and there were insufficient data to extract diagnostic characteristics separately. The reported sensitivity of cytogenetic testing ranged widely across included studies. Specificity, however, was consistently high (usually close to 100%), with very few false

positive cytogenetic results reported, although data from population surveys where subjects were re-tested with molecular tests retrospectively indicated a high rate of false positive results with cytogenetic testing (see 'Background'). This indicates that a positive cytogenetic result is likely to rule in a diagnosis of fragile X. Increasing the cut-off value for a positive diagnosis of fragile X from fewer than 4% of cells with the mutation to more than 4% of cells with the mutation on cytogenetic testing resulted in a decrease in sensitivity and an increase in specificity. Variable sensitivities may have been related to the spectrum of subjects tested, as those carrying a premutation may express a very low proportion of cells with the mutation that are able to be detected by cytogenetic testing. Sensitivities of cytogenetic testing were particularly low in prenatal diagnosis. Thus, a negative cytogenetic result does not necessarily rule out a diagnosis of a premutation, or even a full mutation. For cytogenetic testing compared to Southern blot, the likelihood ratios for positive tests could only be calculated from four larger studies that reported false positive results. These likelihood ratios were high, indicating that compared to the probability of having the disorder prior to testing, after taking the test, an individual with a positive result has an increased likelihood of having the disorder. Likelihood ratios for a negative test were between 0.5 and one, indicating little change in post-test probability of having the disorder if a negative result is obtained. Thus, a negative cytogenetic result is not necessarily indicative of the true fragile X status and further testing with molecular techniques is required.

Studies comparing PCR to Southern blot reported high sensitivity and specificity. It should be noted that PCR may not reliably amplify full mutations and Southern blot is usually necessary to reliably demonstrate a full mutation.

Item 2 Family cascade testing

Family cascade testing and genetic counselling

Cascade testing for fragile X identifies individuals within families at high risk of transmitting the mutation. This type of testing is aimed at informing reproductive choice by systematic testing within families of affected individuals and may help to facilitate appropriate management strategies. In Australia, fragile X cascade testing is seldom administered without appropriate genetic counselling which is regarded as best practice. The purpose of counselling is to provide information and support to the individuals and their families regarding the implications of carrying fragile X.

Item 2.1 Fragile X testing and informed reproductive decision-making in affected individuals diagnosed with fragile X mutations

Two studies were identified which investigated the effects of fragile X testing and genetic counselling on reproductive decision making (Curtis et al 1994; McConkie-Rosell et al 1997). These studies present case series data derived from interviews and surveys of fragile X carriers.

The study by McConkie-Rosell et al (1997) interviewed 28 women who were fragile X carriers and had undergone genetic counselling. These women were interviewed with regards to how their carrier status would affect their reproductive decisions. This study reported that 67% of women felt that knowing about their fragile X status changed their plans about having more children since they felt that their risk of having an affected child was too high. Eighty-nine per cent of women also reported that had they known that

fragile X was in their families prior to having children they would have reduced the size of their families or not had any biological children.

Curtis et al (1994) reported the results of 27 women who were known to be fragile X carriers or possible fragile X carriers. These women were categorised as having low (0–10%), medium (11–39%) or high (40–100%) carrier risk (premutation or full mutation as diagnosed by cytogenetic, DNA linkage or direct mutational analysis). In this group there were 10 pregnancies to 10 women, seven in the high-risk group and three in the medium to low risk group. Among those in the high risk category, four women took steps to avoid the birth of an affected son. Of the pregnancies in the medium to low risk group, one affected child was born. In addition, six women chose to be sterilised as a result of either their carrier status or a diagnosis of fragile (X) in a family member.

The results of these studies indicate that women at risk of having children with fragile X carefully consider their reproductive choices. However, it is important to note the results of these studies are based on case series data which are subject to a number of biases, and therefore, any conclusions drawn from these results should be considered with caution.

Items 2.2 and 2.3 The effect of cascade testing on health and psychosocial outcomes of carriers and non-carriers

The psychological burden of fragile X can impact emotionally on individuals, especially those with a positive diagnosis (Cronister 1995). A number of studies have investigated the emotional response of women following carrier testing (Ryynanen et al 1994;McConkie-Rosell et al 2000).

McConkie-Rosell et al (2000, 2001) have investigated the emotional response of women after fragile X diagnosis for carriers and non-carriers. The authors measured the responses of women at the time of DNA testing (time 1) and approximately six months after learning the results of their carrier test (time 2). The sample consisted of 20 carriers and 22 non-carriers. The measures used in this study were a fragile X visual analogue scale, a structured interview (McConkie-Rosell et al 2000, 2001) and Tennessee self-concept scale (McConkie-Rosell et al 2000). Since McConkie-Rosell et al (2001) is an extension of McConkie-Rosell et al (2000), only the results of the more recent study will be discussed when presenting the results for the fragile X visual analogue scale (feeling upset and emotional).

These studies reported that the response of carriers and non-carriers was the same, with the majority of women (67%) feeling upset after learning of their carrier risk. After six months, however, the emotional response of carriers and non-carriers diverged. Non-carriers were significantly less upset by the outcome of their carrier testing ($p<0.05$), with 82% of women reporting no feelings of being upset and 95% expressing positive emotions such as relief and happiness that their children would not be affected. In contrast, carrier women experienced no significant change in their level of being upset from time 1 to time 2, and expressed strong negative emotions about their carrier status. Carriers were also concerned about how their positive test would affect their children and grandchildren as well as their own reproductive choices. Feelings of self-concept, presented in McConkie-Rosell et al (2000), were not significantly different between carriers and non-carriers for either time period. In fact the mean self-concept score (total and subscales) for the whole group was within the normal range.

Similar results were also reported in a study by Ryannen et al (1999), which investigated the attitudes of women with regards to their fragile X carrier test results. The study sample consisted of 72 women - 54 non-carriers and 18 carriers. The results of this study reported that a higher proportion of carrier women (75%) were very anxious after receiving a positive test, compared to controls (4%).

Some of the limitations associated with these studies are that the samples were not randomly selected and may not be representative of those seeking fragile X diagnosis as a result of selection bias. In the McConkie-Rosell studies (McConkie-Rosell et al 1995, 1997, 2000, 2001) the study group were exclusively Caucasian, the majority of which were married and had children. In addition, the studies did not allow for the analysis of additional variables such as reproductive stage and number of affected individuals within families.

However, despite these limitations, the results of these studies have demonstrated that the emotional impact of carrier testing is greatest for those who are diagnosed with a fragile X mutation. A positive diagnosis has been shown to cause feelings of anxiety, upset and concern, both in the long and short term. These feelings may be ameliorated with the provision of information and genetic counselling.

Item 2.4 For cascade testing, when is the most appropriate age to screen for fragile X syndrome in terms of improved health and psychosocial outcomes?

Parents sometimes request that their children be tested for late-onset diseases, disease susceptibilities, and carrier status so that they can address many of the psychosocial issues.

The Working Party of the Clinical Genetics Society (Clarke 1994) argues that non-medical use by parents is one of the most controversial issues in testing children. While some authors argue that parents should be able to obtain such information, others such as Harper and Clarke (1990) contend that such information should be restricted or prohibited if the children will gain no immediate medical benefit. There is no consensus on this issue in Australia or internationally, and no evidence to direct practice. In general, a cautious approach is advised, with postponement of testing until the child is old enough to give informed consent.

Item 2.5 What is the effectiveness of genetic counselling for patients undergoing fragile X testing?

One of the primary goals of genetic counselling is to help individuals and their families make informed decisions with regards to having children. In addition, genetic counselling also provides a means to offer emotional support for families dealing with the psychological burden of the disease (Cronister 1995). The major components of genetic counselling are the provision of information and support surrounding the medical aspects of fragile X syndrome, inheritance patterns, available testing and family planning for immediate and extended family (Cronister 1995). In addition, genetic counselling may also facilitate referral to specific treatment and management strategies for carriers. Specific guidelines for the dissemination of genetic risk information for fragile X syndrome have been published (McConkie-Rosell et al 1995).

Only two comparative studies were identified which demonstrated the impact of genetic counselling on reproductive outcomes (Turner et al 1992; Robinson et al 1996). One case series study was identified which reported the effects of genetic counselling on information exchange and emotional support (Roy et al 1995).

The studies by Robinson et al (1996) and Turner et al (1992) present Australian data from a statewide testing program in New South Wales (Turner et al 1992; Robinson et al 1996). Both studies reported the effect of genetic counselling on reproductive patterns of full mutation and premutation carrier women using a case control design. They identified 303 matched pairs born between 1945–1975 who had a first degree relative with fragile X syndrome. Cases were defined as women in this group and the study period was from the year of genetic counselling to the year of follow-up. Controls were defined as those women that had received genetic counselling at an age that was older than that of their matched cases at follow-up. Therefore, the reproductive pattern of cases during a particular age span was compared with the same age span of controls whom had yet to receive counselling. Cases and controls were matched for level of intellectual functioning, whether they lived with an affected male and whether they had given birth to at least one affected child.

The result of this study showed that women undergoing carrier testing with counselling had a significantly lower birth rate (26%) than those not receiving counselling ($\chi^2=4.03$, $p<0.05$). This result was unrelated to the risk of being a carrier with reproductive patterns being the same between intellectually normal carriers and women with low carrier risk. However, level of intellectual functioning may affect reproductive patterns. Carriers of impaired intellectual functioning were shown to have significantly more pregnancies than their matched controls whereas carriers of normal intellect had fewer pregnancies than controls ($\chi^2=11.0$, $p<0.001$).

The effect of genetic counselling on information exchange and emotional support was reported in a case series study by Roy et al (1995). This study surveyed 151 families of children diagnosed with fragile X syndrome by cytogenetic or DNA analysis. Regarding the benefits of genetic counselling, 38% responded that it was a source of emotional support, 65% stated that it helped in their understanding of behavioural aspects of the disease, 83% stated that it helped in their understanding of the inheritance patterns of the disease and 65% responded that it helped in their understanding of DNA testing.

Although these studies highlight the importance of genetic counselling they are not without their limitations. The studies by Robinson et al (1996) and Turner et al (1992) use historical controls to determine the effect of counselling which may introduce confounding since external factors unique to a specific time period (such as societal factors) may have changed during the two different time periods Such factors may directly effect reproductive outcomes, independent of the counselling. The study by Roy et al (1995) is cross-sectional and may be subject to selection and reporter bias.

Despite these limitations these studies have demonstrated that genetic counselling has the potential to help people affected by fragile X syndrome understand the facts surrounding the disease and its heritability in order for them to cope with the emotional burden of a positive diagnosis and make informed reproductive decisions.

What are the economic considerations?

General Framework

The framework for the economic evaluation of any medical technology considered by MSAC is the comparison of the costs and benefits of that technology compared with the current alternative treatment for patients. The ideal approach is to calculate an incremental cost-effectiveness ratio $(C_I-C_c)/(O_I-O_c)$ where C_I is the total cost of resources used associated with the intervention, C_c is the total cost of resources used by the comparator, O_i is the output associated with the intervention, and O_c is the outcome associated with the comparator. The broad perspective is a societal one that includes costs borne by governments and individuals.

Where there are two comparators or patient groups, a weighted average of cost and outcome can be calculated where the weights are the proportion of patients who are likely to receive each of the comparator treatments.

One difficulty in evaluating genetic testing is the lack of a single standardised outcome measure. A common outcome measured in the literature is the number of affected births prevented (Turner et al 1986). This is used to demonstrate the net long term benefits of testing and the analysis often includes the lifetime cost of care of affected individuals as a potential cost saving from testing. In these studies, no account is taken of either the value of life, or the value of information to patients, and the analysis is therefore only partial. It could be argued that a critical outcome of genetic testing is the information provided to patients that in some cases allows them to make a more informed choice of therapies, taking into account the consequences of each. This approach suggests that an appropriate outcome to be measured (and valued) is the incremental number of cases detected by the testing intervention, for a given number of false positives. This would allow comparison with other testing programs and if a value could be placed on the information it would allow comparison with other medical interventions. The cost-effectiveness analysis in this report calculates the cost per extra case detected with genetic testing for fragile X syndrome.

The value of information, however, is a complex issue and in this case involves considerations such as the risk of an affected birth and associated disability as well as the desire for re-assurance and the avoidance of regret. If there were no possible interventions to prevent the transmission of the genetic defect the value of the information would presumably be less than where something can be done. This becomes an even greater issue where testing has implications for other family members who may be given information on their fragile X carrier status that they did not choose to know. This suggests that information and choice, while an important part of the outcome of the testing program, are not the only considerations. The risk to other family members is also part of the outcome of a genetic testing program.

The cost-effectiveness of testing expressed in terms of the average cost of detecting one affected individual can be estimated using the unit costs for each part of the testing process. This may include the provision of information, DNA testing, genetic counselling prenatal diagnosis, and abortion. The average cost is then estimated for given detection and false positive rates.

Economic evaluation

For fragile X testing, the quality of evidence is weak, although two UK Health Technology Assessment (HTA) programs have been reported (Murray et al 1997; Pembrey et al 2001). Pembrey et al (2001) reports the type of costs which may be involved. These are divided into the costs of diagnosis and the costs of testing. Costs of diagnosis may include costs to the individual and family, including stigmatisation, of both the individual diagnosed with the syndrome and their family. Another cost to the mother may be guilt at transmitting the syndrome, albeit without knowing, to her child. The emotional stress of having a handicapped child may be similar to a bereavement reaction. Build-up of stress and other factors associated with having a handicapped child may lead to relationship problems, less time spent with other children and less opportunities for paid employment. There may also be high costs of care. It should be noted that most of these costs are not specific to fragile X syndrome.

The HTA report (Pembrey et al 2001) notes there may be costs to the community and National Health Service (NHS) in terms of education, health and social services. Again, these costs are not specific to fragile X. MENCAP (the UK charity for those with learning difficulties) indicates the annual costs for a child with fragile X would be £20,000 (1995 prices) (Pembrey et al 2001). Wildhagen et al (1998) takes account of where people live in the Netherlands; 38 per cent of males and 8 per cent of females are in institutions, 18 per cent of males and females are in family units, 35 per cent of males and 38 per cent of females live with parents, and 9 per cent of males and 36 per cent of females are self supporting.

Anxiety may result from factors affecting costs of testing for the individual and family. Mostly this will be short lived, but for a minority, these effects may continue. It should be noted there may be offsetting benefits from reassurance following a normal result. For women identified as having mutations there may be additional costs associated with additional testing and procedure induced miscarriage. Further costs may be associated with the decision to have an abortion or not. In systematic case findings there can also be anxiety associated with the decision to consent to the test; cascade testing may have consequences in terms of a disturbance to family relationships.

Pembrey et al (2001) state that current good practice would involve offering genetics services to relatives at high risk through family cascade counselling. Additional costs of systematic case finding and cascade counselling in the paediatric population are difficult to predict because of uncertainty as to how widespread testing is in current practice.

Experience in New South Wales (Pembrey et al 2001) suggests systematic case finding and cascade counselling programs require one dedicated counsellor per regional genetics centre, support costs, and additional laboratory costs. Whatever the testing strategy there will be additional costs, including costs of counselling before and after testing, costs from obstetrics services providing tests, and laboratory costs. Pembrey et al (2001) conclude that: costs are mainly human rather than financial; costs for unaffected individuals from testing include anxiety, which for all but a minority will not persist beyond a normal result; and that testing strategies may be cost saving, although once a certain number of cases are detected, costs will exceed savings. This may be when the prevalence is greater than 1 in 6,000 males, and 50 per cent of high risk women have been identified.

The literature on the economic costs and benefits of testing for fragile X syndrome is summarised in Appendix D. As stated in the general framework, the chosen surrogate

outcome measure is cost per case detected. The literature review of the economics of testing for fragile X found three cost-effectiveness analyses, three cost benefit analyses, and one cost analysis. None of the cost-effectiveness studies estimate cost per case detected for DNA testing using cascade testing. Instead, two of the studies calculate the cost-effectiveness of cytogenetic testing for cascade testing where the cost for preventing the birth of an intellectually handicapped child was between AUS$14,200 (Turner et al 1986) and US$12,740 (Gabarron et al 1992). The other cost-effectiveness study reports the cost per preventing one affected birth for an antenatal testing program at GB£93,000 (Murray et al 1997). The cost analysis reports a cost per completed analysis at US$64,400 for cascade testing (Nolin et al 1991). Two of the cost benefit papers report savings for 'at risk' couples who present themselves for testing (van der Riet et al 1997) of between DFL118,000 and DFL321,400, and for prenatal, preconceptual, and school testing programs (Wildhagen et al 1998) of between US$2 million and US$9 million, with costs of US$45,000 per case detected. Vintzileos et al (1999) reports costs of between US$10 million and US$195 million for a routine prenatal program, with cost per case detected ranging from US$20,800 to US$770,800, depending on assumptions made.

Cost per case detected for Australia

The economic costs are divided between diagnosis and family cascade testing. The submission to MSAC suggested costs of testing of $200 per test for Southern Blot and $100 per test for PCR testing. As noted earlier there may be costs associated with anxiety caused by testing programs, possibly associated with false positives (reduced by the high accuracy of DNA tests), or associated with the process itself. These are particularly difficult to quantify and may apply to any medical intervention. Also difficult to quantify are ascertainment costs - the costs of identifying those at risk. The literature suggests information and organisational average costs of $50 (Wildhagen et al 1998) for cascade testing. Including counselling ($100) and ascertainment costs ($50) would give a more realistic figure of overall costs.

Table 24 shows the range of costs per case for diagnosis for a population of 1,500 patients. The assumptions made (on the advice of the supporting committee) are that two thirds of those tested are males, and that 95 per cent of males have PCR only, the other 5 per cent having PCR and Southern Blot. For females, 80 per cent are assumed to have PCR only, with 20 per cent having PCR and Southern Blot.

Table 24 Cost per case for diagnosis

Cases detected	Test only	Test with ascertainment	Test with counselling	Test with ascertainment and counselling
26 (1.73%)	$6,346	$8,942	$11,538	$14,135
13 (0.87%)	$12,692	$17,885	$23,077	$28,269

As stated elsewhere in the report, it is important to note there are no published data on detection rates in the general population. Based on the cost assumptions above, if 1,500 high risk people were tested (in Victoria), and 26 cases were detected (1.73% of those screened), the cost per extra case detected would be $6,346, given the assumptions stated. If we include $100 for counselling the cost per case detected rises to $11,538. Including ascertainment costs and counselling would raise cost per case detected to $14,135. However, we do not have confidence in the estimate of the detection rate. The cost per case detected will vary linearly with the number of cases detected (for a given

false positive rate). Thus, if we assume only 13 cases detected (0.87% of those screened) the cost per case detected could be at least $28,269.

The economic evaluation we have undertaken is from the perspective of the individual. There may be consequences beyond the individual in societal terms in terms of the provision of information about others. This is especially the case as genetic testing may involve family members beyond the individual being screened, with potential issues of intergenerational privacy (ie knowing about the health status of your children and relatives).

Key areas of economic uncertainty:

- There is uncertainty as to the number of cases that will be detected as there is little evidence for determining numbers and consequently the number of tests following cascade testing.
- There is uncertainty as to the value of information that has benefits and costs. For example, in terms of anxiety surrounding testing programs in general, costs are very difficult to quantify.
- There is some uncertainty as to which testing program would be most efficient.
- There is uncertainty as to the testing protocol – what is the upper bound for numbers who are screened?
- There is uncertainty as to the effects of adding in the cost of downstream effects to cascade testing, such as costs of testing beyond the immediate family and the extra cost of care for affected children born in the family of those with fragile X. This includes the lifetime costs of individuals born with learning disabilities.

Likely number of patients per year:

The submission suggests up to 7,500 tests per year, as a result of cascade testing.

Financial cost

Given the suggested number of test procedures, and using the costs presented in the submission and the assumptions made concerning numbers of patients who would receive PCR only, and PCR and southern blot, total costs for cascade testing in Australia would be $2.1 million for 7,500 initially tested, assuming a detection rate of 1.73%. As there is no definitive data for numbers to be tested initially, Table 25 reports sensitivity analysis of up to 15,000, which would result in costs of $4.2 million.

Table 25 Total costs of diagnosis and family testing

Number tested initially	Total costs of initial diagnosis	Family testing: 4 relatives	Family testing: 6 relatives	Diagnosis & cascade (4 relatives)	Diagnosis & cascade (6 relatives)
1,500	$367,500	$36,300	$54,500	$403,800	$422,000
2,500	$612,500	$60,500	$90,800	$673,000	$703,300
5,000	$1.225m	$121,100	$181,600	$1.346m	$1.407m
7,500	$1.838m	$181,600	$272,500	$2.019m	$2.110m
10,000	$2.45m	$242,200	$363,300	$2.692m	$2.813m
12,500	$3.063m	$302,700	$454,100	$3.365m	$3.517m
15,000	$3.675m	$363,300	$544,900	$4.038m	$4.220m

This does not include the costs of those found to have a fragile X syndrome mutation who proceed to pregnancy, and may require antenatal testing (estimated package $2,600). There is no information on the percentage of women at risk who require antenatal testing, or on the consequences, and hence value, placed on such information.

Summary

A cascade testing program is estimated to cost up to $4 million annually, and would result in a cost per initial case detected of between $14,000 and $28,000, depending on assumptions made, especially those of the detection rates in the population. This does not account for the costs of anxiety surrounding testing programs, although there may be certain benefits associated with reassurance to be balanced against these costs. Nor does it take into account the social costs and consequences of providing the information to individuals, such as the decision to abort a pregnancy or the additional costs to society of caring for a disabled person.

Costs may be greater downstream, in terms of the costs of choices individuals make. These may include further diagnosis, abortion, or lifetime costs of having a child with fragile X.

Conclusions

Safety

An extensive literature search did not identify any reports of adverse events associated with testing individuals suspected of having fragile X syndrome or cascade testing of relatives of affected individuals. Similarly, no reports of adverse events specific to prenatal diagnosis of fragile X were identified in the literature, however, potential adverse events associated with prenatal diagnosis due to the invasive nature of amniocentesis and chorionic villus sampling required to obtain foetal DNA are well documented. Minor medical events following amniocentesis such as transient vaginal spotting or amniotic fluid leakage have been reported to occur in two to three per cent of women. The exact rate of foetal loss following amniocentesis is difficult to quantify due to the relatively high background rate of spontaneous abortion of three to four per cent in mid-trimester pregnancy; the excess rate of foetal loss is usually stated to be between 0.5 and one per cent above the background rate. The additional rate of spontaneous abortion associated with chorionic villus sampling is similarly difficult to quantify precisely, but is believed to be comparable to amniocentesis.

Effectiveness

Item 1 Diagnostic characteristics

Two factors were considered in determining the effectiveness of genetic tests for fragile X syndrome: accuracy; and usefulness in improving outcomes for people undergoing the test. Accuracy is measured by diagnostic characteristics such as sensitivity and specificity. The ideal method for assessing the usefulness of the test in improving patient outcomes is a randomised controlled trial comparing outcomes of patients undergoing the test to patients not exposed to the test. No such trials were identified. Evidence of the accuracy of the tests from the published literature indicates that cytogenetic testing is not as accurate as molecular techniques in detecting the fragile X full mutation and cytogenetic testing is unable to accurately detect a premutation at all. Sensitivity of cytogenetic testing varied across studies, but specificity was consistently high with few false positive cytogenetic results reported. Thus, a positive cytogenetic test result is likely to rule in a diagnosis of fragile X, but a negative cytogenetic result is not indicative of the true fragile X status particularly in prenatal testing, and thus, testing with molecular techniques is required.

Item 2 Family cascade testing

Cascade testing for fragile X identifies individuals within families at high risk of having an affected child. This type of testing is aimed at providing informed reproductive choice, with a number of studies demonstrating that women at risk of having children with fragile X carefully consider their options when faced with the prospect of having an affected child.

Due to the complex nature of the disease and the emotional impact of having a positive diagnosis, fragile X testing is seldom administered without genetic counselling. A number of studies have shown that genetic counselling can help those affected by fragile X

syndrome understand the nature of the disease and its heritability, in order to cope with the emotional burden and make informed reproductive decisions. It is desirable that genetic counselling and informed consent be included in the process of cascade testing.

One of the issues surrounding cascade testing is the testing of children, where parents may request that their child be tested for late-onset disease, disease susceptibilities and carrier status. The Human Genetics Society of Australasia advocates that 'testing of children under 18 years of age only be considered where the result is likely to be of direct benefit to the child though medical surveillance or intervention'.

Cost-effectiveness

A cascade testing program is estimated to cost up to $4 million annually, and would result in a cost per initial case detected of between $14,000 and $28,000, depending on assumptions made, especially that of the detection rates in the population.

This does not account for the costs of anxiety surrounding testing programs, although there may be certain benefits associated with reassurance to be balanced against these costs. Nor does it take into account the social costs and consequences of providing the information to individuals such as the decision to abort a pregnancy or the additional costs to society of caring for a disabled person.

Costs may be greater downstream, in terms of the costs of choices individuals make. These may include further diagnosis, abortion, or lifetime costs of having a child with fragile X.

Recommendation

MSAC recommended that on the strength of the evidence pertaining to Genetic Test for Fragile X Syndrome using DNA analysis, public funding should be supported for the use of:

Nucleic Acid Amplification (NAA) in those with specific clinical features of Fragile X (A) syndrome, including intellectual disabilities, and in first and second degree relatives of individuals with the Fragile X (A) mutation and Southern Blot where the results of NAA testing are inconclusive."

- The Minister for Health and Ageing accepted this recommendation on 20 August 2002.

Appendix A MSAC terms of reference and membership

MSAC's terms of reference are to:

- advise the Minister for Health and Ageing on the strength of evidence pertaining to new and emerging medical technologies and procedures in relation to their safety, effectiveness and cost-effectiveness and under what circumstances public funding should be supported;
- advise the Minister for Health and Ageing on which new medical technologies and procedures should be funded on an interim basis to allow data to be assembled to determine their safety, effectiveness and cost-effectiveness;
- advise the Minister for Health and Ageing on references related either to new and/or existing medical technologies and procedures; and
- undertake health technology assessment work referred by the Australian Health Ministers' Advisory Council (AHMAC), and report its findings to AHMAC.

The membership of MSAC comprises a mix of clinical expertise covering pathology, nuclear medicine, surgery, specialist medicine and general practice, plus clinical epidemiology and clinical trials, health economics, consumers, and health administration and planning:

Member	Expertise or Affiliation
Dr Stephen Blamey (Chair)	general surgery
Professor Bruce Barraclough	general surgery
Professor Syd Bell	pathology
Dr Paul Craft	clinical epidemiology and oncology
Professor Ian Fraser	reproductive medicine
Associate Professor Jane Hall	health economics
Dr Terri Jackson	health economics
Ms Rebecca James	consumer health issues
Professor Brendon Kearney	health administration and planning
Mr Alan Keith	Assistant Secretary, Diagnostics and Technology Branch, Commonwealth Department of Health and Ageing
Associate Professor Richard King	internal medicine
Dr Ray Kirk	health research
Dr Michael Kitchener	nuclear medicine
Mr Lou McCallum	consumer health issues
Emeritus Professor Peter Phelan	paediatrics
Dr Ewa Piejko	general practice
Dr David Robinson	plastic surgery
Professor John Simes	clinical epidemiology and clinical trials

Professor Richard Smallwood	Chief Medical Officer, Commonwealth Department of Health and Ageing
Professor Bryant Stokes	neurological surgery, representing the Australian Health Ministers' Advisory Council
Associate Professor Ken Thomson	radiology
Dr Douglas Travis	urology

Appendix B Supporting committee

Supporting committee for MSAC application 1035 Genetic Test for Fragile X Syndrome

Emer Prof Peter Phelan (Chair) BSc, MBBS, MRACP, MD, FRACP Emeritus Professor of Paediatrics University of Melbourne	Member of MSAC
Mrs Denise Birney BAppSc, MC Secretary, Queensland Fragile X Association, Inc.	Nominated by the Consumers' Health Forum
Dr Ross Brookwell BSc (Hons), PhD, HGSACC Laboratory Manager Genetics Department Sullivan Nicolaides Pathology, Qld	Nominated by the Pathology Services Table Committee
Dr Michael Buckley FRCPA, PhD Director of Molecular and Cytogenetics South Eastern Area Laboratory Services, NSW	Nominated by the Pathology Services Table Committee
Dr Jonathan Cohen MBBS, FRACGP, MFamMed Senior Lecturer Centre for Developmental Disability Health, Vic Monash University Department of General Practice Medical Director, Fragile X Alliance Clinic	Nominated by the Royal Australian College of General Practitioners
Assoc Prof Eric Haan AccClinGenet, BMedSc, MBBS, FRACP Head, Clinical Genetics Service Women's and Children's Hospital Adelaide, SA	Co-opted member
Ms Emily Lancsar BEc, BA, PostGradDip Health Econ Senior Health Economist Centre for Health Economics, Research and Evaluation, NSW	Nominated by the Centre for Health Economics, Research and Evaluation
Dr Anne Turner AccClinGenet, MBBS (Hons), FRACP Head of Clinical Genetics Service South Eastern Area Health Service, NSW	Nominated by the Royal Australian College of Physicians

Appendix C Studies included in the review

Item 1

1.1 What are the diagnostic characteristics of PCR compared to cytogenetic testing?

Brown WT, Houck GE, Jr., Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS & Jenkins EC (1993). Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. *Journal of American Medical Association* **270:** 1569-1575

1.2 What are the diagnostic characteristics of Southern blot compared to cytogenetic testing?

Brown WT, Houck GE, Jr., Jeziorowska A, Levinson FN, Ding X, Dobkin C, Zhong N, Henderson J, Brooks SS & Jenkins EC (1993). Rapid fragile X carrier screening and prenatal diagnosis using a nonradioactive PCR test. *Journal of American Medical Association* **270:** 1569-1575.

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Appendix D Literature on economic costs of fragile X testing

Publication	Assumptions	Costs	Outcomes	Results
(Murray et al 1997)	DNA testing; Antenatal screening (based on population model); Baseline: no-one refuses screen, prenatal diagnosis, or termination, and two pregnancies	Information giving: £2 Genetic counselling: £25 Prenatal diagnostic procedures: £275 DNA test: £25	Preventing one affected birth	Average cost of preventing one affected birth is £93,000. Reduced uptake does not alter this markedly as screening and diagnostic tests make up most of the costs. Cost does increase in proportion to the number who do not want diagnosis or termination. If there is 75% uptake and 75% diagnosis, cost of preventing one affected death rises to £124,000.
(Gabarron et al 1992)	Cytogenetic test: Tested mentally retarded males identified through examining medical records in special schools and sheltered workshops, then family studies were undertaken to identify mutations and women at risk, who were then given genetic counselling including prenatal diagnostic information.	Overall program: US$200,000	Preventing birth of one affected male	Cost per preventing birth of one affected male is US$12,740 (1992 prices). Authors comment that given a more definite approach, such as DNA testing, cost-effectiveness would improve.
(Nolin et al 1991)	Cytogenetic test; Identified males at risk using a screening form identifying features such as physical, behavioural or family history, for mentally retarded males with living relatives in state operated developmental centres, or community residences.	Cytogenetic analysis: US$400	Detection of affected males and possible females with mutations	Cost per completed analysis: US$64,400
(Turner et al 1986)	Cytogenetic test; Cascade testing; tested intellectually handicapped identified through public schools and sheltered workshops. Family studies were then undertaken to identify those at risk with mutations under 35 with no children, who were then given genetic counselling, and alerted to the availability of antenatal diagnosis	Staffing costs: AUS$300,000; Lifetime costs of raising child with fragile X: AUS$1 million (there may be further costs including continuing counselling, the availability of antenatal diagnostic facilities, screening in schools)	Prevention of birth of intellectually handicapped boy	AUS$14,200 (1986 prices)(including a chorionic villus biopsy investigation) to prevent the birth of one intellectually handicapped boy (women are assumed to request antenatal diagnosis)
(van der Riet et al 1997)	DNA tests; Identification from at risk couples who ask for information, who are related to an affected person who want identification as possibly having mutations or who want prenatal diagnosis.	Test: DFL1,200; Delivery: DFL3,916; Abortion: DFL987; Curettage: DFL668; Lifetime costs of care: DFL 4.1m (1994 exchange rate to US$ 1.92)	Risk of son with fragile X; Information about genetic risks available to family, (giving better chance of health child, but higher risk of termination); savings/costs from avoiding lifetime costs.	At prior risk of 45%, DNA diagnosis resulted in savings per couple of DFL321,417; at 22.5% risk saving is DFL118,034. Actively screening for families at high risk would be beneficial, compared to couples presenting themselves.

Publication	Assumptions	Costs	Outcomes	Results
(Vintzileos et al 1999)	DNA tests; Routine prenatal mutation testing using a cost benefit equation (see Appendix 2); assumptions include: Therapeutic Abortion Rate 50%-80%; Prevalence 1:4000; 1:250 pregnant women test positive for mutation status; 2 pregnancies.	Lifetime costs US$500,000; DNA rest: US$250; Amnio package: US$1,300	Savings/costs from avoiding the lifetime costs of an individual with fragile X	Depending on assumptions prenatal screening leads to losses overall of US$10 million to US$195 million in USA. The cost per case detected would range from US$20,833 (mature program with 100% TAB), to US$770,833 per case (first year of program, 50% TAB). In addition between 46 and 115 foetal lives would be lost annually through amniocentesis.
(Wildhagen et al 1998)	DNA tests; Prenatal screening, preconceptional screening, and school mutation screening (based on population model); decision analysis model; lack of knowledge therefore large number of assumptions made; women targeted; 1:4000 prevalence; premutation frequency 1:435; 90% of detected male and 45% female aborted.	Information prior to screening: media/leaflet: US$6, organisational cost US$19; DNA extraction:US$9; Cost of test: US$99; Counselling those with mutations: US$114; Prenatal diagnosis: USD1,436; Abortion: US$78-502; Lifetime cost of care US$750,000	Costs/savings of precluding lifetime medical costs if not born; detected with mutation	US$45,000 per detected with mutation; screening cost saving overall – US$2 million for school screening; US$9 million for preconceptional screening; US$14 million for prenatal screening.

Abbreviations

CGG	cytosine-guanine-guanine
CVS	chorionic villus sampling
DNA	deoxyribonucleic acid
FM	full mutation
FMR	fragile site mental retardation
FMRP	FMR protein
FN	false negative
FP	false positive
FPR	false positive rate
FRAX	Fragile X
HTA	Health Technology Assessment
IQ	Intelligence Quotient
LR	likelihood ratio
NAA	nucleic acid amplification
NHS (UK)	National Health Service (UK)
PCR	polymerase chain reaction
PM	pre-mutation
Sne	sensitivity
Spe	specificity
TN	true negative
TP	true positive

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