

Medical Services Advisory Committee (MSAC) Public Summary Document

Application No. 1675 – Whole Genome Sequencing for the diagnosis of mitochondrial disease

Applicant: Australian Mitochondrial Disease Medical Network Ltd

Date of MSAC consideration: 24-25 November 2022

Context for decision: MSAC makes its advice in accordance with its Terms of Reference, [visit the MSAC website](#)

1. Purpose of application

An application requesting public funding of virtual panel-based whole genome sequencing (WGS) or whole exome sequencing (WES) and mitochondrial DNA (mtDNA) sequencing, for the diagnosis of mitochondrial disease (MD) in patients who are suspected of having either acute or chronic disease, and cascade testing of their biological relatives and testing of their reproductive partners, was received from the Australian Mitochondrial Disease Medical Network Ltd by the Department of Health and Aged Care.

2. MSAC's advice to the Minister

After considering the strength of the available evidence in relation to comparative safety, clinical effectiveness, cost-effectiveness and total cost, MSAC supported the creation of new Medicare Benefits Schedule (MBS) items for genetic testing for mitochondrial disease. MSAC advised that this was an area of significant unmet clinical need, and that genetic testing was safer and more effective than the current diagnostic process, including muscle biopsy. MSAC considered that this testing would increase diagnostic certainty and provide benefits in changing patient management. It would also inform the risk of disease in relatives, and support informed reproductive decision-making. Publicly funding this testing would support equitable access to targeted therapies, and identify when mitochondrial donation may be appropriate. MSAC advised the testing was cost-effective, and that the financial cost to the MBS was modest and acceptable, and there would also be cost-offsets to healthcare funded by the states and territories.

MSAC supported MBS items for testing affected individuals using singleton and trio virtual gene panel-based analysis of whole exome or genome data, data re-analysis, mitochondrial DNA deletion testing, cascade testing of biological relatives, reproductive partner testing, and fetal testing.

Consumer summary
<p>This was an application from the Australian Mitochondrial Disease Medical Network Ltd requesting Medicare Benefits Schedule (MBS) listing of genetic testing to diagnose mitochondrial diseases.</p> <p>Mitochondria make energy within cells of the human body. They are often called the powerhouses of the cell. There can be hundreds or thousands of mitochondria within a single</p>

Consumer summary

human cell. Mitochondrial disease is rare and affects a person's ability to make enough energy for the body. Mitochondrial disease can affect children and adults. It can affect different organs with different severity. Patients with mitochondrial disease can have a wide range of symptoms. Mitochondrial disease is usually diagnosed through tests such as muscle biopsies (minor surgery to take a sample of muscle tissue), but these do not provide a definite result without additional genetic testing.

There are variants in at least 350 genes that are known to cause mitochondrial disease. Most of these genes are in the main DNA in the nucleus of the cell (called nuclear DNA), but some of the genes are located in separate DNA within the mitochondria (also called mitochondrial DNA, or the mitochondrial genome). MSAC considered that it was important that genetic testing for mitochondrial disease looks for variants in both nuclear DNA and mitochondrial DNA.

This application proposed different options for genetic testing in patients suspected of having mitochondrial disease: next generation sequencing with analysis of only genes known to cause mitochondrial disease, known as virtual panel testing, plus testing for deletion variants in mitochondrial DNA. Virtual gene panel testing can be done by looking at all the coding parts of a patient's genetic make up (whole exome sequencing, WES) or by sequencing all of a patient's genetic make up (whole genome sequencing, WGS). WGS also looks at the mitochondrial DNA, which is generally not included in WES.

Because there are so many mitochondria in each cell, it can be difficult to detect disease-causing genetic variants in mitochondrial DNA if they are only present in a small proportion of mitochondria or only in some tissues. Although virtual panel analysis using WGS is more effective because it includes mitochondrial DNA, MSAC considered both WGS and WES with mitochondrial DNA sequencing were similarly effective at detecting genetic variants that could cause mitochondrial disease, so supported both types of virtual gene panel test. MSAC considered genetic testing for mitochondrial diseases to be good value for money.

If testing the affected person finds a genetic variant causing their mitochondrial disease, then the patient's relatives may choose to have testing to see if they also have the genetic variant. This is called cascade testing. For variants in nuclear DNA, it may also be useful to test reproductive partners for variants in the same gene that could result in their child being born with mitochondrial disease. This allows couples whose genetic tests show these variants to make informed reproductive choices, including choosing to have pre-implantation or prenatal fetal testing if they wish to.

MSAC considered genetic testing for mitochondrial disease to be safer than current testing, as it only requires a blood sample. It is also more effective as it can provide a more accurate diagnosis than current test methods, and sooner. People with correctly diagnosed mitochondrial diseases may be able to avoid tests such as biopsies.

Public funding for genetic testing would support better access to the treatments that are available for some patients. MSAC advised that genetic testing for mitochondrial disease would have a modest and acceptable cost to the MBS and that it will reduce the cost to the State and Territories' health budgets because tests such as biopsies are able to be avoided.

MSAC's advice to the Commonwealth Minister for Health and Aged Care

MSAC supported listing genetic testing for mitochondrial disease on the MBS. MSAC considered the testing to be safe, effective, good value for money, and to have an acceptable financial cost.

3. Summary of consideration and rationale for MSAC's advice

MSAC noted that this application from the Australian Mitochondrial Disease Medical Network Ltd was a request for MBS funding of virtual gene panel-based analysis of whole genome sequencing (WGS) or whole exome sequencing (WES) and mitochondrial DNA (mtDNA) sequencing data, and also mitochondrial deletion testing, for the diagnosis of mitochondrial disease (MD) in patients who are suspected of having either acute or chronic disease. The application also included cascade testing of the patient's biological relatives, as well as reproductive partner testing and fetal testing. MSAC noted that it has not previously considered genetic testing for mitochondrial disease.

MSAC noted that MDs are the most common group of inheritable disorders caused by genetic variants in either mtDNA or nuclear DNA (nDNA), affecting both children and adults. There are currently more than 350 genes identified in which variants can cause MD. MDs have widely heterogeneous clinical syndromes and clinical manifestations; they can range from mild or oligo-symptomatic disease affecting only a single organ, through to severe or life-threatening multi-organ dysfunction. MDs can follow different modes of inheritance including maternal, autosomal recessive, autosomal dominant, X-linked recessive, or X-linked dominant.

MSAC noted that the evidence is limited given the rarity of MD, though an estimated 120,000 Australians carry a disease-causing mtDNA variant, and approximately one affected Australian child is born each week, so considered there to be a high clinical need for this testing. The current diagnostic pathway for MD combines muscle, liver, or other tissue biopsy (MBS item 30075, and age-appropriate anaesthetic items), which are then analysed using MBS-subsidised diagnostic tests such as complex histology (MBS item 72380), enzyme histochemistry (MBS item 72844) and immunohistochemistry (MBS item 72846). MSAC considered the current diagnostic process to be complex, potentially painful, and noted that it may not yield a definitive result. MSAC considered the main benefit of genetic testing was that it may allow affected individuals to avoid biopsies and other investigations, and also considered genetic testing is more effective and can provide more certainty than the current pathway to diagnose MD. MSAC noted delays in diagnosing MD using the current pathway, and considered that MBS-funded genetic testing is faster so would shorten the diagnostic odyssey. MSAC considered this is reflective of a broader paradigm shift from a biopsy-first approach followed by targeted genetic testing, to a genetics-first approach using NGS technology followed by functional validation¹. MSAC also considered the effectiveness of cascade and reproductive testing to be superior to current care, and that it would improve confidence for reproductive decision-making. MSAC considered that genetic testing in relation to MD may allow access to (variably effective) targeted therapies, inform family members whether they also carry MD-causing variants, and in the context of reproductive decision-making could potentially in future also support the use of donor mitochondria under the Mitochondrial Donation Law Reform (Maeve's Law) Act 2022. MSAC considered this testing would also have secondary benefits such as access to clinical trials, and non-health benefits including access to disability services. MSAC noted the applicant's pre-MSAC response also commented in relation to non-health benefits, and stated more than 200 relevant clinical trials are registered at present. MSAC noted patient and consumer comments on this application also stated that a diagnosis of MD can take years at present, and that genetic testing for MD would reduce the diagnostic odyssey and its associated strain on patients and their families.

¹ Watson E, Davis R, Sue CM (2020). New diagnostic pathways for mitochondrial disease. *J Transl Genet Genom*, 4: 188-202.

MSAC noted that the four proposed populations were limited to patients with specific indications:

- Population A – affected individuals: adults and children suspected of having either acute or chronic MD, based on clinical signs and symptoms
- Population B – biological relatives who may have the pathogenic or likely pathogenic (P/LP) variant identified in the proband
- Population C – reproductive partners of people with a recessive P/LP variant
- Population D – fetuses at risk of MD due to the parents' genotypes.

MSAC noted that there were six proposed MBS items:

- AAAA – singleton testing of the affected individual, once per lifetime (fee = \$2,100)
- BBBB – trio testing of the affected individual and their biological parents, once per lifetime (fee = \$2,900)
- CCCC – re-analysis of WGS/WES plus mtDNA data, at least 18 months after previous genetic testing for the duration of the patient's illness or until a diagnosis is confirmed (fee = \$500)
- GGGG – testing of a fetus at risk of having MD based on the parents' genotypes, once per fetus (fee = \$400)
- HHHH – mtDNA deletion testing for patients strongly suspected of having a mtDNA deletion and in whom WGS or mtDNA sequencing and analysis was non-informative, once per lifetime (fee = \$450)
- IIII – whole gene sequencing of the relevant gene(s) in the reproductive partner of an individual with a recessive P/LP variant for MD, once per gene per partner per lifetime (fee = \$1,200)
- JJJJ – mtDNA sequencing and analysis (using other tissue such as muscle), once per lifetime (fee = \$1,200)
- KKKK – cascade testing of biological relatives, once per variant per lifetime (fee = \$400).

MSAC noted that virtual panel testing using both WES and WGS were proposed at the same fee, but that since WES does not typically include mtDNA sequencing, if WES is used then additional testing is required to sequence mtDNA if no genetic diagnosis was made based on nuclear DNA. MSAC noted that ESC had therefore advised WGS appeared to be both superior and more cost-effective than WES+mtDNA sequencing, and noted the applicant in the pre-MSAC response also supported limiting virtual panel testing to WGS. MSAC noted there are techniques that enrich mitochondrial DNA and allow sequencing of mtDNA at greater read depth, and that detection of mtDNA variants also depends on the bioinformatic pipeline to be used, as pipelines can discard mtDNA results. MSAC also noted ESC's advice that the evidence in the DCAR showed there was no significant difference in diagnostic yield (DY) for a diagnosis of MD between WGS and WES±mtDNA analysis. MSAC also noted that similar DY was also shown in unpublished data from the Australian Genomics Mitochondrial Disease Flagship², which reported a DY of 49% for WGS (n = 68) versus 46% for WES ±mtDNA sequencing (n=72). MSAC considered the evidence did not support WGS having superior effectiveness compared to WES+mtDNA sequencing, and therefore it supported both WES and WGS as backgrounds for virtual panel testing. MSAC advised that testing should be performed at a read depth that is adequate to detect mitochondrial variants present at low levels of heteroplasmy, and that this should be addressed in a practice note. MSAC considered this to be particularly important for virtual panel testing in adults, in whom P/LP variants are more often found in mtDNA.

² Prof John Christodoulou's slides presented at Mito Foundation meeting 7 May 2022: "Smashing the diagnostic odyssey: updates from the Australian Genomics Mitochondrial Disease Flagship project". Available at: <https://www.mito.org.au/wp-content/uploads/2022/05/Christodoulou-Slides-Melbourne-2022.pdf>

MSAC noted that the order in which WES and mtDNA sequencing are conducted could differ depending on the patient's age. MSAC considered that it created unnecessary complexity to have a separate MBS item for mtDNA sequencing (JJJJ), and advised mtDNA sequencing should be included with WES/WGS in the virtual gene panel testing items (AAAA and BBBB). MSAC considered that WGS with sufficient read depth to detect mtDNA variants present at low heteroplasmy levels would cost around \$2,100 for a singleton analysis. MSAC noted that a fee of \$2,100 for singleton virtual panel analysis had been proposed based on the childhood syndromes WES/WGS item 73358. MSAC noted that the bulk billing rate for MBS items 73358 and 73359 is almost 100% in the public setting and about 93% in the private setting, that patients are not incurring out-of-pocket costs, and that actual utilisation was under the predicted utilisation suggesting that the current MBS fee levels were not driving unnecessary testing. MSAC considered that the laboratory cost to conduct standard exome or genome testing is approximately \$900, and that because virtual gene panels are pre-curated lists of relevant genes only approximately \$300 of curation and reporting time is required in addition, making \$1,200 the appropriate fee for virtual gene panel testing of nDNA alone. MSAC considered that analysing mtDNA is also important in diagnosing MD, and that mtDNA sequencing costs approximately \$900 in other laboratories, such as \$947 at SA Pathology³. MSAC therefore advised that a fee of \$2,100 is appropriate for singleton virtual panel testing for MD using either WGS to the read depth required to detect mtDNA variants at low heteroplasmy, or WES±mtDNA sequencing (AAAA). MSAC considered that the cost to conduct virtual gene panel testing on an exome background is lower than the Victorian Clinical Genetics Services (VCGS) charges for this service. MSAC considered that if a genetic diagnosis could be made based on either WES or mtDNA sequencing alone, then for that patient the second method (mtDNA sequencing or WES) would not be required. MSAC confirmed that where either WES or mtDNA sequencing had a negative or inconclusive result, then the second method would be required. MSAC considered that trio testing should cost \$1,200 more than singleton testing, as library preparation and analysis for a sample costs a minimum of \$600. MSAC therefore advised trio virtual panel testing, using either WGS or WES ±mtDNA sequencing (BBBB), should have a fee of \$3,300.

MSAC agreed with ESC that the appropriate fee for fetal testing for familial variants (GGGG) was \$1,600 and noted the DCAR's sensitivity analysis of a fee of \$1600 for GGGG showed that raising the fee for fetal testing (GGGG) to \$1,600 had very little effect on the cost-effectiveness and financial estimates.

MSAC noted that the proposed MBS item descriptors listed several types of specialists that could request the genetic testing. MSAC considered that requestors improve in DY as they gain experience in recognising the signs of mitochondrial disease and that listing a defined set of specialties was not necessary. MSAC advised that a more pragmatic way to address requestors who have the relevant expertise ordering these tests would be for the descriptor to simply describe the requestor as a specialist with expertise in mitochondrial disease.

MSAC noted that the applicant proposed using PanelApp Australia⁴ to define the list of genes for virtual panel analysis, and advice from the Department that Australian legislation does not allow a website to be referred to in MBS item descriptors. MSAC considered that PanelApp UK or another curated and recognised reference source for a comprehensive list of relevant genes would be appropriate, so advised a practice note referring generically to "a recognised test directory" should be used instead to allow the pathologist to choose an appropriate high-quality reference source to use in determining the genes to be assessed on the virtual panel.

³ SA Pathology's listed cost to conduct "Mitochondrial gene mutation screen" in blood, tissue or urine. Source: <https://www.sapathology.sa.gov.au/wps/wcm/connect/sa+pathology+internet+content+new/content/clinicians/pathology+collection+guide> [Accessed 6 February 2023]

⁴ PanelApp Australia – available at: <https://panelapp.gha.umccr.org/>

MSAC noted that CCCC was proposed by the applicant to have a minimum timeframe for re-analysis of 18 months in line with similar previously supported re-analysis items, but that ESC had proposed the minimum interval be extended to 24 months based on newly published systematic review (k=29) of re-analysis in Australian populations⁵. MSAC agreed that the appropriate minimum timeframe for re-analysis was 24 months.

MSAC considered that re-analysis is for previously unreported variants, so would not apply to HHHH as that is for testing a single mtDNA deletion or other variant, and advised HHHH should be removed from re-analysis item CCCC.

MSAC noted that frequency restrictions such as 'once per lifetime' can be enforced in an automated manner through Medicare payment systems prior to the payment of benefits, but that others such as 'once per gene per partner per lifetime' are may not be automatically enforced, and are typically enforced through post-payment compliance activity. MSAC considered that stating the intended frequency may guide requestors, and on balance considered it more appropriate to include frequency restrictions that cannot be enforced in an automated manner prior to the payment of benefits as guidance in a practice note. MSAC noted the genes listed in the PanelApp Australian mitochondrial disease panel included some genes with an X-linked mode of inheritance. MSAC considered that fetal testing would also be relevant where the familial variant(s) have an X-linked mode of inheritance, and advised this should also be included in fetal testing item GGGG.

MSAC's supported item descriptors are provided at the end of this section (Table 1).

MSAC agreed with ESC that the comparator of no genetic testing did not accurately reflect testing funded by the States and patients under current clinical practice, though considered that the comparator should be a health technology with established cost-effectiveness. MSAC considered that existing publicly funded genetic testing (i.e., in public hospitals funded by the States and Territories), which was common gene panels and single gene tests, already provides some of the benefits that are claimed to be associated with virtual gene panel testing, and also incurs costs.

MSAC noted the proposed clinical management algorithm and considered genetic testing would simplify the current diagnostic process for patients suspected of having MD.

MSAC considered that there were no significant safety concerns with genetic testing, and considered genetic testing to have non-inferior safety in adults and superior safety in children, because at present children often have a muscle biopsy under a general anaesthetic, which may be able to be avoided through genetic testing.

MSAC considered that overall there was relatively limited evidence (mostly limited to case series) for most components of the assessment, except for test accuracy in detection of P/LP variants. However, due to the rarity of MDs MSAC considered it unlikely that better evidence would become available. MSAC noted that in three Australian studies, 2.3–9.0% of patients had a change in clinical management, including 6.7% (9/130) receiving a variant-specific targeted treatment or avoiding contraindicated medication. In Davis 2022⁶, 2.3% (3/130) of patients were also diagnosed with a treatable non-MD condition that mimics MD and received condition-specific treatment. MSAC also noted that 84% (37/44) of paediatric patients with a MD diagnosis had some form of change in management, including diet and exercise changes, and avoided additional investigations. MSAC considered the largest advantage of earlier accurate diagnosis of

⁵ Dai P, Honda A, Ewans, L, et al. (2022). Recommendations for next generation sequencing data reanalysis of unsolved cases with suspected Mendelian disorders: a systematic review and meta-analysis. *Genetics in Medicine*, **24**(8): 1618–29.

⁶ Davis, RL, Kumar, KRR, Puttick, C, et al. 2022, 'Use of whole genome sequencing for mitochondrial disease diagnosis', *Neurology*, 99(7): pp. e730-e742.

MDs (or phenocopies) through genetic testing is a likely reduction in the need for invasive investigations such as muscle biopsies.

MSAC noted that the economic evaluation was a cost-effectiveness analysis and cost-consequences analysis, and the economic model took the form of a decision tree analysis incorporating the estimates of definitive molecular diagnosis achieved in patients suspected of having MD using WGS, using scenarios to show the marginal cost-effectiveness of adding cascade testing and reproductive-related testing. MSAC noted the incremental cost-effectiveness ratios (ICERs) reported in the DCAR for the adult and paediatric affected individual populations:

- \$1,832 (adults) and \$6,721 (children) per proband detected (population A)
- \$1,301 (adults) and \$2,076 (children) per person with a positive genotype detected (populations A and B)
- \$1,320 (adults) and \$2,075 (children) per person (including a fetus) with a positive genotype detected (populations A, B, C and D).

MSAC considered the cost-effectiveness of genetic testing for MD to be acceptable, and in line with previously supported ICERs using similar measures of effectiveness.

MSAC noted that the main driver of the ICER was DY (currently 50–60%; lower DY increases the ICER), which was influenced by factors including syndromic/non-syndromic presentation, singleton/trio testing proportion and adult/paediatric proportion. Other factors that sensitivity analyses showed affected the cost-effectiveness in the adult and/or paediatric affected individual populations included:

- the proportion of people having muscle biopsies in the absence of WGS (higher proportion reduced the ICER)
- the proportion of virtual panel tests that use WGS rather than WES±mtDNA sequencing (more WGS decreased the ICER)
- the appropriate fees (lower fees decreased the ICER)
- the cost of muscle biopsy (lower biopsy cost increased the ICER)
- the cost of pre- and post-test genetic counselling (excluding the cost reduced the ICER).

MSAC noted that the application estimated that 400 adults and 52 children nationally will seek virtual panel testing for the diagnosis of MD within the first year of listing, including a backlog of 100 adult patients and 20 children waiting for this service in 2021. MSAC noted that previous assessments of genetic tests had estimated three first-degree relatives would be tested per proband.

MSAC noted that the proposed financial cost of genetic testing for MD to the MBS was \$1.2 million in Year 1, decreasing to \$1.0 million in Year 5, which it considered to be modest. MSAC further noted that updates to the financial analyses to reflect its advice on fees and items reduced the financial cost to the MBS to \$997,000 in Year 1, decreasing to \$834,000 in Year 5 (italicised rows in Table 24). MSAC also noted that there would be a cost-offset to the States and Territories (\$870,000 in Year 1) due to biopsies avoided, resulting in a net financial cost of genetic testing for MD across all health budgets of \$91,000 to \$154,000 per year taking into account updated costs to the MBS.

MSAC considered the listing should be reviewed after two years, including to review uptake, diagnostic yield, associated MBS items (such as for biopsy), and the requesting specialty groups.

Table 1 MSAC's supported item descriptors

Category 6 – PATHOLOGY SERVICES Group P7 – GENETICS
<p>MBS item AAAA</p> <p>Characterisation by whole genome sequencing or either or both whole exome sequencing and mitochondrial DNA sequencing, of germline variants present in nuclear DNA and in mitochondrial DNA, from a comprehensive list of phenotypically driven genes associated with mitochondrial disorders, of a patient with a strong suspicion of a mitochondrial disease if:</p> <p>(a) the characterisation is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and</p> <p>(b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:</p> <ul style="list-style-type: none"> (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or (ii) evident mitochondrial dysfunction or decompensation, and/or (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or (vi) cardiomyopathy and/or cardiac arrhythmias, and/or (vii) rapid hearing or painless visual loss or ptosis, and/or (viii) stroke-like episodes or nonvasculitic strokes, and/or (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or (x) external ophthalmoplegia, and/or (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or (xii) family history of mitochondrial disease, or any of the above; and <p>(c) the characterisation is not performed in conjunction with a service to which items BBBB, 73358 or 73359 applies</p> <p>Applicable only once per lifetime</p> <p>Fee: \$2,100.00 Benefit: 75% = \$1,575.00 85% = \$2,006.80</p>
<p>MBS item BBBB</p> <p>Characterisation by whole genome sequencing or either or both whole exome sequencing and mitochondrial DNA sequencing of germline variants present in nuclear DNA and in mitochondrial DNA, from a comprehensive list of phenotypically driven genes associated with mitochondrial disorders, of a patient with a strong suspicion of a mitochondrial disease if:</p> <p>(a) the characterisation is performed using a sample from the patient and a sample from each of the patient's biological parents; and</p> <p>(b) the request for the characterisation states that singleton testing is inappropriate; and</p> <p>(c) the characterisation is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and</p> <p>(d) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:</p> <ul style="list-style-type: none"> (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or (ii) evident mitochondrial dysfunction or decompensation, and/or (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or (vi) cardiomyopathy and/or cardiac arrhythmias, and/or

(vii) rapid hearing or painless visual loss or ptosis, and/or
 (viii) stroke-like episodes or nonvasculitic strokes, and/or
 (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
 (x) external ophthalmoplegia, and/or
 (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
 (xii) family history of mitochondrial disease, or any of the above; and
 (e) the characterisation is not performed in conjunction with a service to which item AAAA, 73358 or 73359 applies.
 Applicable only once per lifetime
 Fee: \$3,300.00 Benefit: 75% = \$2,475.00 85% = \$3,206.80

MBS item CCCC
 Re-analysis of whole genome or whole exome or mitochondrial DNA data obtained in performing a service to which item AAAA or BBBB applies, for characterisation of previously unreported germline variants related to the clinical phenotype, if:
 (a) the re-analysis is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and
 (b) the patient is strongly suspected of having a monogenic mitochondrial disease; and
 (c) the re-analysis is performed at least 24 months after:
 (i) a service to which item AAAA or BBBB applies; or
 (ii) a service to which this item applies
 Applicable twice per lifetime.
 Fee: \$500.00 Benefit: 75% = \$375.00 85% = \$425.00

MBS item GGGG
 Testing of a pregnant patient for detection of gene variant/s present in the parents for diagnostic purpose, in the fetus, if
 (a) the gene variant/s is:
 (i) a variant in the mitochondrial genome identified in the oocyte donating parent; or
 (ii) autosomal recessive variants identified in both biological parents within the same gene; or
 (iii) an autosomal dominant or X-linked variant identified in either biological parent; or
 (iv) identified in a biological sibling of the fetus; and
 (b) the causative variant/s for the condition of the fetus's first-degree relative have been confirmed by laboratory findings; and
 (c) the results of the testing performed for the first-degree relative are made available for the purpose of providing the detection for the fetus; and
 (d) the detection is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and
 (e) the detection is not performed in conjunction with a service to which item KKKK, 73361, 73362 or 73363 applies
 Fee: \$1,600.00 Benefit: 75% = \$1,200.00 85% = \$1,506.80

MBS item HHHH
 Characterisation of a single mitochondrial DNA deletion or variant for diagnostic purposes in a patient suspected to have mitochondrial disease based on the following criteria:
 (a) the characterisation is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and
 (b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:
 (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
 (ii) evident mitochondrial dysfunction or decompensation, and/or
 (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
 (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
 (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
 (vi) cardiomyopathy and/or cardiac arrhythmias, and/or

<p>(vii) rapid hearing or painless visual loss or ptosis, and/or</p> <p>(viii) stroke-like episodes or nonvasculitic strokes, and/or</p> <p>(ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or</p> <p>(x) external ophthalmoplegia, and/or</p> <p>(xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or</p> <p>(xii) family history of mitochondrial disease, or any of the above; and</p> <p>(c) the characterisation is performed following the performance for the patient of a service to which items 73292, AAAA, BBBB, 73358 or 73359 applies for which the results were non-informative; and</p> <p>Applicable three times per lifetime</p> <p>Fee: \$450.00 Benefit: 75% = \$337.50 85% = \$382.50</p>
<p>MBS item IIII</p> <p>Whole gene testing of a person for the characterisation of all germline gene variants within the same gene in which the person's reproductive partner has a pathogenic or likely pathogenic germline recessive gene variant for mitochondrial disease confirmed by laboratory findings; and the characterisation is:</p> <p>(a) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or</p> <p>(b) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease.</p> <p>Fee: \$1200.00 Benefit: 75% = \$900.00; 85% = 1,106.80</p>
<p>MBS item KKKK</p> <p>Testing of a person (the person tested) for the detection of a single gene variant, if:</p> <p>(a) the person tested has a biological relative with a known pathogenic or likely pathogenic mitochondrial disease variant confirmed by laboratory findings that can be plausibly shared between them; and</p> <p>(b) the results of the testing performed for the person tested are made available for the purpose of providing the detection; and</p> <p>(c) the detection is requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; and</p> <p>(d) the detection is not performed in conjunction with a service to which item 73361, 73362 or 73363 applies</p> <p>Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00</p>

85% benefit reflects the 1 November 2022 Greatest Permissible Gap (GPG) of \$93.20. All out-of-hospital Medicare services that have an MBS fee of \$621.50 or more will attract a benefit that is greater than 85% of the MBS fee – being the schedule fee less the GPG amount. The GPG amount is indexed annually on 1 November in line with the Consumer Price Index (CPI) (June quarter).

Practice notes:

AAAA, BBBB: The list of phenotypically driven genes should be based on a recognised test directory.

AAAA, BBBB: The read depth should be sufficient to detect mitochondrial variants that are present at a low level of heteroplasmy. Where whole exome sequencing is performed first, if no genetic diagnosis is made using whole exome sequencing, then mitochondrial DNA sequencing should also be performed.

GGGG, HHHH, IIII: new practice note:

Genomic testing for mitochondrial disease

Item GGGG: Testing should not be required more than once per fetus; additional testing should only be performed if it is clinically relevant.

Item HHHH: Testing should not be required more than once per tissue type; additional testing should only be performed if it is clinically relevant.

Item IIII: Testing should not be required more than once per gene per lifetime; additional testing should only be performed if it is clinically relevant.

4. Background

MSAC has not previously considered WGS/WES for MD diagnosis in individuals with suspected MD, and cascade testing of their biological relatives and testing of their reproductive partners.

The terms WGS or WGS/WES used throughout this document refer to analysis of whole genome and/or exome DNA sequence data using a virtual panel of MD-related genes. The proposed use of WGS/WES for the diagnosis of MD in patients suspected of having the disease and cascade testing of their biological relatives and testing of their reproductive partners in Australian clinical practice was outlined in a PICO confirmation that was presented to, and accepted by, the PICO Advisory Sub-Committee (PASC).

The purpose of WGS/WES is to detect genetic variants in either mtDNA or nDNA for the diagnosis of MD in all patients who are suspected of having either an acute or chronic MD presentation. The current diagnostic pathway for patients with MD is complex, including multiple clinical consultations and various biochemical, histopathological, and enzymological tests supported by the analysis of a tissue biopsy, predominantly from muscle. Patients with clinical indicators suggestive of MD require a muscle biopsy and complex pathology testing of the sample to confirm that the patient is likely to have a phenotypic diagnosis of MD. However, due to the invasive nature of the muscle biopsy, a firm phenotypic diagnosis of MD in children is often delayed until adulthood. Instead, a 'strongly suspected' MD phenotypic diagnosis is more often given. Moreover, the clinical and phenotypical diversity in the environment of its two genome complexities and heterogeneities makes the MD diagnosis process complicated, arduous, and expensive, with multiple tests involved. In the absence of genetic testing (i.e. based on a phenotypic diagnosis alone), no information on the heritable nature of a MD diagnosis is available.

WGS provides comprehensive sequencing of nDNA and mtDNA simultaneously and is becoming the method of choice as it can also detect intronic P/LP variants. WES provides comprehensive sequencing of nDNA exons. Although standard WES methods amplify both nDNA exons and mtDNA, the read depth for mtDNA is often too low. A higher read depth is required for mtDNA than for nDNA in order to detect P/LP mtDNA variants with a low level of heteroplasmy. The literature reports variable success in sequencing mtDNA data using WES, even if a method of mtDNA enrichment is used. A similar result to WGS can be achieved using WES combined with mtDNA sequencing using either next generation sequencing (NGS) or Sanger sequencing.

Diagnosis is provided by analysis of the raw sequence data using a curated list of MD-related genes (i.e. a "virtual panel"). The proposed item descriptors refer specifically to virtual panel analysis of, at minimum, the 'green genes' in the MD gene panel published on PanelApp Australia or PanelApp UK. This is in line with MSAC's previous support for gene panel testing referring to PanelApp gene lists, and testing including the 'green genes' at a minimum. The MD virtual panel gene list in PanelApp Australia contains 377 genes, of which 305 are 'green'⁷. Testing for the MD virtual panel may be followed by symptom-specific virtual panels in those for whom no P/LP variant was identified. Variants would be classified using the American College of Medical Genetics and Genomics (ACMG) five-tier system of classification for germline variants⁸. The classifications are (i) pathogenic, (ii) likely pathogenic, (iii) variants of uncertain significance (VUS), (iv) likely benign, or (v) benign.

⁷ PanelApp Australia mitochondrial disease virtual panel. <https://panelapp.gha.umccr.org/203/> [Accessed 27 July 2022]

⁸ Richards S, Aziz N, Bale S, et al. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*, 17(5):405-24. doi: 10.1038/gim.2015.30.

PASC has advised that where WGS is not used, sequential rather than simultaneous mtDNA and nDNA WES should be considered. PASC's recommendation is that mtDNA analysis should occur first.

If no P/LP variants are identified and clinicians are highly suspicious that the patient may have a mtDNA deletion, testing for a large deletion would occur using samples such as saliva, urinary epithelial cells, or muscle tissue with next generation sequencing (NGS) or polymerase chain reaction (PCR) methodologies.

Biological relatives of patients with a P/LP variant causing MD, who may have inherited the variant, will be eligible for cascade testing. The tests would be variant-specific and are likely to use techniques such as PCR, Sanger sequencing or multiplex ligation-dependent probe amplification (MLPA). Reproductive partners of individuals carrying a recessive P/LP variant will then be eligible for single gene sequencing for reproductive decision-making purposes. This would occur using NGS or Sanger sequencing,

The applicant indicates that virtual panel based WGS/WES is superior in effectiveness and superior in safety compared to no genetic testing for diagnosis of MD in individuals with suspected MD and cascade testing of their biological relatives and reproductive partners.

5. Prerequisites to implementation of any funding advice

In Australia, WGS/WES is available from laboratories that have joint National Association of Testing Authorities (NATA) and Royal College of Pathologists Australasia (RCPA) accreditation, and are specifically accredited to provide genetic testing via WGS/WES.

There are limited number of pathology providers with accreditation for WGS, therefore WGS-based testing is likely to remain limited to these few centres of excellence. However, the number of laboratories with appropriate technology and accreditation to undertake WGS is likely to increase over time.

6. Proposal for public funding

Eight MBS items related to the detection of P/LP nDNA or mtDNA variants causative of MD in patients suspected of having MD, biological relatives and reproductive partners were proposed in the Ratified PICO Confirmation (Table 2) and are summarised as follows:

- WGS or WES analysis of germline variants, from a phenotypically driven gene list for patients (children and adults) who are suspected of having either acute or chronic MD:
 - If WES is to be used, the patient must first undergo mtDNA sequencing and analysis (proposed MBS item JJJJ) and WES will only be performed in a patient for whom mtDNA analysis was non-informative;
- WGS or WES can occur as either:
 - Singleton testing of the affected individual (proposed MBS item AAAA); or,
 - Trio testing of the affected individual and their biological parents for segregation analysis (proposed MBS item BBBB);
- mtDNA deletion testing has been proposed for patients strongly suspected of having a mtDNA deletion and in whom WGS or mtDNA sequencing and analysis was non-informative (proposed MBS item HHHH);

- Re-analysis of WGS/WES plus mtDNA data can occur every 18 months in patients for whom no causative P/LP variant was identified using items AAAA, BBBB, JJJJ and/or HHHH (proposed MBS item CCCC);
- Cascade testing of biological relatives for diagnostic purposes, segregation analysis or reproductive decision-making purposes (proposed MBS item KKKK);
- Prenatal cascade testing of a fetus at risk of having MD for diagnostic purposes (proposed MBS item GGGG);
- Whole gene testing of the reproductive partner of someone with a P/LP recessive variant for MD (proposed MBS item IIII).

Table 2 Proposed MBS items for patients suspected of having MD, their relatives and reproductive partners

Category 6 – PATHOLOGY SERVICES
<p>MBS item AAAA</p> <p>Characterisation via whole genome sequencing or whole exome sequencing and analysis of germline variants, from a phenotypically driven gene list including at least the ‘green genes’ on the relevant PanelApp Australia or PanelApp UK panel present in nuclear DNA (and also those present in mitochondrial DNA if captured by the methodology) of a patient with a strong suspicion of a mitochondrial disease based on the following criteria:</p> <p>(a) the characterisation is:</p> <ul style="list-style-type: none"> (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and <p>(b) if a methodology that does not include sequencing the mitochondrial genome is used, then the characterisation must be performed following the performance of mitochondrial sequencing for the patient in a service to which item JJJJ applies, and for which the results were non-informative; and</p> <p>(c) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:</p> <ul style="list-style-type: none"> (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or (ii) evident mitochondrial dysfunction or decompensation, and/or (iii) unexplained hypotonia or weakness, profound hypoglycaemia or ‘failure to thrive’ in the presence of a metabolic acidosis, and/or (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or (vi) cardiomyopathy and/or cardiac arrhythmias, and/or (vii) rapid hearing or painless visual loss or ptosis, and/or (viii) stroke-like episodes or nonvasculitic strokes, and/or (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or (x) external ophthalmoplegia, and/or (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or (xii) family history of mitochondrial disease, or any of the above; and <p>(d) the characterisation is not performed in conjunction with a service to which items BBBB, 73358 or 73359 applies</p> <p>Applicable only once per lifetime</p> <p>Fee: \$2,100.00 Benefit: 75% = \$1,575.00 85% = \$2,012.10</p>
<p>MBS item BBBB</p> <p>Characterisation via whole genome sequencing or whole exome sequencing combined with mitochondrial DNA sequencing and analysis, of germline variants, from a phenotypically driven gene list including at least the ‘green genes’ on the relevant PanelApp Australia or PanelApp UK panel present in nuclear DNA (and also those present in mitochondrial DNA if captured by the methodology) of a patient with a strong suspicion of a mitochondrial disease based on the following criteria:</p>

- (a) the characterisation is:
- (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 - (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and
- (b) if a methodology that does not include sequencing the mitochondrial genome is used, then the characterisation must be performed following the performance of mitochondrial sequencing for the patient in a service to which item JJJJ applies, and for which the results were non-informative; and
- (c) the request for the characterisation states that singleton testing is inappropriate; and
- (d) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:
- (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
 - (ii) evident mitochondrial dysfunction or decompensation, and/or
 - (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
 - (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
 - (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
 - (vi) cardiomyopathy and/or cardiac arrhythmias, and/or
 - (vii) rapid hearing or painless visual loss or ptosis, and/or
 - (viii) stroke-like episodes or nonvasculitic strokes, and/or
 - (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
 - (x) external ophthalmoplegia, and/or
 - (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
 - (xii) family history of mitochondrial disease, or any of the above; and
- (e) the characterisation is performed using a sample from the patient and a sample from each of the patient's biological parents; and
- (f) the characterisation is not performed in conjunction with a service to which item AAAA, 73358 or 73359 applies.
- Applicable only once per lifetime
 Fee: \$2,900.00 Benefit: 75% = \$2,175.00 85% = \$2,812.10

MBS item CCCC

Re-analysis of whole genome or whole exome plus mitochondrial DNA data obtained in performing a service to which item AAAA, BBBB or HHHH (and also JJJJ where applicable) applies, for characterisation of previously unreported germline variants related to the clinical phenotype, if:

- (a) the re-analysis is:
- i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 - (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and
- (b) the patient is strongly suspected of having a monogenic mitochondrial disease; and
- (c) the re-analysis is performed at least 18 months after:
- (i) a service to which item AAAA or BBBB applies; or
 - (ii) a service to which this item applies
- Applicable for the duration of the patient's illness or until a diagnosis is confirmed.
 Fee: \$500.00 Benefit: 75% = \$375.00 85% = \$425.00

MBS item GGGG

Testing of a pregnant patient for detection of gene variant/s present in the parents for diagnostic purpose, in the fetus, if

- (a) the gene variant/s has been:
- (i) identified in the biological mother and is of mitochondrial genome lineage; or
 - (ii) identified in both biological parents within the same gene, present in the Mendeliome as autosomal recessive; or

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(iii) identified in either biological parent, present in the Mendeliome as autosomal dominant; or
 (iv) identified in a biological sibling of the fetus; and
 (b) the causative variant/s for the condition of the fetus's first-degree relative have been confirmed by laboratory findings; and
 (c) the results of the testing performed for the first-degree relative are made available for the purpose of providing the detection for the fetus; and
 (d) the detection is:
 (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and
 (e) the detection is not performed in conjunction with a service to which item KKKK, 73361, 73362 or 73363 applies
 Applicable only once per fetus
 Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00

MBS item HHHH
 Characterisation of a single mitochondrial DNA deletion or variant for diagnostic purposes in a patient suspected to have mitochondrial disease based on the following criteria:
 (a) the characterisation is:
 (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and
 (b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:
 (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
 (ii) evident mitochondrial dysfunction or decompensation, and/or
 (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
 (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
 (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
 (vi) cardiomyopathy and/or cardiac arrhythmias, and/or
 (vii) rapid hearing or painless visual loss or ptosis, and/or
 (viii) stroke-like episodes or nonvasculitic strokes, and/or
 (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
 (x) external ophthalmoplegia, and/or
 (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
 (xii) family history of mitochondrial disease, or any of the above; and
 (c) the characterisation is performed following the performance for the patient of a service to which items 73292, AAAA, BBBB, 73358 or 73359 applies for which the results were non-informative; and
 Applicable only once per lifetime
 Fee: \$450.00 Benefit: 75% = \$337.50 85% = \$382.50

MBS item IIII
 Whole gene testing of a person for the characterisation of germline gene variant(s) within the same gene in which the person's reproductive partner has a pathogenic germline recessive gene variant for mitochondrial disease confirmed by laboratory findings; and the characterisation is:
 (a) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 (b) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease.
 Applicable only once per gene per partner per lifetime

Fee: \$1200.00 Benefit: 75% = \$900.00; 85% = 1,115.30

MBS item JJJJ

Characterisation via mitochondrial DNA sequencing and analysis, of germline variants, from a phenotypically driven gene list including at least the 'green genes' on the relevant PanelApp Australia or PanelApp UK panel present in mitochondrial DNA of a patient with a strong suspicion of a mitochondrial disease based on the following criteria:

(a) the characterisation is:

- (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
- (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and

(b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:

- (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
- (ii) evident mitochondrial dysfunction or decompensation, and/or
- (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
- (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
- (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
- (vi) cardiomyopathy and/or cardiac arrhythmias, and/or
- (vii) rapid hearing or painless visual loss or ptosis, and/or
- (viii) stroke-like episodes or nonvasculitic strokes, and/or
- (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
- (x) external ophthalmoplegia, and/or
- (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
- (xii) family history of mitochondrial disease, or any of the above; and

Applicable only once per lifetime

Fee: \$1200.00 Benefit: 75% = \$900.00; 85% = 1,112.10

MBS item KKKK

Testing of a person (the person tested) for the detection of a single gene variant for diagnostic purposes, segregation analysis in relation to another person, or for the purpose of reproductive decision making, if:

(a) the person tested has a biological relative with a known mitochondrial disease variant confirmed by laboratory findings that can be plausibly shared between them; and

(b) the results of the testing performed for the person tested are made available for the purpose of providing the detection; and

(c) the detection is:

- (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
- (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and

(d) the detection is not performed in conjunction with a service to which item 73361, 73362 or 73363 applies

Applicable only once per variant per lifetime

Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00

Source: Table 1 of the DCAR

The proposed fees for these MBS items were agreed to by PASC in the ratified PICO.

7. Population

The population is divided into two groups:

- Population 1
 - Population A (adults and children suspected of having either acute or chronic MD)
- Population 2
 - Population B (biological relatives who may have the P/LP variant identified in the proband),
 - Population C (reproductive partners of a person with a recessively heritable P/LP MD variant) and
 - Population D (a fetus at risk of MD due to the parents' genotypes)

Population 1

MDs are the most common group of inheritable disorders caused by genetic variants in either mtDNA or nDNA. MD results from defects in oxidative phosphorylation (OXPHOS) activity or to integral mitochondrial functions. MD may present with widely heterogeneous clinical syndromes and clinical manifestations, affecting only a single organ, mild or oligo-symptomatic disease through to severe or life-threatening multi-organ dysfunction. Acute or chronic symptoms and signs may overlap with more common conditions or progress throughout an individual's lifespan, affecting both children and adults

More than 350 MD-causing genes have been identified to date and have been divided into six subsets according to their functional roles: (1) OXPHOS subunits, assembly factors, and electron carriers; (2) mtDNA maintenance, expression, and translation; (3) mitochondrial dynamics, homeostasis, and quality control; (4) metabolism of substrates; (5) metabolism of cofactors; and (6) metabolism of toxic compounds.

The majority of P/LP gene variants for MD are autosomal recessive, many are maternal, and some are autosomal dominant, X-linked dominant or X-linked recessive. Some variants and deletions causing MD are not inherited but arise spontaneously (de novo). The genes encoded on the mtDNA are almost always only inherited from the mother. Additionally, in some cases, different P/LP variants in the same gene can cause different MD syndromes, which may also differ in the inheritance pattern, such that one variant causes recessive disease and another variant in the same gene causes dominant disease.

There is limited epidemiologic data available on primary MDs. While individually rare, the collective prevalence of all P/LP variants in both nDNA and mtDNA is 23 per 100,000. Prevalence studies have estimated 1 in 200 – 250 people (or approximately 120,000 Australians) carry a disease-causing mtDNA variant that puts them at risk of developing a MD or other related symptoms. According to the Mito Foundation, approximately one affected Australian child is born each week – or 52 children every year – will develop a severe or life-threatening form of MD (1 in 5000 people).

Population 2

A definitive genetic diagnosis allows cascade testing of biological relatives at risk of disease to enable early interventions, and also testing of the reproductive partners of those carrying a recessive P/LP variant for reproductive planning, and testing of fetuses that are at risk of MD based on the parents' genotypes.

If the P/LP variant is autosomal or X-linked dominant, assisted reproductive technologies (ART) and pre-implantation genetic diagnosis (PGD) options can be considered. Females with P/LP mtDNA variants can consider the use of donor oocytes, in their entirety or given the recent passage of Maeve's Law, in the future mitochondrial donation may be an additional option. For individuals with autosomal recessive P/LP variants, whole (single) gene testing of the reproductive partner (Population C) improves the confidence in any family planning decisions the couple make. If both parents are carriers of an autosomal recessive variant for a MD, then PGD and ART are also in scope for their reproductive-decision-making.

Cascade testing can also be performed on a fetus (Population D) if P/LP variant(s) in the mother, father and/or sibling indicate that the fetus may be affected by MD.

Expected size of the population to be tested

The application projected that 400 adults and 52 children nationally will seek WGS/WES for the diagnosis of MD within the first year of listing, including a backlog of 100 adult patients and 20 children waiting for this service in 2021 (an estimated total of 1000 WGS/WES tests in the first three years). These estimates reflect the number of affected individuals, i.e. are unaffected by whether singleton or trio testing is used.

Previous assessments of genetic tests had estimated three first-degree relatives would be tested per proband (e.g. applications 1476, 1598), therefore the number of relatives eligible for cascade testing will vary depending on the diagnostic yield in affected individuals.

The sizes of Population C (reproductive partners) and Population D (prenatal testing) are uncertain.

8. Comparator

PASC considered that the most appropriate comparator to WGS detection of MD P/LP variants is no genetic testing.

The current diagnostic pathway of MD combines muscle, liver or other tissue biopsy (MBS item 30075, and age-appropriate anaesthetic items), which are then analysed using MBS-subsidised diagnostic tests such as complex histology (MBS item 72380), enzyme histochemistry (MBS item 72844) and immunohistochemistry (MBS item 72846). These comparators do not provide a definitive genetic diagnosis for MD, nor inform the need for cascade testing, nor inform family planning.

9. Summary of public consultation input

Consultation input was received from ten (10) organisations and one (1) individual consumer. The feedback was overall supportive of the application. The organisations that provided input were: Australian Genomics (AG), Australian Pathology (AP), Childhood Dementia Initiative (CDI), GUARD Collaborative Australia (GUARD), Human Genetics Society of Australasia (HGSA), Murdoch Children's Research Institute (MCRI), Mito Foundation (Mito), Public Pathology Australia (PPA), The Royal College of Pathologists of Australasia (RCPA), and Rare Voices Australia (RVA).

Benefits

The main benefit of the proposed testing is that genomic diagnosis can remove the need for invasive testing, as genomic sequencing is less costly and more effective when compared to conventional care. The reduction in invasive testing would lead to savings in hospital and pathology costs, and more broadly to the health system. The proposed intervention would provide the opportunity to avoid risks and pain associated with current invasive testing methods. Harms from genomic testing are currently experienced by the community to be minimal.

A confirmed genetic diagnosis allows for appropriate patient management measures such as targeted therapies for the subset of MDs that respond to high doses of specific vitamins, improving quality of life for patients, and potentially access to gene therapy. It could also provide non-health benefits including facilitating access to disability services, and enabling enrolment in clinical trials.

The proposed intervention would shorten the diagnostic odyssey, allow cascade testing of relatives, inform family planning options (potentially including mitochondrial donation) and restore reproductive confidence, allow institution of surveillance measures for complications associated with MD, and support consistent service and equity of access. The proposed intervention could lead to improved care by confirming an alternate diagnosis to MD.

The proposed intervention would improve patient confidence in diagnosis, including providing significant 'value of knowing'. Value of knowing could include psychological benefit, alleviating feelings of uncertainty and guilt, and reducing the psychological impact on caregivers. A genetic diagnosis could create a foundation for more accurate information on prognosis and likely severity.

Publicly funding the proposed intervention may provide an incentive for laboratories to invest in genomic testing, increasing providers of the service.

Disadvantages

There are no cures for and very limited pharmacological interventions for MD, though management approaches can reduce symptoms or slow progression and health decline.

There is an emotional burden of diagnosis, and also a chance of recurrence. There is an emotional toll on caregivers of children with MD. Discovery of any genetic condition presents communication challenges and may lead to communication breakdown. Biological parental lineage and custodial parent roles may be redefined.

Limiting panel testing to known MD genes may result in false negatives for patients with causative variants in a non-mitochondrial gene.

The ability for WES to detect mtDNA variants is variable. WGS represents a far superior approach for MD as it is best practice, and has demonstrated advantages over WES for MD.

Restrictions of providers is likely to limit the population.

There are few diagnostic laboratories that can offer RCPA/NATA accredited analysis of the whole genome sequencing data. Publicly funding this item could de-centralise the provision of care and dilute the expertise of managing clinicians.

Other Comments

Other services identified in the feedback are genetic counselling, metabolic physicians, psychological services and social workers.

The proposed intervention should be offered to anyone regardless of age, however, the Nijmegen criteria should be used to define the population as those <16 years and that those >16 year with more than one feature associated with MD. For patients aged <16 years, increasing Nijmegen score tracks correlatively with genomic diagnosis, so could be used for this cohort or incorporated with current defined clinical features/indicators.

To avoid test creep, ordering this item should be done in consultation with a physician with specialist knowledge of MD. Similarly, prenatal testing should be made in consultation with a clinical geneticist and/or other clinician experienced in pre-natal testing. Pre-test and post-test genetic counselling should be delivered in association with this intervention by a qualified genetic counsellor. If the applicant does not plan to offer formal education and credentialling in relation to MD, then similar to 73358, requestors for WGS could be specialists in consultation with a clinical geneticist.

mtDNA mutant load testing should be added either as a standalone item or as part of item HHHH. Item DDDD should be applied for relevant first-degree relatives and not just siblings. Item GGGG should also include X-linked disorders and should be expanded to include pre-implantation testing. The frequency restriction on item GGGG should be clarified as the application states the test is limited to “once per lifetime” however fetal testing is generally registered as a maternal sample in pathology laboratories.

If analysis of the MD virtual panel is negative, this should be expanded to the Mendeliome. An estimated quarter of patients with suspected MD can have a genomic diagnosis in a non-MD gene. Some patients have genetic diagnoses involving two or more disease loci.

Follow-up testing where WGS fails to identify a variant is likely to use muscle tissue as the preferred choice of biological sample. Laboratories are not yet accredited to use samples such as saliva and epithelial tissue for WGS. Blood will probably suffice to detect mtDNA deletions in children, though not in adults, where muscle is preferred.

The proposed fees may be too high (GGGG) or too low and not realistic (AAAA, BBBB, JJJJ). WES should not be used to calculate the cost of the proposed intervention, as WGS is more costly.

The setting of the service should be changed to accredited pathology laboratories.

Admitted patients should be included in the population as patients often present to hospital in acute decompensation.

10. Characteristics of the evidence base

Overall, there was relatively limited evidence for every component of the assessment report, except for test accuracy in detection of P/LP MD variants. A summary of the key features of the evidence is shown in Table 3.

Table 3 Key features of the included evidence

Criterion	Type of evidence supplied	Extent of evidence supplied	Overall risk of bias in evidence base
Test accuracy	Diagnostic yield for detection of P/LP variants for WGS, WES plus mtDNA analysis	☒ k=23 n=5,941	Low risk of bias [QUADAS-2 risk of bias tool - modified as appropriate]
	Ratified PICO Confirmation does not list a reference standard or clinical utility standard against which the accuracy of the intervention can be measured		
	Concordance between NGS and WGS, WES plus mtDNA analysis or SS plus RFLP in detecting P/LP mtDNA variants	☒ k=6 n=140	Low risk of bias [QUADAS-2 risk of bias tool - modified as appropriate]
	Mean difference in determining heteroplasmy between NGS and WGS, WES plus mtDNA analysis or SS plus RFLP	☒ k=6 n=140	Low risk of bias [QUADAS-2 risk of bias tool - modified as appropriate]
Change in patient management	Proportion of patients who had a change in management	☒ k=2 n=174	Low risk of bias [IHE Quality Appraisal Tool for Case Series]
	Change in supportive care e.g. lifestyle interventions and prevention measures	☒ k=1 n=44	Moderate risk of bias [IHE Quality Appraisal Tool for Case Series]
	Proportion who have a change in treatment	☒ k=4 n=229	Case series data with very limited applicability
Health outcomes	Evidence of MD targeted treatment effectiveness	☒ k= 11 n= 321	Low risk of bias [RoB.2]
	Quality of life	☒ k=2 n= 58	Moderate risk of bias [NHLBI Cohort and Cross-Sectional checklist]
	Uptake of genetic testing	☒ k=1 n= 312	Low risk of bias [NHLBI Cohort and Cross-Sectional checklist]
	Health outcomes of prenatal and PGD testing	☒ k=2 n= 9	Case series data with very limited applicability
Safety	Safety of WGS/WES compared to muscle biopsy	☒ k=8 n= 4,981	Not assessed
	Safety of targeted treatments compared symptom-based treatments	☒ k=3 n= 160	Low risk of bias [RoB 2.0]
	Safety of pre-implantation genetic diagnosis	☒ k=1 n= 35,117	Not assessed

Source: Table 2 of the DCAR

IHE = Canadian Institute of Health Economics; k=number of studies; MD = mitochondrial diseases; mtDNA = mitochondrial DNA; n=number of patients; NGS = Next generation sequencing; NHLBI = National Heart, Lung, and Blood Institute; QUADAS = Quality Assessment of Diagnostic Accuracy Studies; RFLP = Restriction fragment length polymorphism; RoB = Risk of Bias; SS = Sanger Sequencing; WES = Whole exome sequencing; WGS = Whole genome sequencing

11. Comparative safety

The comparative safety of genetic testing compared with no genetic testing for populations 1 and 2 is summarised in Table 4.

Overall, the safety of venepuncture for WGS/WES is non-inferior/superior compared with the current diagnostic pathway involving a muscle biopsy. The safety of prenatal testing compared with women with similar risk profiles who did not have prenatal testing is non-inferior. The safety of a pre-implantation genetic diagnosis (PGD) pregnancy is inferior compared with in vitro fertilisation (IVF) and spontaneous pregnancies.

Table 4 Safety of genetic testing compared with no genetic testing

Safety issue	Outcome
Population 1	
Safety of WGS/WES compared to a muscle biopsy in adults	The safety profiles of venepuncture to obtain a blood sample for WGS/WES and muscle biopsies appear to be similar. The safety of venepuncture for WGS/WES compared with the current diagnostic pathway (muscle biopsy) is likely to be non-inferior.
Safety of WGS/WES compared to a muscle biopsy in children (performed under general anaesthesia)	The safety profiles of venepuncture to obtain a blood sample for WGS/WES and muscle biopsies appear to be similar. General anaesthesia (required to perform muscle biopsy in children) carries an increased risk compared to no anaesthetic. The safety of venepuncture for WGS/WES compared with the current diagnostic pathway (muscle biopsy under general anaesthetic) is likely to be superior.
Safety of targeted treatments compared to no targeted treatment	Three studies indicated that targeted MD treatments are well tolerated and associated with no serious adverse events compared with placebo. The safety of targeted treatment is non-inferior to current symptom-based treatment.
Population 2	
Safety of prenatal testing compared with no prenatal testing in women with similar risk profiles	There is a small increased risk of miscarriage after amniocentesis (0.12%) or CVS (0.11%) compared to women with similar risk profiles who did not have prenatal testing. The safety prenatal testing is non-inferior to no prenatal testing in women with similar risk profiles.
Safety of pre-implantation genetic diagnosis (PGD) compared to natural conception	Spontaneous miscarriage occurred in 13% of clinical pregnancies arising from PGD compared to 0.91% for amniocentesis procedures and 1.39% for CVS. PGD resulted in a higher rate of hypertensive disorders of pregnancy (6.9%) compared with women having IVF (4.7%) or with spontaneous conception (2.3%). Neonates born after PGD had a higher rate of being small-for-gestational age (12.4%) compared with those born after IVF (4.5%) or spontaneous conception (3.9%). The safety of PGD is inferior to IVF and spontaneous pregnancies.

Source: Table 3 of the DCAR

CVS = chorionic villus sampling; IVF = in vitro fertilisation; MD = mitochondrial disease; PGD = pre-implantation genetic diagnosis; PTS = post traumatic stress; tWES = whole exome sequencing; WGS/= whole genome sequencing

12. Comparative effectiveness

Population 1

Diagnostic yield for detection of P/LP MD variants

WGS

Two Australian studies reported the diagnostic yield in children and in adults, and a third study, from the UK, reported on the yield in both adults and children.

Given the unexplained discrepancies between the Australian and UK studies (with a lower diagnostic yield reported in the UK study), only the Australian studies were used to calculate the diagnostic yield for the detection of P/LP MD variants using WGS (Table 5).

Table 5 Median diagnostic yield of P/LP variants in patients with suspected MD using WGS

Intervention	mtDNA	nDNA	mtDNA + nDNA
Paediatric populations			
WGS	MD Dx: 10.0% (k=1) Any Dx: 12.5% (k=1)	MD Dx: 27.5% (k=1) Definite Dx: 50.0% (k=1) Any Dx: 61.1% (k=1)	MD Dx: 37.5% (k=1) Definite Dx: 55.0% (k=1) Any Dx: 67.5% (k=1)
Adult populations			
WGS	MD Dx: 30.2% (k=1)	MD Dx: 29.6% (k=1) Definite Dx: 33.7% (k=1) Any Dx: 40.2% (k=1)	MD Dx: 50.8% (k=1) Definite Dx: 53.7% (k=1) Any Dx: 61.2% (k=1)

Source: Table 4 of the DCAR

Note: Definite Dx refers to a genetic diagnosis of a MD or non-MD condition and Any Dx refers to any diagnosis including a VUS considered to be the most likely disease-causing candidate.

DNA = deoxyribonucleic acid; Dx = diagnosis; MD = mitochondrial disease; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; P/LP = pathogenic or likely pathogenic; VUS = variant of unknown significance; WGS = whole genome sequencing

WES in the Paediatric population

The ratified PICO indicated that patients who will undergo WES instead of WGS under the proposed MBS item numbers AAAA or BBBB should be tested for P/LP mtDNA variants prior to WES. The median diagnostic yield for patients without P/LP mtDNA variants could be calculated using the results from eight studies (Table 6). The overall median diagnostic yield for detecting P/LP mtDNA and/or nDNA variants is also summarised in Table 6.

The diagnostic yield for P/LP mtDNA variants detected by WES, NGS or Sanger sequencing was reported in six studies. Two studies used WES, two studies used NGS and two studies used Sanger sequencing to identify mtDNA variants. The two studies using Sanger sequencing also used either Southern blotting or long range-PCR to detect large mtDNA deletions. The median diagnostic yield for identifying P/LP mtDNA variants responsible for MD in children is summarised in Table 6.

WES is not routinely used for this purpose even though mtDNA reads are generated during standard WES, because standard clinical WES bioinformatic pipelines do not report disease causing mtDNA variants. Both studies that used WES used bioinformatics pipelines specifically designed to analyse the mtDNA reads. However, the accuracy of this method when compared to the standard methods used to identify mtDNA variants, such as Sanger sequencing and NGS, is not known, and may be lower due to the higher read depth required to detect variants in mtDNA that are present at a low level of heteroplasmy.

The median diagnostic yield for identifying P/LP mtDNA variants responsible for MD in unselected children using Sanger sequencing (23.2%; range 15.9–30.4%) or NGS (22.8%; range 7–38.6%) were similar. This cannot be directly compared to the median diagnostic yield for identifying P/LP mtDNA variants responsible for MD in pre-screened children using WES, which was much lower (4.5%; range 3.6–5.3%; k=2). The children in the two studies using WES to detect mtDNA variants had previously undergone routine testing, such as targeted mtDNA and single nuclear gene analysis, with no findings.

Table 6 Median diagnostic yield of P/LP variants in paediatric patients with suspected MD using WES

Intervention	mtDNA	nDNA
nDNA-WES of patients without P/LP mtDNA variants		MD Dx: 25.1% (14.8–61.5); k=8 Definite Dx: 40.3% (24.6–59.3); k=6 Any Dx: 63.6% (58.0–90.4); k=3
mtDNA-WES	MD Dx: 4.5% (3.6–5.3); k=2	
mtDNA-NGS	MD Dx: 22.8% (7.0–38.6); k=2 Any Dx: 9.9%; k=1	
mtDNA-SS plus LR-PCR or SB	MD Dx: 23.2% (15.9–30.4); k=2 Any Dx: 69.6%; k=1	
mtDNA analysis (any method)	MD Dx: 11.5% (3.6–38.6); k=6 Any Dx: 39.8% (9.9–69.6); k=2	
mtDNA analysis plus nDNA WES		MD Dx: 35.6% (18.5–57.4); k=4 Definite Dx: 59.3% (35.2–63.0); k=3 Any Dx: 69.6%; k=1

Source: Table 5 of the DCAR

Note: Definite Dx refers to a genetic diagnosis of a MD or non-MD condition and Any Dx refers to any diagnosis including a VUS considered to be the most likely disease-causing candidate.

DNA = deoxyribonucleic acid; Dx = diagnosis; LR-PCR = long range polymerase chain reaction; MD = mitochondrial disease; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; NGS = next generation sequencing; P/LP = pathogenic or likely pathogenic; SB = Southern blot; SS = Sanger sequencing; WES = whole exome sequencing; WGS = whole genome sequencing

WES in a mixed adult and paediatric population

Of the six studies that included a mixed paediatric and adult cohort, three reported on the diagnostic yield of both mtDNA and nDNA P/LP variants and three reported on P/LP mtDNA variants alone.

The diagnostic yield of P/LP mtDNA variants was determined using WES in three studies and NGS in three studies (Table 7). One study also used Sanger sequencing plus Southern blotting to identify mtDNA variants. The median diagnostic yield for identifying P/LP mtDNA variants responsible for MD in the mixed cohorts using either WES (6.3%; range 2.3–19.7%) or NGS (7.9%; range 5.4–12.1%) were higher than for Sanger sequencing (3.5%). Overall, the median diagnostic yield for P/LP mtDNA variants in the mixed cohorts was lower than for the paediatric cohorts discussed above. The reason for this is unknown and contradicts the published literature, which reports that P/LP mtDNA variants are more common in adults than in children.

The median diagnostic yield for P/LP nDNA variants identified by WES in the mixed cohorts without P/LP mtDNA variants was 31.4% (range 8.3–53.2%; k=3). This was similar to that reported above for paediatric populations (25.1%; range 14.8–61.5; k=8). Overall, the median diagnostic yield for a MD diagnosis due to either mtDNA or nDNA variants in a mixed cohort was 39.7% (range 10.4–62.4%; k=3), which is similar to that in a paediatric population (35.6%; range 18.5–57.4; k=4).

Table 7 Median diagnostic yield of P/LP variants in a mixed adult and paediatric population with suspected MD using WES

Intervention	mtDNA	nDNA
nDNA-WES of patients without P/LP mtDNA variants		MD Dx: 31.4% (8.3–53.2); k=3 Any Dx: 60.6%; k=1
mtDNA-WES	MD Dx: 6.3% (2.3–19.7); k=3 Definite Dx: 8.2%; k=1	
mtDNA-NGS	MD Dx: 7.9% (5.4–12.1); k=3	
mtDNA-SS plus SB	MD Dx: 3.5%; k=1	
mtDNA analysis (any method)	MD Dx: 7.1% (2.3–19.7); k=4 Definite Dx: 8.2%; k=1	
mtDNA analysis plus n-DNA WES	MD Dx: 39.7% (10.4–62.4); k=3 Any Dx: 68.4%; k=1	

Source: Table 6 of the DCAR

Note: Definite Dx refers to a genetic diagnosis of a MD or non-MD condition and Any Dx refers to any diagnosis including a VUS considered to be the most likely disease-causing candidate.

DNA = deoxyribonucleic acid; Dx = diagnosis; LR-PCR = long range polymerase chain reaction; MD = mitochondrial disease; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; NGS = next generation sequencing; P/LP = pathogenic or likely pathogenic; SB = Southern blot; SS = Sanger sequencing; WES = whole exome sequencing; WGS = whole genome sequencing

WES in patients with specific MD syndromes

Four studies reported on the diagnostic yield of P/LP nDNA and/or mtDNA variants in patients clinically diagnosed with one of four different MD syndromes using WES and/or Sanger sequencing, respectively (Table 8).

Table 8 Median diagnostic yield of WES and/or mtDNA analysis in detecting P/LP variants causing a specific MD

Intervention	SS mtDNA	WES nDNA
MELAS	MD Dx: 63.6%; k=1 Definite Dx: 72.7%; k=1	MD Dx: 45.5%; k=1 Definite Dx: 63.6%; k=1 Any Dx: 90.9%; k=1
Leigh syndrome	MD Dx: 29.0%; k=1	MD Dx: 35.5%; k=1
LHON	MD Dx: 72.7%; k=1	
CPEO	MD Dx: 51.1%; k=1	
Overall WES nDNA with or without SS mtDNA	MD Dx: 57.4% (29.0–72.7); k=4 Definite Dx: 72.7%; k=1	MD Dx: 40.5% (35.5–45.5); k=2 Definite Dx: 63.6%; k=1 Any Dx: 90.9%; k=1
	MD Dx: 73.2% (64.5–81.8); k=2	

Source: Table 7 of the DCAR

Note: Definite Dx refers to a genetic diagnosis of a MD or non-MD condition and Any Dx refers to any diagnosis including a VUS considered to be the most likely disease-causing candidate.

CPEO = chronic progressive external ophthalmoplegia; DNA = deoxyribonucleic acid; Dx = diagnosis; LHON = Leber's hereditary optic neuropathy; LR-PCR = long range polymerase chain reaction; MD = mitochondrial disease; MELAS = mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; NGS = next generation sequencing; P/LP = pathogenic or likely pathogenic; SB = Southern blot; SS = Sanger sequencing; WES = whole exome sequencing; WGS = whole genome sequencing

Summary of the diagnostic yield for WGS and WES in paediatric, adult and mixed populations

The median diagnostic yield for detecting P/LP mtDNA or nDNA variants in paediatric, adult and mixed populations are summarised in Table 9.

The median diagnostic yield for WES plus mtDNA analysis is very similar to that for WGS in a paediatric population for an MD diagnosis (35.6% versus 37.5%), a definite diagnosis of MD or

non-MD (59.3% versus 55.0%) and for any diagnosis (including VUS suspected to be disease causing; 67.5% versus 69.0%).

As expected, based on the published literature, more adult patients than children were diagnosed with P/LP mtDNA variants using WGS (30.2% versus 10.0% of all patients tested). Among patients who were identified as having a P/LP MD variant, more adults (65.0%; 80/123) had a P/LP mtDNA variant compared with paediatric patients (26.7%; 4/15). The diagnostic yield of P/LP nDNA variants causing MD using WGS was similar in both paediatric and adult populations (27.5% versus 29.6%). Consequently, more adult patients had a genetic diagnosis of MD than paediatric patients (50.8% versus 37.5%).

However, more paediatric cases than adult cases were diagnosed with non-MD P/LP nDNA variants and nDNA-VUSs that were considered to be disease-causing based on further investigations such as segregation testing.

The median diagnostic yield of P/LP mtDNA variants identified by any method in conjunction with WES was much lower than expected in the mixed population cohort studies (7.1%) when compared to WGS/WES plus mtDNA analysis for both the paediatric and adult populations (10–11.5% and 30.2%, respectively). The reason for this cannot be determined. However, the median diagnostic yield of P/LP nDNA variants by WES in the mixed populations (31.4%) was comparable to that seen for both paediatric and adult populations (25.1–27.5% and 29.6%, respectively).

Seven studies reported on the correlation between MDC score and the likelihood of obtaining a genetic diagnosis. Five studies found that patients with a MDC score of 2–4 (possible MD) were less likely to receive a genetic diagnosis than those with a MDC score of ≥ 5 (probable or definite MD). However, two studies found that the 'definite MD' group, which had MDC scores ≥ 8 , had a lower diagnostic yield than the 'possible MD' group, which had the lowest MDC scores. In fact, only a median of 38% (range 34.4–75.0; $k=5$) of patients with a clinical diagnosis of 'definite MD' had a genetic diagnosis. The reasons for this could not be determined and may simply be due to chance.

One study found that the diagnostic yield varied depending on the presenting clinical phenotype rather than by MDC score. The highest diagnostic yields were achieved when patients presented with clear clinical MD phenotypes, such as optic atrophy (95.8%) or stroke-like episodes (60.1%). The diagnostic yield for non-syndromic complex phenotypes (defined as >5 clinical features listed in the Nijmegen criteria) were much lower (23.3%).

Two studies reported that the diagnostic yield for detecting P/LP variants using WGS/WES was higher for trio testing (31.0% and 41.8%; proposed MBS item number BBBB) than with singleton testing (22.5% and 23.6%; proposed MBS item number AAAA). The authors speculated that this was largely due to an enhanced ability to detect de novo and compound heterozygous variants with trio testing.

One study noted that there was no difference in the diagnostic yield between consanguineous (61.9%) and non-consanguineous (61.8%) families.

The diagnostic yield was higher in patients with a clinical diagnosis of mitochondrial myopathy, encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS), Leigh syndrome, Leber's hereditary optic neuropathy (LHON) or chronic progressive external ophthalmoplegia (CPEO) compared to patients with variable and possibly non-specific symptoms and a clinical diagnosis of 'possible MD' (73.2% versus 39.7%). This is not surprising, as a genetic diagnosis should be more likely if a patient fits the profile for a specific syndrome, rather than presenting with symptoms that could fit a number of different syndromes.

In conclusion, this data suggests that WGS has a comparable diagnostic yield for detection of P/LP MD variants to WES when combined with an established methodology for mtDNA variant analysis, such as NGS or Sanger sequencing combined with a method for detecting large-scale deletions, such as restriction-fragment length polymorphism (RFLP) or LR-PCR.

Table 9 Median diagnostic yield for WGS versus WES for different patient populations

Intervention	mtDNA	nDNA	mtDNA + nDNA
Paediatric populations			
WGS	MD Dx: 10.0% (k=1) Any Dx: 12.5% (k=1)	MD Dx: 27.5% (k=1) Definite Dx: 50.0% (k=1) Any Dx: 61.1% (k=1)	MD Dx: 37.5% (k=1) Definite Dx: 55.0% (k=1) Any Dx: 67.5% (k=1)
WES ± mtDNA analysis (any method)	MD Dx: 11.5% (3.6–38.6); k=6 Any Dx: 39.8% (9.9–69.6); k=2	MD Dx: 25.1% (14.8–61.5); k=8 Definite Dx: 40.3% (24.6–59.3); k=6 Any Dx: 63.6% (58.0–90.4); k=3	MD Dx: 35.6% (18.5–57.4); k=4 Definite Dx: 59.3% (35.2–63.0); k=3 Any Dx: 69.6%; k=1
Adult populations			
WGS	MD Dx: 30.2% (k=1)	MD Dx: 29.6% (k=1) Definite Dx: 33.7% (k=1) Any Dx: 40.2% (k=1)	MD Dx: 50.8% (k=1) Definite Dx: 53.7% (k=1) Any Dx: 61.2% (k=1)
WGS/WES (singleton)			Dx: 23.1% (22.5–23.6); k=2
WGS/WES (trio)			Dx: 36.4% (31.0–41.8); k=2
WGS/WES with 'possible MD' clinical Dx			Dx: 23.3% (0–47); k=7
WGS/WES with 'probable or definite MD' clinical Dx			Dx: 46.8% (23.0–87.5); k=7
Mixed paediatric and adult populations			
WES ± mtDNA analysis (any method)	MD Dx: 7.1% (2.3–19.7); k=4 Definite Dx: 8.2%; k=1	MD Dx: 31.4% (8.3–53.2); k=3 Any Dx: 60.6%; k=1	MD Dx: 39.7% (10.4–62.4); k=3 Any Dx: 68.4%; k=1
Mixed paediatric and adult patients clinically diagnosed with a specific MD			
WES ± SS mtDNA	MD Dx: 57.4% (29.0–72.7); k=4 Definite Dx: 72.7%; k=1	MD Dx: 40.5% (35.5–45.5); k=2 Definite Dx: 63.6%; k=1 Any Dx: 90.9%, k=1	MD Dx: 73.2% (64.5–81.8); k=2

Source: Table 8 of the DCAR

Note: Definite Dx refers to a genetic diagnosis of a MD or non-MD condition and Any Dx refers to any diagnosis including a VUS considered to be the most likely disease-causing candidate.

DNA = deoxyribonucleic acid; Dx = diagnosis; MD = mitochondrial disease; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; SS = Sanger sequencing; WES = whole exome sequencing; WGS = whole genome sequencing

Concordance in detecting P/LP mtDNA variants and determining heteroplasmy levels

Identification of P/LP mtDNA variants provides an incomplete clinical picture with respect to MD penetrance and severity. While heteroplasmy levels are dynamic and can change during the patient's lifetime, the quantification of heteroplasmy is also required for a complete genetic diagnosis of inherited MDs caused by P/LP mtDNA variants.

In the included evidence base, four different methods were used to detect P/LP mtDNA variants and measure their heteroplasmy level. Seven of these studies compared the measured heteroplasmy levels between NGS and WGS, WES with mtDNA enrichment or Sanger sequencing

plus a method to detect large deletions. No one method was considered perfect but NGS was considered to be the reference standard in most of these studies.

NGS of long-range PCR amplicons was effective for detecting and quantifying P/LP mtDNA single nucleotide variants (SNVs). NGS was used to measure the heteroplasmy levels of P/LP mtDNA variants in eight studies. Three studies included patients with low heteroplasmy P/LP mtDNA variants and NGS was able to detect these variants down to between 1–3.5% in five of these studies. None of the studies reported ‘missed’ P/LP mtDNA variants that were not detected by NGS.

Overall, the best method for detection and determining heteroplasmy of P/LP mtDNA variants appears to be WGS, with the mean difference of the proportion of mtDNA molecules having the variant between WGS and NGS being 1.6% (range 0–12) for SNVs and 4.3% (range 0–78) when large deletions were included (Table 10). WGS was able to detect low heteroplasmy P/LP mtDNA variants down to 0.29%. One study found that WGS analysis that includes polymorphic insertions of mitochondrial sequences into the nuclear genome (NUMTs) may very occasionally result in low heteroplasmy variant calls (false positives).

Other issues with detecting, or not detecting, variants can occur according to the type of sample used (i.e. blood versus muscle tissue), and can result in false positives or false negatives.

When NGS is compared to the standard techniques of Sanger sequencing and RFLP, it was considered to be superior. WES for detection of mtDNA variants was considered to be the least robust method.

Table 10 Detection of P/LP mtDNA variants and quantification of their heteroplasmy level

Intervention/samples	Detection/concordance	Range of heteroplasmy detected
NGS compared to WES with mtDNA enrichment (k=2) 30 SNVs, 1 large deletion	NGS: 100% (45/45) WES: 87.5% (28/32) Concordance = 27/31 (87.1%)	NGS: 3.5–100% WES: 20–100% Mean Difference (n=27 SNVs): 4.5% (0–40)
NGS compared to WGS (k=2) 58 SNVs, 3 large deletions	NGS: 100% (61/61) WGS: 100% (86/86) Concordance = 61/61 (100%)	NGS: 1–99% WGS: 0.29–96% Mean Difference (n=58 SNVs): 1.6% (0–12) Mean difference (n=3 DELs): 56% (31–78) Mean difference (n=61): 4.3% (0–78)
NGS compared to SS plus SNaPshot (k=1) 17 SNVs	NGS: 100% (17/17) SS+ SNaPshot: 88.2% (15/17) Concordance = 15/17 (88.2%)	NGS: 3.5–99% SS + SNaPshot: 5–100% Mean Difference (n=15 SNVs): 4.6% (0–15)
NGS compared to SS plus RFLP±SB (k=2) 39 SNVs, 3 large deletions	NGS: 100% (42/42) SS + RFLP: 100% (42/42) Concordance = 42/42 (100%)	NGS: 2.1–100% SS + RFLP: 5–100% Mean difference (n=39 SNVs): 12.9% (0–38) Mean Difference (n=3 DELs): 6.7% (0–15) Mean difference (n=42): 12.4% (0–38)

Source: Table 9 of the DCAR

DEL = deletion; DNA = deoxyribonucleic acid; mtDNA = mitochondrial DNA; NGS = next generation sequencing; P/LP = pathogenic or likely pathogenic; RFLP = restriction fragment length polymorphism; SB = Southern blotting; SNaPshot = single-nucleotide allele-specific primer extension analysis; SNV = single nucleotide variant; SS = Sanger sequencing; WES = whole exome sequencing; WGS = whole genome sequencing

Change in management

The genetic diagnosis of MD using WGS/WES combined with mtDNA sequencing can lead to changes in clinical management through the commencement of MD-specific therapies, avoiding contraindicated therapies in disorders mimicking MD, and providing clarification of reproductive

options. The results of the included studies providing evidence for change in management are summarised in Table 11.

Proportion of patients who had a change in management

Three studies provided evidence on the proportion of patients receiving a definite genetic diagnosis who experienced a change in management. The applicant provided additional data on the change in management in paediatric patients who received a genetic diagnosis of MD. Overall, a median of 9% (range 7–34%) of patients who received a definitive genetic diagnosis had a change in treatment that may result in improved health outcomes.

In a study by Davis et al, 53.7% (130/242) patients with suspected MD received a definitive genetic diagnosis. The genetic diagnosis led to a change in management in 18% (24/130) of these patients. However, only 6.7% (9/130) of patients received variant-specific treatments. A further 2.3% of patients were identified with other treatable conditions mimicking MD, which also led to a change in clinical management. The identification of P/LP variants in the *POLG* gene resulted in some patients avoiding contraindicated medications. Similarly, in two other studies, 7% and 9% patients commenced on targeted biotin and thiamine therapies after receiving a definite MD genetic diagnosis.

The additional data provided by the applicant indicated that 84% of paediatric patients who received a definite MD genetic diagnosis had some form of change in management. This included 73% who had a change in supportive care, 52% who were managed with change in diet and exercise regime, 15% who had a change in surgical/pharmacological interventions, and 9% who avoided extensive investigation such as muscle biopsies – however it was not clear that changes such as vitamin therapy were not part of routine care and could be attributed to the test itself.

Table 11 Proportion of patients with clinically diagnosed MD who had a change in management after a genetic diagnosis using WGS/WES plus mtDNA analysis

Study	Population	Proportion with change in management
Davis et al (2022) ⁹	N=130 patients who had definite genetic diagnoses	18% (24/130) of adult patients who had a definite genetic diagnosis had a change in management 6.9% (9/130) obtained variant-specific targeted treatment 2.3% (3/130) were diagnosed with treatable conditions mimicking MD and received condition-specific treatment Other patients avoided contraindicated medications (n not reported)
Riley et al (2020) ¹⁰	N=15 paediatric patients obtained definite genetic diagnosis using WGS	7% (1/15) of paediatric patients who had a definite genetic diagnosis
Lee et al (2020) ¹¹	N=22 patients suspected of having Leigh Syndrome obtained a genetic diagnosis	9% (2/22) families who were clinically diagnosed with LS

⁹ Davis, RL, Kumar, KRR, Puttick, C, et al. 2022, 'Use of whole genome sequencing for mitochondrial disease diagnosis', *Neurology*, **99**(7): pp. e730-e742.

¹⁰ Riley LG, Cowley MJ, Gayevskiy V, et al. (2020). 'The diagnostic utility of genome sequencing in a pediatric cohort with suspected mitochondrial disease', *Genetics in Medicine*, **22**(7): pp. 1254-1261.

¹¹ Lee JS, Yoo T, Lee M, et al. (2020). 'Genetic heterogeneity in Leigh syndrome: Highlighting treatable and novel genetic causes', *Clin Genet*, **97**(4): pp. 586-594.

Study	Population	Proportion with change in management
Additional information provided by applicant that includes paediatric patients in the study by Riley et al (2020)	N=44 paediatric patients with suspected MD who had a definite genetic diagnosis	84% (37/44) had some form of a change in management 73% (32/44) children had a change in supportive care such as additional of mitochondrial cocktail, exercise regime and lifestyle interventions and prevention measures 52% (25/44) children were managed with specific therapies (change in diet, exercise regime and targeted nutraceutical regimes) 34% (15/44) had a change in pharmacological / surgical intervention 21% (9/44) children avoided extensive investigations such as muscle biopsies for clinical diagnosis of MD

Source: Table 10 of the DCAR

Case Reports

An additional seven case reports/series reported on patients diagnosed with specific P/LP variants associated with MD who had a subsequent change in management. Five studies indicated that molecular diagnosis of MD led to a potential change in clinical management in eight paediatric patients. It included therapy redirected to palliative care in 29% (n=2) of patients, diet modification and co-factor supplementation in 71% (n=5) of patients and initiation of supportive care such as lifestyle interventions & preventative measures and addition of 'mitochondrial cocktail' in 14% (n=1) of patients.

Two studies reported commencement of targeted therapies such as co-factor supplementation in four adult patients who were genetically diagnosed with MD. These studies illustrate that a genetic diagnosis can lead to a beneficial change in management, however, the proportion of patients suspected of having MD with a genetic diagnosis suitable for targeted therapies cannot be determined from these studies.

Health outcomes

Treatment effectiveness

There is no single effective and standard treatment available for MD due to the phenotypic diversity of MD. There is also a sparsity of high-quality clinical trials. A total of 11 clinical trials reported evidence on the effectiveness of optimal MD targeted treatments in patients with specific variants. Eight trials followed randomised, placebo-controlled design, whereas three were open-label, single arm trials. Three of the studies were crossover studies with varying washout periods depending on the pharmacokinetic profile of the drug under study. There were eight different treatments in the included studies. The participants in the included studies harboured varying P/LP variants such as MELAS patients with m. 3243A >G, 3271T>G, 3244G>A, 3258T>C or 3291T>C and LHON patients with m.3460G>A, m.11778G>A, and m.14484T>C. The primary outcomes of the studies were quite different. Overall, studies reported improvement in gait parameters, locomotor function and muscle strength, symptoms and biomarkers related to stroke-like episodes, visual acuity, and quality of life in patients after the treatment. However, most of these studies failed to observe statistically significant differences between treatment and control arms. The studies show that for patients with particular variants, an effective targeted treatment is available that may improve health outcomes.

Uptake of Genetic Testing

A study conducted in Europe assessed the uptake and utility of diagnostic genetic testing in patients clinically diagnosed with a range of genetic diseases including MD, blindness, deafness, movement disorders and colorectal cancer. The authors found that after receiving pre-test counselling, 97.8% of MD patients offered WES agreed to undertake the test and only 2.2% declined. Overall, WES was declined by 10% of the heterogeneous population especially among patients with deafness (22.4%), colorectal cancer (11.9%) and blindness (8.9%). The main reason identified for declining WES in young adults and children was fear of receiving unsolicited findings.

Perceived Utility of Genetic Testing

One study discussed how parents perceived the utility of genetic testing and its influence on treatment and care decisions. As a part of a randomised controlled trial, a survey on parental perceptions of rapid WGS and WES in critically ill infants was conducted, following enrolment in the study and within a week of receiving genetic test results.

Although only 23% (27) of 117 infants received a genetic diagnosis in this study, the majority of the 161 parents who responded perceived genetic diagnosis to be beneficial for informed decision making for their child's care and future reproductive planning.

Population 2

Cascade testing

Only four published studies and some additional information provided by the applicant provided any data on cascade testing of P/LP variants in biological relatives.

Additional information

At the Garvan Institute, in Sydney, the current best practices for the utilisation of cascade testing in children for a P/LP MD variant are as follows:

- nDNA variants – both parents and any symptomatic children are tested, and as per the parents' request, unborn fetuses as well
- mtDNA variants – both the mother and siblings are tested (regardless of health status), unless refused due to potential 'un-insurable' consequences

The applicant provided data on twenty-five families that had some cascade testing performed. The results were:

- 'Trio' testing was performed in 14 sets of parents
- Confirmatory mtDNA 'cascade' testing was performed in 10 mothers, three of whom were displaying symptoms
- Cascade testing was performed in at least 14 siblings confirming a diagnosis in 7
- Cascade testing diagnosed a family cluster of three siblings (via second degree relatives)
- Cascade testing confirmed the diagnosis in a total of 10 children

The mean time from symptom onset to genetic confirmation via targeted NGS/WES/WGS testing was 51 months (range 0–6 years). If cascade testing was included, the diagnosis was hastened by an average of 5 months (mean 46 months; range 0–14 years).

Change in management

Reproductive decision making

Only one published paper provided evidence that a definite genetic diagnosis and refutation of heritable MD can enable informed reproductive decisions and give confidence to proceed without need for in vitro fertilisation (IVF) or preimplantation genetic diagnosis (PGD).

In the additional data provided by applicants, 4.2% (3/72) of adult patients who received definite genetic diagnosis for MD received certainty for reproductive decisions and 42.9% (6/14) of families who had trio testing were offered family planning strategies.

Prenatal Testing and PGD

Three case series/reports indicated that for people at risk of having a child affected by MD, prenatal testing and pre-implantation genetic diagnosis can be effective strategies, with families able to make informed decisions about their pregnancies, and to choose to implant unaffected embryos when available.

Clinical claim

Population 1:

The use of WGS/WES testing for detecting P/LP MD variants results in superior effectiveness compared with the current clinical diagnostic pathway.

The use of WGS/WES testing for detecting P/LP MD variants results in superior safety compared with the current clinical diagnostic pathway for paediatric patients.

The use of WGS/WES testing for detecting P/LP MD variants results in non-inferior/superior safety compared with the current clinical diagnostic pathway for adult patients.

Population 2:

The use of cascade testing and reproductive testing have superior effectiveness compared to no genetic testing for all reproductive outcomes.

The use of cascade testing and reproductive testing results in inferior safety compared to no genetic testing for all pregnancy outcomes. However, the potential benefits outweigh the safety concerns.

13. Economic evaluation

A cost-effectiveness analysis estimating the incremental cost per proband detected (MD only) was performed for the affected individuals. An extended cost-effectiveness analysis estimating the incremental cost per positive genotyping (including probands and genetic carriers identified with MD variant(s) in subsequent cascade and reproductive partner testing) was also performed, with sensitivity analyses presented around the key uncertainties.

The economic model takes the form of decision tree analysis incorporating the estimates of definitive molecular diagnosis achieved in patients suspected of having MD using WGS. Analyses are performed separately for affected individuals who are children and those who are adults using the same model structure but with different input values. The decision tree extends to integrate estimates of cascade testing (biological relatives and prenatal) and reproductive partner testing. Costs captured in the model include those associated with specialist consultations, WGS or other sequential genetic testing and muscle/tissue biopsies and complex

pathology testing of the sample. Costs associated with the post-diagnosis change in clinical management are not included in the economic evaluation.

A scenario analysis is also presented where the comparator is changed to reflect existing clinical practice (i.e. array/common gene panel/single gene testing) currently funded by State or Territory governments.

The effectiveness measures included in the model to quantify the clinical benefit of WGS to the diagnostic odyssey of MD are incremental cost per proband detected and incremental cost per positive genotyping for MD. The additional health outcomes of interest are presented as cost consequences.

A summary of the economic evaluation is provided in Table 12.

Table 12 Summary of the economic evaluation

Perspective	Australian health care system (base-case: retrospective, scenario analysis: current)
Population	A. Affected individuals with suspected MD B. Biological relatives (including prenatal) of proband with molecular diagnosis of MD C. Eligible reproductive partner of an individual identified with recessive P/LP variant for MD
Prior testing	Complete clinical workup including neuromuscular, hearing, and visual tests, etc. Laboratory investigations including full biochemical, haematological and metabolic workup. Imaging may include MRI, CT, ultrasound, etc.
Intervention:	<ul style="list-style-type: none"> WGS/mtDNA ± WES in patients with clinical suspicion of MD. mtDNA deletion testing using long-range PCR or Southern blot analysis to determine the presence of mtDNA single deletions, if patient is suspected of a single mtDNA deletion and WGS/WES was non-informative Re-analysis of raw WGS/WES data for testing of previously unreported variants Cascade testing for known variant in biological relatives of the proband Whole single gene testing in reproductive partners of someone with a P/LP recessive gene variant for MD
Comparator	<p><u>Assessment base case:</u> Affected individuals: no genetic testing, with management directed by symptoms. Relatives: no genetic testing, with regular monitoring for identification of MD symptoms <u>Scenario analysis:</u> common gene panel testing and single gene testing in affected individuals and specific variant testing in relatives</p>
Type of economic evaluation	Cost-effectiveness analysis, cost-consequences analysis
Outcomes	<ul style="list-style-type: none"> Affected individuals with genetically confirmed status (proband) detected People with positive genotyping identified (proband and family members identified with P/LP variant - either symptomatic/asymptomatic cases or carriers) People with clinically actionable P/LP variants identified (MD or non-MD) Impact on clinical management
Time horizon	Time to test results (typically less than 12 months)
Methods used to generate results	Cohort analysis using decision-tree
Generation of the base case	<p>Modelled stepped economic evaluation:</p> <p><u>Step 1:</u> Direct costs and outcomes of testing in affected individuals <u>Step 2:</u> Step 1 + inclusion of cascade testing in biological relatives <u>Step 3:</u> Step 2 + family planning (reproductive partner testing and prenatal testing)</p>
Transition probabilities	<ul style="list-style-type: none"> Proportion of tests that use WGS Diagnostic yield in affected individuals Number of relatives tested per proband Diagnostic yield in cascade testing of family members
Software packages used	TreeAge Pro 2022 and Excel 2016

Source: Table 11 of the DCAR

MD = mitochondrial disease; mtDNA = mitochondrial DNA; P/LP = pathogenic or likely pathogenic; QALY = quality-adjusted life year; WES = whole exome sequencing; WGS = whole genome sequencing

Table 13 summarises the possible outcomes (modelled and not modelled) associated with WGS testing in individuals with high suspicion of MD and cascade testing in the biological family members of a proband who may have inherited the P/LP variant.

Table 13 Summary of the possible health-related outcomes (modelled and not modelled ^a) in individuals with high suspicion of MD and cascade testing in the eligible family members of a proband

	Genetic status	No genetic testing ^b	Genetic testing available ^b
Affected individual	Genotype positive	<u>Modelled</u> <ul style="list-style-type: none"> Muscle/tissue biopsies and complex pathology testing of the sample for confirmation of MD diagnosis <u>Not modelled</u> <ul style="list-style-type: none"> Management according to symptoms and presentation 	<u>Modelled</u> <ul style="list-style-type: none"> Muscle or tissue biopsies and complex pathology testing of the sample avoided Cascade testing in eligible family members (step 2) <u>Not modelled</u> <ul style="list-style-type: none"> Shortened diagnostic odyssey Eligibility to enroll in clinical trials Targeted disease management
	Genotype negative	<u>Modelled</u> <ul style="list-style-type: none"> Muscle/tissue biopsies and complex pathology testing of the sample for confirmation of MD diagnosis <u>Not modelled</u> <ul style="list-style-type: none"> Management according to symptoms and presentation 	<u>Modelled</u> <ul style="list-style-type: none"> Muscle/tissue biopsies and complex pathology testing of the sample for confirmation of MD diagnosis in some cases where mtDNA deletion testing is performed <u>Not modelled</u> <ul style="list-style-type: none"> Management according to symptoms and presentation Re-analysis of WGS/WES raw data for the presence of newly identified P/LP MD gene variants
Cascade testing in eligible family members of a proband	Proband (genotype positive)	<u>Not modelled</u> <ul style="list-style-type: none"> Clinical investigations including biopsies and complex pathology testing of the sample where necessitated Management according to symptoms in symptomatic family members Periodic surveillance in asymptomatic family members if clinically indicated General family planning advice 	<u>Modelled</u> <ul style="list-style-type: none"> Persons identified with P/LP variants (diagnostic or predictive) Reproductive partner testing for the individuals identified with the recessive variant. <u>Not modelled</u> <ul style="list-style-type: none"> Clinical investigations including biopsies and complex pathology testing of the sample where necessitated Targeted management and monitoring of family members identified with familial P/LP MD variant. Periodic surveillance not needed in family members with no familial variant identified Accurate family planning advice based on the inheritance pattern observed.

Source: Table 12 of the DCAR

^a While some of the relevant health outcomes are not modelled due to lack of evidence, these are discussed as cost-consequences.

^b All persons (affected individuals and family members) will still need to have clinical assessment irrespective of the availability of genetic test.

MD = mitochondrial disease; P/LP = pathogenic or likely pathogenic; WES = whole exome sequencing; WGS = whole genome sequencing

Assumptions used in the model

- The final diagnostic yield following singleton testing in the affected individual and subsequent segregation analysis in selected cases is similar to trio testing of affected individuals and both biological parents.
- Trio testing of the affected individual and biological parents reduces downstream cascade genetic testing.
- The probability of a P/LP familial variant in biological relatives of a proband is estimated based on relatedness to the family member tested (parent/sibling), mode of inheritance (autosomal, X-linked, maternal) and (for nuclear genes) a Mendelian inheritance pattern.
- Inheritance of mtDNA variants (except for *de novo* variants) is 90% in maternally linked relatives (mother, siblings, maternal aunts, uncles and cousins, etc.) – based on mtDNA variants being theoretically 100% maternally inherited, but only 90% are detectable based on differing heteroplasmy-load thresholds in testing capabilities.
- The time to diagnosis of the affected individual is less than a year; in reality, the diagnostic odyssey may span multiple years.

Results

Two scenarios are presented:

- The base case scenario assumes no genetic testing happens in affected individuals or their family members in the absence of WGS.
- An alternate scenario assumes some form of single or multi-gene testing happens in affected individuals and their family members in the absence of WGS.

The modelled economic evaluation is run separately for affected individuals categorised as children and adults. The economic analyses initially present the costs and outcomes aggregated across the affected individuals, the biological relatives of probands, reproductive partner testings and prenatal testing. A stepped analysis is then presented incrementally expanding the proposed population from affected individuals only to affected individuals plus biological relatives, then to include reproductive partners and prenatal testing. ICERs are presented for these incremental expansions.

Adults with suspected MD

Cost of intervention (affected individuals only) is \$3,269 per affected adult patient and includes costs associated with proposed tests and biopsies. Cost of intervention is \$4,576 per affected adult patient and family members, and includes costs associated with proposed tests in the affected patient and cascade testing in biological relatives and reproductive partner testing, and biopsies performed in affected individuals.

The modelled results are presented in a stepped manner in Table 14.

Table 14 Stepped presentation of results, adult individuals suspected of MD and their family members: average costs and outcomes per affected individual tested.

Stepped analysis	Incremental cost	Incremental outcome	ICER
Step 1 models affected individuals suspected of MD. Costs are associated with WGS and other proposed genetic tests in the affected individual and biopsies performed. Outcomes modelled are definite diagnosis, MD diagnosis and number of biopsies avoided.	\$1,031	56.28% of affected individuals have positive MD genotype	\$1,832 per proband
Step 2 integrates affected individuals suspected of MD and their biological relatives. Costs are associated with WGS and other proposed genetic tests in the affected patient and cascade testing in their biological relatives. Outcome modelled is positive genotyping for MD in affected patients and relatives.	\$2,297	1.766 persons with positive MD genotype	\$1,301 per person with positive genotype
Step 3 extends Step 2 to include costs and outcomes associated with reproductive partner tests and prenatal cascade tests.	\$2,338	1.772 persons/fetuses with positive MD genotype	\$1,320 per person (including fetuses) with positive genotype

Source: Table 13 of the DCAR

ICER = incremental cost effectiveness ratio; MD = mitochondrial disease

The base case result for affected individuals only, indicates that compared with no genetic testing, the proposed testing pathway (WGS/mtDNA sequencing ± WES and other genetic tests) costs an additional \$1,031 per affected individual and results in a definitive diagnosis in an additional 59% of affected individuals (57% being an MD diagnosis), and 63% fewer biopsies. This results in ICERs of \$1,832 per additional proband confirmed with MD, or \$1,743 per additional genetic diagnosis (of MD or another condition).

The base case result for affected individuals and eligible family members, indicates that compared with no genetic testing, the proposed testing pathway costs an additional \$2,338 per affected individual and results in identification of approximately 1.8 genotype-positive cases (affected individual/biological family members/fetuses). This results in ICER of \$1,320 per additional positive genotyping.

Table 15 summarises the total cost and incremental costs for an average person in a cohort of affected individuals suspected of MD and expected important clinical outcomes, as predicted by the model.

Table 15 Disaggregated costs per affected individual and outcomes as a percentage of the cohort eligible for testing, with respect to WGS or proposed testing of MD variants

	Proposed tests	No genetic test	Increment
Affected individual			
Cost per affected individual	\$3,269	\$2,238	\$1,031
Costs associated with tests	\$3,025	\$0	\$3,025
Cost associated with biopsy and analysis of sample	\$245	\$2,238	-\$1,943
Overall definite diagnoses	59.18%	0.00%	59.18%
MD diagnoses	56.28%	0.00%	56.28%
Muscle or tissue biopsies avoided	7.77%	71.00%	63.23%
Affected individual + biological family members of proband			
Cost per affected individual and family members of proband	\$4,576	\$2,238	\$2,338
Costs associated with tests in affected individual	\$3,269	\$2,238	\$1,031
Costs associated with cascade tests	\$1,307	\$0	\$1,307
Positive genotyping for MD	1.772	0.000	1.772

Source: Table 14 of the DCAR

ICER = incremental cost effectiveness ratio; MD = mitochondrial disease

The applicant provided additional data for adult patients included in the study by Davis et al (2022). Out of 48 MD patients who had a genetic diagnosis with WGS, 12 patients (25%) had a change in clinical management (shown in Table 16).

Table 16 Change in clinical management following WGS compared with no genetic testing in adults

Description	Value	Clinical benefit
Incremental cost per affected individual tested	\$1,031	Additional definite diagnosis
Proportion of affected individuals who have had change in clinical management as a result of genetic diagnosis with WGS	25%	More appropriate treatment
<ul style="list-style-type: none"> • Commencement of Prophylactic L-arginine 	12.5%	Reduction of the severity and frequency of stroke-like episodes in MELAS patients
<ul style="list-style-type: none"> • Commencement of Idebenone 	4.2%	Promotes vision recovery and preventing further damage to retinal ganglion cells in LHON patients
<ul style="list-style-type: none"> • Cessation of Epilim (Sodium valporate) 	4.2%	Reduced risk of liver failure in patients with presence of P/LP POLG variant
Reproductive decisions	4.2%	Offspring with no MD

Source: Table 15 of the DCAR

LHON = Leber Hereditary Optic Neuropathy; MD = mitochondrial disease; MELAS = Mitochondrial encephalopathy, lactic acidosis, stroke/stroke-like syndrome; P/LP = pathogenic or likely pathogenic; WGS = whole genome sequencing

Scenario analyses

Given that clinical practice in Australia utilises state-funded genetic testing, a scenario analysis is also provided where the comparator reflects this existing practice (i.e. including common gene panel and single gene tests). The results of this analysis are summarised in Table 17.

Table 17 Scenario analysis: Estimated costs and outcomes for adults with suspected MD utilising proposed genomic testing vs conventional genetic testing.

	Proposed genomic testing	Current genetic testing	Increment
Affected individuals only			
Cost	\$3,269	\$2,784	\$486
Definitive genetic diagnosis	59.18%	30.56%	28.62%
<i>Incremental cost per additional definitive diagnosis</i>			\$1,698
MD diagnosis	56.28%	25.00%	31.28%
<i>Incremental cost per additional proband identified for MD</i>			\$1,553
Muscle biopsies	7.77%	71.00%	63.23% avoided
Affected individuals + biological family members of proband			
Cost	\$4,576	\$3,307	\$1,269
Definitive diagnosis	1.772	0.775	0.997
<i>Incremental cost per additional definitive diagnosis</i>			\$1,273

Source: Table 16 of the DCAR
MD = mitochondrial disease

Sensitivity analysis

Univariate sensitivity analyses were conducted by varying the key inputs and assumptions to understand the impact on the ICER. Only the primary outcomes, MD diagnosis and positive genotyping are considered to calculate the ICERs. Table 18 summarises the key drivers of the model.

Table 18 Key drivers of the model

Description	Method/Value	Impact (Base case: \$1,832/additional proband detected for MD)
Biopsies performed in the absence of WGS	Proportion of patients having biopsies in the absence of WGS varied from 50% to 90%	<i>High, higher proportion of biopsies in the comparator arm favors intervention</i> <i>Use of 90% biopsies in no genetic testing arm decreased the ICER to \$768/additional proband detected for MD.</i>
Proportion of tests that are WGS	Proportion of tests that are WGS varied from 30% to 100%	<i>High, higher use of WGS tests in the intervention arm decreases the ICERS</i> <i>Use of 100% WGS in the intervention arm decreased the ICER to \$1,087/additional proband detected for MD.</i>
Diagnostic yield of WGS/mtDNA and WES	Diagnostic yield of WGS/mtDNA and WES decreased by 50% or increased by 25%	<i>High, lower diagnostic yield of proposed tests increases the ICERS</i> <i>Reducing the diagnostic yield by 50% increased the ICER to \$4,273/additional proband detected for MD.</i>
Fees for proposed tests	A fee of \$1,200 for AAAA and \$1,800 for BBBB	<i>High, lower fees for AAAA (singleton test in affected individual) and BBBB (trio tests) reduced the ICER to \$444/additional proband detected for MD.</i>

Source: Table 17 of the DCAR
ICER = incremental cost-effectiveness ratio; Inc = incremental; mt = mitochondrial; WES = whole exome sequencing; WGS = whole genome sequencing

Children with suspected MD

The cost of the genomic testing intervention (affected individuals only) is \$4,062 per child tested (or trio) for the diagnosis of MD and includes costs associated with proposed tests and biopsies in the proportion of patients with inconclusive test results.

Cost of the intervention, including cascade testing, for an affected child and biological family members, and reproductive partner testing, is \$4,542 per affected child. This includes costs associated with the proposed genomic test in the affected individual and cascade genetic testing in biological relatives of probands and testing in partners, and biopsies and complex pathology of sample performed in affected individuals.

The modelled results are presented in a stepped manner in Table 19.

Table 19 Stepped presentation of results, children suspected of MD and their family members: average costs and outcomes per affected child tested.

Stepped analysis	Incremental cost	Incremental outcome	ICER
Step 1 models affected individuals suspected of MD. Costs are associated with WGS and other proposed genetic tests in the affected individual and biopsies performed. Outcomes modelled are definite diagnosis, MD diagnosis and number of biopsies avoided.	\$2,486	39.65% of affected individuals have positive MD genotype	\$6,721 per proband
Step 2 integrates affected individuals suspected of MD and their biological relatives. Costs are associated with WGS and other proposed genetic tests in the affected individual and cascade testing in the biological relatives of probands . Outcome modelled is positive genotyping for MD in affected individuals and biological relatives of probands.	\$2,958	1.425 persons with positive MD genotype	\$2,076 per person with positive genotype
Step 3 extends Step 2 to include costs and outcomes associated with partner tests and prenatal cascade tests .	\$2,965	1.429 persons/fetuses with positive MD genotype	\$2,075 per person (including fetuses) with positive genotype

Source: Table 18 of the DCAR

ICER = incremental cost effectiveness ratio; MD = mitochondrial disease

Table 20 summarises the total cost and incremental costs for an average person in a cohort of affected individuals suspected of MD and expected important clinical outcomes, as predicted by the model.

Table 20 Costs and outcomes with respect to WGS or proposed testing of MD variants, as a percentage of the cohort eligible for testing, estimated by the economic model in the proposed setting

	Proposed tests	No genetic test	Increment
Affected individuals			
Cost per affected individual	\$4,062	\$1,576	\$2,486
Costs associated with tests	\$3,857	\$0	\$3,857
Cost associated with biopsy and complex pathology of sample	\$205	\$1,576	-\$1,371
Overall definite diagnoses	57.15%	0.00%	57.15%
MD diagnoses	39.65%	0.00%	39.65%
Muscle or tissue biopsies avoided	6.50%	50.00%	43.50%
Affected individuals + biological family members of probands			
Cost per affected individual and family members of the proband	\$4,542	\$1,576	\$2,965
Costs associated with tests in affected individuals	\$4,062	\$0	\$4,062
Costs associated with cascade tests	\$479	\$0	\$479
Positive genotyping for MD	1.429	0.000	1.429

Source: Table 19 of the DCAR

ICER = incremental cost effectiveness ratio; MD = mitochondrial disease

The base case result for affected individuals only, indicates that compared with no genetic testing pathway, the proposed testing pathway (WGS/mtDNA sequencing ± WES and other genetic tests in affected individuals) costs additional \$2,486 and results in definitive diagnosis in additional 57% of the affected individuals (including 40% additional MD diagnosis) and 44% fewer biopsies. This results in ICERs of \$6,721 per additional proband detected for MD, or \$4,350 per additional definitive diagnosis.

The base case result for affected individuals and eligible family members, indicates that compared with no genetic testing pathway, the proposed testing pathway costs an additional \$2,965 and results in an additional 1.4 genotype-positive cases (affected individuals/biological family members/fetuses). This results in ICER of \$2,075 per additional positive genotyping.

The applicant provided additional data for paediatric patients, most of whom were included in the study by Riley et al. (2020)¹². Out of 44 children who had confirmed genetic diagnosis, 37 patients (84%) had a change in clinical management (presented in Table 21).

Table 21 Change in clinical management following WGS compared with no genetic testing in children

Description	Value
Change in Management	84%
Change in supportive care	73%
Management with targeted therapies	52%
Change in pharmacological/ surgical intervention	34%
Avoided extensive investigations for MD in family members	36%

Source: Table 20 of the DCAR

MD = mitochondrial disease; WGS = whole genome sequencing

Source: Data provided by the applicant

Scenario analysis

Given that clinical practice in Australia currently utilises state-funded genetic testing, a scenario analysis where the comparator reflects existing practice (i.e. including common gene panel and single gene tests) is provided. Results of this analysis are summarised in Table 22.

Table 22 Results for scenario analysis, children suspected with MD and their family members

	Proposed testing pathway	Conventional pathway	Increment
Affected individuals only			
Cost	\$4,062	\$2,053	\$2,009
Definitive diagnosis	57.15%	25.86%	31.28%
<i>Incremental cost per additional definitive diagnosis</i>			\$6,421
MD diagnosis	39.65%	13.79%	25.85%
<i>Incremental cost per additional proband identified for MD</i>			\$7,770
Muscle biopsies	6.50%	50.00%	43.50% avoided
Affected individuals + biological family members of probands			
Cost	\$4,542	\$2,335	\$2,207
Definitive diagnosis	1.429	0.519	0.911
<i>Incremental cost per additional definitive diagnosis</i>			\$2,423

Source: Table 21 of the DCAR

MD = mitochondrial disease

¹² Riley LG, Cowley MJ, Gayevskiy V, et al. (2020). The diagnostic utility of genome sequencing in a pediatric cohort with suspected mitochondrial disease, *Genetics in Medicine*, **22**(7): pp. 1254-1261.

The scenario analysis result is for affected individuals only and indicates that compared with conventional genetic testing pathway, the proposed testing pathway (WGS/mtDNA sequencing ± WES and other genetic tests in affected individuals) costs an additional \$2,009 and results in a definitive diagnosis in an additional 31% of affected individuals (including 26% additional MD diagnosis) and results in 44% fewer biopsies and complex pathology of sample. This results in ICERs of \$7,770 per additional proband detected for MD and \$6,421 per additional definite diagnosis.

The scenario analysis result for affected individuals and biological family members of probands, indicates that compared with conventional genetic testing pathway, the proposed testing pathway costs an additional \$2,207 and results in an additional 0.911 affected individuals and biological family members with positive genotyping. This results in an ICER of \$2,423 per additional positive genotyping.

Sensitivity analysis

Univariate sensitivity analyses were conducted by varying the key inputs and assumptions to understand the impact on the ICER. Sensitivity analyses are presented in Table 23.

Table 23 Sensitivity Analyses: Results for affected individual (child suspected with mitochondrial disease)

	Incremental cost	Incremental effect	\$ per proband detected	% change
Base-case	\$2,486	39.65%	\$6,271	
<i>Diagnostic yield of WGS/mtDNA and WES</i>				
Reduced by 50%	\$2,688	22.56%	\$11,918	90.06%
Increased by 25%	\$2,387	47.92%	\$4,981	-20.57%
<i>Proportion of tests that are WGS (base case 50%)</i>				
30%	\$2,747	39.65%	\$6,929	10.49%
100%	\$1,834	39.65%	\$4,625	-26.24%
<i>Number of biopsies in the absence of WGS</i>				
30%	\$3,116	39.65%	\$7,861	25.36%
70%	\$1,856	39.65%	\$4,680	-25.36%
<i>Lower fee for AAAA and BBBB (base case \$2,100 and \$2,900 respectively)</i>				
AAAA: \$1,200 and BBBB: \$1,800	\$1,469	39.65%	\$3,707	-40.89%
<i>Cost of biopsy per patient (base case \$3,152)</i>				
\$1,576	\$3,172	39.65%	\$8,000	27.58%
\$4,728	\$1,800	39.65%	\$4,541	-27.58%
<i>Cost of pretest and post-test genetic counselling (base case: fees for MBS items 132 and 133)</i>				
Not included	\$1,870	39.65%	\$4,716	-24.79%

Source: Table 22 of the DCAR

mtDNA = mitochondrial DNA; WES = whole exome sequencing; WGS = whole genome sequencing

It is noted that the proposed listing and fee is identical for both WGS and WES, despite WES being a less resource intensive test procedure. Further, the outcomes of each are only considered equivalent when WES is supplemented with the mtDNA analysis that is additionally funded. In the sensitivity analysis for both adults and children, it is observed that when all testing uses the WGS method, the cost per diagnosis is reduced substantially. Economic theory suggests that an equivalent price for tests that have different resource inputs, effectiveness and cost-effectiveness sends incorrect price signals and creates non-optimal service selection incentives.

14. Financial/budgetary impacts

A market share based approach is used in estimating the use of proposed MBS services for patients suspected with MD and their family members. The applicant provided an estimate of current utilisation of WGS for diagnosing MD in children and adults. These cohort estimates (number of children or adults suspected with MD who will be seeking diagnosis) were then applied to the decision analytic model presented in Section 3 to estimate use of the proposed services. The estimated proportion of singleton versus trio tests is based on 85% trio testing in the paediatric patient population and 10% trio testing in the adult patient population.

The financial implications to the MBS resulting from the proposed listing of genomic/genetic tests for MD are summarised in Table 24.

Table 24 Net financial implications of proposed genomic testing to the MBS

Parameter	Year 1 2023	Year 2 2024	Year 3 2025	Year 4 2026	Year 5 2027
Estimated use and cost of the proposed health technology					
Number of people eligible for genomic testing	452	397	342	347	352
Proposed item AAAA (WGS/WES singleton test)	270	238	207	210	213
Proposed item BBBB (WGS/WES trio test)	65	55	45	46	47
Proposed item CCCC ¹ (WGS/WES reanalysis >18 months)	0	0	93	0	144
Proposed item JJJJ (mtDNA sequencing)	226	198	171	173	176
<i>Proposed item JJJJ (mtDNA sequencing)</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>
Proposed item HHHH (mtDNA deletion test)	34	30	26	27	27
Proposed item KKKK (cascade testing: biological relative)	643	568	493	500	507
Proposed item IIII (reproductive partner testing)	8	7	6	6	6
Proposed item GGGG (cascade testing: prenatal test)	5	4	4	4	4
Cost to the MBS (co-payments excluded)	\$1,219,065	\$1,069,334	\$959,229	\$933,206	\$1,008,155
<i>Cost to the MBS (co-payments excluded)</i>	<i>\$997,385</i>	<i>\$873,931</i>	<i>\$790,069</i>	<i>\$761,579</i>	<i>\$834,025</i>
Change in use and cost of other health technologies					
Change in number of muscle biopsies	-276	-243	-210	-213	-216
Net cost offset to State and Territory health budgets	\$868,602	\$764,588	\$660,710	\$670,345	\$680,121
Net financial impact to the MBS	\$1,219,065	\$1,069,334	\$959,229	\$933,206	\$1,008,155
Net financial impact to the MBS	\$997,385	\$873,931	\$790,069	\$761,579	\$834,025
Net financial impact to the government (MBS + State and Territory + other health budgets)	\$350,463	\$304,746	\$298,519	\$262,861	\$328,033
Net financial impact to the government (MBS + State and Territory + other health budgets)	\$128,782	\$109,343	\$129,359	\$91,233	\$153,903

Source: Table 23 of the DCAR. Italics indicate recalculated figures to reflect MSAC's advice that the fee for BBBB should be increased to \$3,300, the fee for GGGG increased to \$1,600, and that JJJJ was not supported as mtDNA sequencing is to form part of the service provided under AAAA and BBBB, and to update the 85% benefits to use the 1 November 2022 Greatest Permissible Gap.

MBS = Medicare Benefits Schedule; WES = whole exome sequencing; WGS = whole genome sequencing

Sensitivity analyses were conducted to examine sources of uncertainty associated with the use of the proposed services and their cost. The sensitivity analysis of the net financial implications to the MBS for increasing the fee for GGGG to \$1,600 is shown below (Table 25).

Table 25 Sensitivity analyses of the net financial implications to the MBS

	Year 1 2023	Year 2 2024	Year 3 2025	Year 4 2026	Year 5 2027
Base case					
Net costs to MBS	\$1,219,065	\$1,069,334	\$959,229	\$933,206	\$1,008,155
<i>A fee of \$,1600 for GGGG (prenatal testing)</i>					
Net costs to MBS	\$1,224,491	\$1,074,109	\$963,355	\$937,392	\$1,012,402

Source: Table 94 of the DCAR
MBS = Medicare Benefits Schedule

15. Other relevant information

The use of no genetic testing as the comparator does not reflect true clinical practice. mtDNA sequencing and deletion analysis, and NGS nuclear panels for the most common genes, such as *POLG*, *SURF1*, *OPA1*, *SLC22A5*, *SPG7*, *ACADM*, *BTD*, *ACADVL*, *CPT2*, *TRMT5*, *CLPB*, *HADHA*, *COQ8A*, *CEP89* and *LIPT1*, are available from some Australian diagnostic laboratories. These tests are not funded by the MBS and the cost would be incurred by the patient or by the State Government funded hospital system.

The existing unfunded genetic testing already provides some of the benefits (genetic diagnoses and downstream benefits of diagnoses) that are claimed to be associated with genomic testing (vs no genetic testing). Likewise, existing genetic tests also incur additional costs.

16. Key issues from ESC to MSAC

Main issues for MSAC consideration

Clinical issues:

- The evidence was weak and largely came from case series. There were no comparative studies, and the case studies had high levels of bias except for some effectiveness outcomes. Further, most of the evidence for diagnostic yield and clinical outcomes were from only two case series.
- The WES±mtDNA sequencing option for virtual gene panel testing may not be necessary. WGS was superior to WES, costs less than WES±mtDNA sequencing (given the same fee was proposed for AAAA/BBBB regardless of whether WES or WGS is used), and therefore was more cost-effective. Also, mtDNA sequencing is not currently widely available in Australia, so requiring it may reduce access. MSAC could consider not supporting WES±mtDNA sequencing, in preference of WGS alone for virtual panel testing.
- If WES is retained for AAAA/BBBB, it may be appropriate to differ the proposed order of sequential testing by age. The proposed item descriptors require that if WES rather than WGS is used for virtual panel testing under AAAA/BBBB, then mtDNA sequencing (JJJJ) needs to be done first. This order is reasonable for the adult population where many variants are in mtDNA, however in the paediatric population most variants are in nDNA, so it would be more appropriate to perform WES first in children.

Economic issues:

- WGS and WES were proposed to have the same fee, so the economic model did not consider different costs for the two methods. The economic model, and financial impact, would be more certain if WES and WGS were priced differently.
- The cost of a muscle biopsy is uncertain.

Financial issues:

- The appropriate fees for AAAA and BBBB are unclear. The proposed fees align with some previously supported fees, however there are two sets of precedents for virtual panel WES/WGS and work to align the two sets of precedents is underway. WGS and WES are combined in the proposed items though different fees appear more appropriate. Different laboratories provide different services and diagnostic yield may vary if the set of genes included in the virtual panel can vary.
- The financial estimates had significant uncertainty, as data inputs were based on two small studies and data provided by the applicant. The evidence was scarce and uncertain (in terms of cost and number) for cost offsets from muscle biopsies avoided. High-quality resource use data are needed for future applications of genomic testing (especially when health outcomes are not being considered).
- Mitochondrial disease (MD) has multiple complex phenotypes and presentation varies between patients, with no single pathology test able to form a diagnosis; therefore, resource use is also likely to be varied. No good evidence was provided on resource use before and after diagnosis. Downstream clinical investigations and treatments for those with MD after genetic testing were similarly varied.

Other relevant information:

- WGS is offered by five laboratories in Australia, and this is expected to grow in the future. In contrast, only one laboratory currently performs mtDNA sequencing.

ESC discussion

ESC noted that this application from the Australian Mitochondrial Disease Medical Network was for Medicare Benefits Schedule (MBS) listing of virtual gene panel-based analysis of WGS or WES data to diagnose MD in patients who are suspected of having MD. The application also included mtDNA deletion testing and mtDNA sequencing for affected individuals, as well as cascade testing, reproductive partner testing, fetal testing, and re-analysis.

ESC noted that the prevalence of MDs is about 1/5,000 people, and most patients with MD are diagnosed with LHON or MELAS. MDs are multisystem disorders with substantial genotypic and phenotypic diversity, and are difficult to diagnose. Genomic testing using virtual panels is proposed to offer an alternative, potentially definitive, diagnostic option and address a clinical need.

ESC noted that MDs can be caused by variants in genes located in nDNA or mtDNA, so virtual panel analysis requires both genomes to be examined. ESC noted that WGS interrogates both nDNA and mtDNA, whereas WES typically does not detect mtDNA variants, so if WES is used for virtual panel testing then additional mtDNA sequencing is needed to examine all genes on the virtual panel. ESC noted a further challenge with genetic testing for mtDNA variants is that each cell has multiple mtDNA copies and the variant may not be present in all mitochondria within a cell (“heteroplasmy”). An advantage of WGS over WES is that it can detect P/LP mtDNA variants at low levels of heteroplasmy due to its higher read depth, whereas mitochondrial variants

present at a low level of heteroplasmy are missed by WES unless ‘spike in’ baits for mtDNA sequences are used.

ESC noted the feedback from the targeted consultation, which was supportive of the application. Respondents claimed that MBS listing of this testing would improve equity of access. Some organisations suggested that genomic diagnosis can remove the need for invasive testing (muscle biopsy and general anaesthesia required concomitantly in children), and the reduction in invasive testing would lead to savings in hospital and pathology costs. It would also avoid risks and pain associated with current testing methods, and increased risk from anaesthesia in some individuals with MD. In addition, respondents stated that a confirmed genetic diagnosis can shorten the diagnostic odyssey, restore reproductive confidence, facilitate access to disability services, enable enrolment in clinical trials and allow for appropriate patient management measures, improving quality of life for patients. ESC noted consumer concerns regarding difficulties of accessing testing, false negative results, and issues around data storage and re-analysis and patient access to that data. ESC noted that access to genetic counselling is a potential equity issue because patients in the public system will receive genetic counselling before and after the test, whereas for private patients genetic counselling will be an out-of-pocket cost.

ESC noted that the application proposed eight MBS items:

Item	Description	Proposed fee	Proposed frequency restriction
AAAA	Singleton virtual panel testing	\$2,100	Once per lifetime
BBBB	Trio virtual panel testing	\$2,900	Once per lifetime
CCCC	Re-analysis	\$500	At least 18 months after AAAA/BBBB/CCCC, for the duration of the patient's illness or until a diagnosis is confirmed
GGGG	Fetal testing	\$400	Once per fetus
HHHH	mtDNA deletion testing	\$450	Once per lifetime
IIII	Reproductive partner testing	\$1,200	Once per gene per partner per lifetime
JJJJ	mtDNA sequencing and analysis	\$1,200	Once per lifetime
KKKK	Cascade testing	\$400	Once per variant per lifetime

ESC considered that reproductive partner testing and fetal testing would support improved reproductive decision-making, and noted that for MD this also includes mitochondrial donation, with the recent passage of Maeve’s law opening up the future possibility of where the biological mother has MD, swapping the mitochondria in the egg to those from a mitochondrial donor who does not have MD.

ESC noted that WGS and WES are included in the same proposed items at the same fee, in line with previous virtual panel items that also permit both methods (e.g., 73358 and 73359), but that the DCAR commented that identical pricing when the testing procedures have different input costs and outcomes sends incorrect price signals and creates non-optimal service selection incentives. ESC considered that WGS and WES having the same MBS fee was inappropriate as WGS requires substantially more resources. ESC considered that WGS has superior effectiveness, and at the proposed equal fees is cheaper and more cost-effective – however equal fees for WGS and WGS were likely inappropriate. ESC considered that the WES±mtDNA option may not be necessary, and that virtual panel testing for MD could be restricted to WGS. ESC noted the applicant’s comment via email prior to the ESC meeting that WGS was its preferred test method. ESC agreed that WGS is becoming more accessible, with WGS currently being offered by five laboratories in Australia, though more are accredited. ESC noted mtDNA sequencing is only currently provided by one centre in Australia (Victorian Clinical Genetics

Services), so considered that retaining the option to sequentially undergo mtDNA sequencing then WES may in fact reduce access, especially if mtDNA testing is required to be first in all patients. Alternatively, MSAC could consider costing WES and WGS separately and supporting WGS at a higher fee, which ESC noted would be a break from precedent as items supported to date permit both exome and genome backgrounds (and at the same fee), however ESC considered precedents pricing WES and WGS at the same fee were probably flawed. ESC considered that revising the item descriptor to require next-generation sequencing (NGS) methods may not be appropriate given method-agnostic item descriptors are preferred where possible, and also this would not be relevant if MSAC supported WGS alone.

ESC noted the proposed item descriptors state that if WES rather than WGS is used for virtual panel testing under AAAA/BBBB, then mtDNA sequencing (JJJJ) needs to be done beforehand. ESC noted that the genetic basis for MD in paediatric patients was predominantly due to nDNA variants, whereas for adult-onset disease more variants are found in mtDNA, and that the pre-ESC response stated 85% of children have an nDNA variant and 80% of adults have a mtDNA variant. ESC considered that mtDNA sequencing before WES is reasonable for the adult population where many variants are in mtDNA, however in the paediatric population most variants are found in nDNA, so ESC considered that in children it would be more appropriate to perform WES first, if the option to conduct WES is to be retained for AAAA/BBBB.

ESC noted the proposed fees, and considered that the appropriate fees for AAAA and BBBB were uncertain. ESC noted the MSAC Executive advice from November 2021 regarding the need for fee alignment across virtual panel testing items. The MSAC Executive had noted that the MBS fee of \$2,100 for a singleton virtual panel performed under WES/WGS (MBS item 73358, and Application 1600 AAAA1/2) is higher than the MBS fee of \$1,200 for method-agnostic gene panel tests (i.e., permitting virtual panel or amplicon-specific panel methods), and considered that its previous advice that unfiltered analysis was appropriate had likely been based on a very small minority of Australian laboratories conducting such testing, and that widespread practice did use virtual panels to restrict the analysis. The MSAC Executive considered that aligning virtual panel testing under these two previously separate categories creates a fee inconsistency, and had advised that the fees for virtual panel testing should be aligned, and that the fee for a singleton virtual panel test should be lower than \$2,100 as the cost to perform genomic tests is reducing over time. ESC noted the Department has been monitoring the utilisation and patient charges associated with virtual panel testing and other genomic tests to assess if there are policy concerns with current MBS fees. For example, for existing virtual panel items 73358 and 73359 for childhood syndromes (at fees of \$2,100 and \$2,900 respectively), ESC noted the bulk billing rate is almost 100% in the public setting and about 93% in the private setting, indicating that patients are not incurring out of pocket costs to any large extent. Additionally, utilisation of these items was under forecast, indicating that current MBS fee levels were not driving unnecessary testing.

ESC noted the fees proposed were in line with some previous items, but that the Department is currently working to align the fees for virtual panel testing and other genomic tests. ESC considered that virtual panels are becoming more automated and reducing bioinformatic costs over time, however that the appropriate fee for a virtual panel analysis on a WES/WGS background should be determined based on component-based costing. If MSAC considers WES should be retained and splitting WES and WGS to be appropriate, then WGS would warrant a higher fee than WES. ESC noted that a fee of \$400 was proposed for fetal testing (GGGG), however considered that MSAC had recently advised a fee of \$1,600 is appropriate for prenatal testing for known variants, so recommended the fee be raised to \$1,600 for GGGG.

ESC noted that the descriptors include a reference to PanelApp Australia¹³ to define the genes to be included on the virtual panel, and there are currently 305 'green' genes listed for MD. However, ESC noted that legislation prevents MBS descriptors referring to websites, so proposed replacing the reference to PanelApp with "all phenotypically driven genes associated with mitochondrial disorders". Another option that could be done in conjunction with revising the item descriptor, would be to refer to PanelApp in an explanatory note.

ESC noted the pre-ESC response commented that it was unclear that BBBB was for trio testing. ESC considered that moving the two criteria relating to trio testing to the start of the criteria list would make this clearer in the item descriptor.

ESC noted that the minimum interval for re-analysis in CCCC was proposed to be 18 months, in line with other previously supported re-analysis items, though the evidentiary basis behind the initial proposal of 18 months was unclear. ESC noted a 2022 systematic review¹⁴ (k=29) of re-analysis had found the average DY of re-analysis was 10%, and had conducted a subgroup analysis dichotomising re-analysis timeframe to <24 months versus ≥24 months, and found the latter was better (though not statistically significantly) and therefore the authors recommended "that reanalysis be delayed to ≥24 months unless there was urgent clinical need to reanalyze earlier". ESC therefore considered that a minimum re-analysis interval of at least 24 months may be more appropriate. ESC also noted re-analysis was proposed to be available "for the duration of the patient's illness or until a diagnosis is confirmed", and considered that this restriction differed from twice per lifetime, as proposed in application 1680 and seen in previously supported items. ESC considered that restricting re-analysis to twice per lifetime would be more appropriate.

ESC noted that with respect to the fetal testing item GGGG, not all people who are able to transmit mtDNA identify as female. ESC considered that while "pregnant patient" was acceptable, "biological mother" should be replaced with gender-neutral language. ESC also noted that the proposed item descriptors used the word 'variant', which it agreed is more appropriate than 'mutation'.

ESC noted consultation comments from the Royal College of Pathologists of Australasia (RCPA) raised that mtDNA variants can be differentially detectable depending on the tissue analysed, and that it proposed changing applicable once per lifetime to "applicable for up to three different tissue types per patient per lifetime". ESC considered that testing for mtDNA variants (i.e., HHHH, JJJJ) in multiple tissues such as blood, muscle and urine may be clinically appropriate, due to the multisystem, heterogenous nature of MDs and clinical preference for different sample types for a particular population (e.g., infants).

ESC considered that the descriptor for KKKK provided unnecessary and potentially confusing detail on the potential purposes of cascade testing, therefore ESC suggested removing "for diagnostic purposes, segregation analysis or reproductive decision-making purposes".

ESC's proposed amendments to the item descriptors are in green below (Table 26; additions in italics, deletions in strikethrough), noting JJJJ would not be required if WES±mtDNA sequencing is not supported. ESC did not propose any revisions to IIII.

¹³ PanelApp Australia mitochondrial disease virtual panel. <https://panelapp.gha.umccr.org/203/> [Accessed 27 July 2022]

¹⁴ Dai P, Honda A, Ewans, L, et al. (2022). Recommendations for next generation sequencing data reanalysis of unsolved cases with suspected Mendelian disorders: a systematic review and meta-analysis. *Genetics in Medicine*, **24**(8):1618–29.

Table 26 ESC's revised item descriptors

Category 6 – PATHOLOGY SERVICES
<p>MBS item AAAA</p> <p>Characterisation via whole genome sequencing or whole exome sequencing and analysis of germline variants, from <i>all</i> phenotypically driven genes <i>associated with mitochondrial disorders list including at least the 'green genes' on the relevant PanelApp Australia or PanelApp UK panel</i> present in nuclear DNA (and also those present in mitochondrial DNA if captured by the methodology) of a patient with a strong suspicion of a mitochondrial disease based on the following criteria:</p> <p>(a) the characterisation is:</p> <ul style="list-style-type: none"> (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and <p>(b) if a methodology that does not include sequencing the mitochondrial genome is used, then the characterisation must be performed following the performance of mitochondrial sequencing for the patient in a service to which item JJJJ applies, and for which the results were non-informative; and</p> <p>(e b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:</p> <ul style="list-style-type: none"> (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or (ii) evident mitochondrial dysfunction or decompensation, and/or (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or (vi) cardiomyopathy and/or cardiac arrhythmias, and/or (vii) rapid hearing or painless visual loss or ptosis, and/or (viii) stroke-like episodes or nonvasculitic strokes, and/or (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or (x) external ophthalmoplegia, and/or (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or (xii) family history of mitochondrial disease, or any of the above; and <p>(e c) the characterisation is not performed in conjunction with a service to which items BBBB, 73358 or 73359 applies</p> <p>Applicable only once per lifetime</p> <p>Fee: \$2,100.00 Benefit: 75% = \$1,575.00 85% = \$2,012.10</p>
<p>MBS item BBBB</p> <p>Characterisation via whole genome sequencing or whole exome sequencing combined with mitochondrial DNA sequencing and analysis of germline variants, from <i>all</i> phenotypically driven genes <i>associated with mitochondrial disorders list including at least the 'green genes' on the relevant PanelApp Australia or PanelApp UK panel</i> present in nuclear DNA (and also those present in mitochondrial DNA if captured by the methodology) of a patient with a strong suspicion of a mitochondrial disease based on the following criteria:</p> <p>(a) the characterisation is performed using a sample from the patient and a sample from each of the patient's biological parents; and</p> <p>(b) the request for the characterisation states that singleton testing is inappropriate; and</p> <p>(a c) the characterisation is:</p> <ul style="list-style-type: none"> (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and <p>(b) if a methodology that does not include sequencing the mitochondrial genome is used, then the characterisation must be performed following the performance of mitochondrial sequencing for the patient in a service to which item JJJJ applies, and for which the results were non-informative; and</p>

Category 6 – PATHOLOGY SERVICES

(d) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:

- (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
- (ii) evident mitochondrial dysfunction or decompensation, and/or
- (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
- (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
- (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
- (vi) cardiomyopathy and/or cardiac arrhythmias, and/or
- (vii) rapid hearing or painless visual loss or ptosis, and/or
- (viii) stroke-like episodes or nonvasculitic strokes, and/or
- (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
- (x) external ophthalmoplegia, and/or
- (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
- (xii) family history of mitochondrial disease, or any of the above; and

(f e) the characterisation is not performed in conjunction with a service to which item AAAA, 73358 or 73359 applies.
 Applicable only once per lifetime
 Fee: \$2,900.00 Benefit: 75% = \$2,175.00 85% = \$2,812.10

MBS item CCCC
 Re-analysis of whole genome or whole exome plus mitochondrial DNA data obtained in performing a service to which item AAAA, BBBB or HHHH (and also JJJJ where applicable) applies, for characterisation of previously unreported germline variants related to the clinical phenotype, if:

- (a) the re-analysis is:
 - i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 - (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and
- (b) the patient is strongly suspected of having a monogenic mitochondrial disease; and
- (c) the re-analysis is performed at least 18 24 months after:
 - (i) a service to which item AAAA or BBBB applies; or
 - (ii) a service to which this item applies

Applicable for the duration of the patient's illness or until a diagnosis is confirmed twice per lifetime.
 Fee: \$500.00 Benefit: 75% = \$375.00 85% = \$425.00

MBS item GGGG
 Testing of a pregnant patient for detection of gene variant/s present in the parents for diagnostic purpose, in the fetus, if

- (a) the gene variant/s has been:
 - (i) identified in the ~~biological mother~~ oocyte donating parent and is of mitochondrial genome lineage; or
 - (ii) identified in both biological parents within the same gene, present in the Mendeliome as autosomal recessive; or
 - (iii) identified in either biological parent, present in the Mendeliome as autosomal dominant; or
 - (iv) identified in a biological sibling of the fetus; and
- (b) the causative variant/s for the condition of the fetus's first-degree relative have been confirmed by laboratory findings; and
- (c) the results of the testing performed for the first-degree relative are made available for the purpose of providing the detection for the fetus; and
- (d) the detection is:
 - (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
 - (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and

(e) the detection is not performed in conjunction with a service to which item KKKK, 73361, 73362 or 73363 applies
Applicable only once per fetus

Fee: ~~\$400.00~~ \$1,600.00 Benefit: 75% = ~~\$300.00~~ \$1,200.00 85% = ~~\$340.00~~ \$1,512.10

MBS item HHHH

Characterisation of a single mitochondrial DNA deletion or variant for diagnostic purposes in a patient suspected to have mitochondrial disease based on the following criteria:

(a) the characterisation is:

- (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
- (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and

(b) onset of one or more clinical features indicative of mitochondrial disease inclusive of at least one or more of the following:

- (i) meeting the clinical criteria with a score of 5 or more in the Nijmegen Mitochondrial Disease Scoring System, for children <16 years, and/or
- (ii) evident mitochondrial dysfunction or decompensation, and/or
- (iii) unexplained hypotonia or weakness, profound hypoglycaemia or 'failure to thrive' in the presence of a metabolic acidosis, and/or
- (iv) unexplained single or multi-organ dysfunction or fulminant failure (in particular but not limited to neuropathies, myopathies, hepatopathy, pancreatic and/or bone marrow failure), and/or
- (v) refractory or atypical seizures, developmental delays or cognitive regression, or progressive encephalopathy or progressive encephalomyopathy, and/or
- (vi) cardiomyopathy and/or cardiac arrhythmias, and/or
- (vii) rapid hearing or painless visual loss or ptosis, and/or
- (viii) stroke-like episodes or nonvasculitic strokes, and/or
- (ix) ataxia, encephalopathy, seizures, muscle fatigue or weakness, and/or
- (x) external ophthalmoplegia, and/or
- (xi) hearing loss, diabetes, unexplained short stature, or endocrinopathy, and/or
- (xii) family history of mitochondrial disease, or any of the above; and

(c) the characterisation is performed following the performance for the patient of a service to which items 73292, AAAA, BBBB, 73358 or 73359 applies for which the results were non-informative; and

Applicable ~~only once~~ for up to three different tissue types per patient per lifetime

Fee: \$450.00 Benefit: 75% = \$337.50 85% = \$382.50

MBS item KKKK

Testing of a person (the person tested) for the detection of a single gene variant ~~for diagnostic purposes, segregation analysis in relation to another person, or for the purpose of reproductive decision making~~, if:

(a) the person tested has a biological relative with a known mitochondrial disease variant confirmed by laboratory findings that can be plausibly shared between them; and

(b) the results of the testing performed for the person tested are made available for the purpose of providing the detection; and

(c) the detection is:

- (i) requested by a specialist or consultant physician experienced in the treatment of mitochondrial disease; or
- (ii) requested by a specialist or consultant physician practising as a neurologist (paediatric or adult), metabolic physician, clinical or metabolic geneticist, or ophthalmologist who have experience in the treatment of mitochondrial disease; and

(d) the detection is not performed in conjunction with a service to which item 73361, 73362 or 73363 applies

Applicable only once per variant per lifetime

Fee: \$400.00 Benefit: 75% = \$300.00 85% = \$340.00

Source: ESC

85% benefit reflects the 1 November 2021 Greatest Permissible Gap (GPG) of \$87.90. All out-of-hospital Medicare services that have an MBS fee of \$586.20 or more will attract a benefit that is greater than 85% of the MBS fee – being the schedule fee less the GPG amount. The GPG amount is indexed annually on 1 November in line with the Consumer Price Index (CPI) (June quarter).

ESC noted the clinical management algorithms and assessment framework in the DCAR. ESC noted that the approach used was in line with other assessments of germline genetic tests, and considered this was appropriate as separately examining each gene of a panel is not practical.

ESC discussed the comparative safety data, which it considered showed no significant safety issues from muscle biopsies done under either local anaesthetic (adults) or general anaesthetic (paediatrics). ESC considered that general anaesthetic has increased risks compared to local anaesthetic, though noted case series data showed biopsies in children were generally safe with a low rate of adverse events, of which none were rated as serious. ESC considered that there was limited data on psychological harms, but there was a high uptake rate after counselling. ESC considered that overall, the risk of psychological harm from genetic testing was low, and the proposed testing likely had non-inferior safety.

ESC noted that DY was examined separately for WGS versus WES±mtDNA sequencing. ESC noted that the clinical evidence for diagnostic yield using WGS was from two Australian case series (Davis 2022¹⁵, $n = 242$ adults; and Riley 2020¹⁶, $n = 40$ children), which looked at definite diagnosis of MD vs any diagnosis (also including genes for other neuromuscular disorders, and where a variant of unknown significance [VUS] was the most likely disease-causing candidate). ESC considered the quality of the available evidence to be low. ESC noted that a UK study (Schon 2021) had been excluded as it had different requirements for diagnosis and inclusion. ESC noted that Riley 2020 used a virtual panel; Davis 2022 used a virtual panel of 249 MD genes, 400 NMD genes, plus the 'mity' analytical pipeline for mtDNA variants in patients who fulfilled Nijmegen Mitochondrial Disease Scoring System criteria, and additional testing was undertaken for patients with a high clinical suspicion but negative panel results. ESC noted that for WGS, the DY for a diagnosis of MD was 37.5% in children (comprised of 10% in mtDNA and 27.5% in nDNA), and 50.8% in adults (comprised of 30.2% in mtDNA and 29.6% in nDNA). ESC considered the DY to be higher in adults than in children. ESC noted that Davis 2022 found some mtDNA variants were not detected by WGS (particularly deletions), as 3% (7/242) adult patients needed further investigations for diagnosis, including muscle biopsies and urine tests.

ESC noted the evidence for DY of WES±mtDNA sequencing was from four case series examining adults and children that used a combination of tests (WES, other NGS methods, Sanger sequencing plus Southern blotting, or long-range polymerase chain reaction (PCR)), using a single (e.g., WES) or multiple techniques, and in different subgroups (e.g., MELAS syndrome or consanguinity). ESC noted that a MD diagnosis was made using WES±mtDNA analysis (any method) in 35.6% of children ($k=4$), and in 39.7% of patients in mixed paediatric and adult populations ($k=3$). ESC noted WES±mtDNA analysis had a DY for any diagnosis of 68.4% in mixed paediatric and adult populations ($k=1$). Overall, ESC considered that there was no significant difference in DY for a diagnosis of MD between WGS and WES±mtDNA analysis. ESC considered DY may be slightly higher in adults than in children.

When considering clinical utility, ESC noted that the evidence was from three Australian studies including the same two used for DY. These data showed that, overall, 2.3–9% of patients demonstrated a change in clinical management, including 6.7% (9/130) receiving a variant-specific targeted treatment, and avoiding contraindicated medications. 2.3% (3/130) of patients in Davis 2022 were also diagnosed with a treatable non-MD condition that mimicks MD, and received condition-specific treatment.

¹⁵ Davis, RL, Kumar, KRR, Puttick, C, et al. 2022, 'Use of whole genome sequencing for mitochondrial disease diagnosis', *Neurology*, **99**(7): pp. e730-e742.

¹⁶ Riley, LG, Cowley, MJ, Gayevskiy, V, et al. 2020, 'The diagnostic utility of genome sequencing in a pediatric cohort with suspected mitochondrial disease', *Genetics in Medicine*, vol. 22, no. 7, pp. 1254-1261.

ESC noted the additional research data provided by the applicant following PASC showing that 84% (37/44) of paediatric patients with an MD diagnosis had some form of change in management, including diet and exercise changes, and avoided additional investigations. Other changes in management following WGS compared with no genetic testing in children, where 73% experiencing change in supportive care, 52% changing management with targeted therapies, 34% change in pharmacological/surgical intervention, and 36% avoided extensive investigations for MD in family members. ESC considered these data had significant discordance from those published in other studies. ESC was not convinced that changes such as vitamin therapy were not part of routine care and could be attributed to the test itself. ESC also considered that the applicant's additional data are non-comparative, so some changes, such as diet and exercise, were likely to occur in the absence of genetic testing.

ESC reviewed the evidence for changes in reproductive decisions after genetic testing, and noted that only 0.4% (1/242) patients in the Davis 2022 study received genetic counselling. ESC noted that in the Riley 2020 study, trio testing informed family planning in 6/44 patients. ESC noted that for fetal testing, there are only very limited case series data to suggest that a genetic diagnosis of MD led to either a termination or continuation of pregnancy, and that no studies included a denominator that can provide quantitative data about the clinical utility of testing. ESC considered the clinical utility for reproductive decisions to be unclear.

ESC noted that of eight randomised controlled studies (RCTs) for treatment effectiveness, 6/8 showed that patients did not show clinical improvements following therapy, and only 2/8 revealed statistically significant improvement. The therapies that led to improvement were gene therapy for LHON and taurine for MELAS. ESC thus considered that a genetic diagnosis of MD does not currently lead to an effective treatment in all cases.

Overall, ESC considered that the clinical evidence was weak (level 4) and largely came from case series. There were no comparative studies, and the case studies had high levels of bias except for those for some effectiveness outcomes. Further, most of the evidence for DY and clinical outcomes came from only two case series. ESC considered that high-quality resource use data are needed for future applications of genomic testing (especially when health outcomes are not being considered).

ESC noted that the economic evaluation was a cost-effectiveness analysis. Paediatric and adult patients were considered separately, and a stepped approach was used for each, starting with affected individuals then expanding to include biological relatives, then fetal and reproductive partner testing. Costs captured in the model include those associated with specialist consultations, WGS or other sequential genetic testing, and muscle/tissue biopsies and complex pathology testing of the sample. However, ESC considered that costs associated with the post-diagnosis change in clinical management were not included in the economic evaluation, though were potentially valuable. ESC noted that the DCAR also conducted a cost-consequence analysis that discussed non-modelled outcomes, which it considered appropriate. ESC considered a different type of model would be necessary to examine the effect of turnaround time.

ESC noted that incremental cost-effectiveness ratios (ICERs) for step 1 (affected individuals only) were \$1,832 per proband for adults and \$6,721 per proband for children. When expanding to include cascade testing (step 2), and reproductive partner/fetal testing (step 3), ICERs were \$1,301 and \$1,321 per positive genotype for adults, and \$2,076 and \$2,075 per positive genotype for children, respectively. ESC noted that sensitivity analysis in the DCAR showed that raising the fee for fetal testing (GGGG) to \$1,600 had very little effect on the ICERs, increasing the ICER for adults by 0.54% and the ICER for children by 0.3%.

ESC noted that the main driver of the ICERs was DY (lower DY increases the ICER). Other factors that sensitivity analyses showed affected the adult and/or paediatric ICERs included:

- the proportion of people having muscle biopsies in the absence of WGS (higher proportion reduces the ICER)
- the proportion of virtual panel tests that use WGS rather than WES±mtDNA sequencing (more WGS decreases the ICER)
- the DY, which was influenced by factors including syndromic/non-syndromic presentation (syndromic defined as >5 clinical features listed in the Nijmegen scale), singleton/trio, and adult/child (lower DY increases the ICER)
- the appropriate fees (lower fees decrease the ICER)
- the cost of muscle biopsy (lower biopsy cost increases the ICER)
- excluding the cost of pre- and post-test genetic counselling reduces the ICER.

ESC also noted that the proposed panel testing resulted in 63% of muscle biopsies being avoided in adults and 44% in children, which was significant as muscle biopsies are costly (\$3,152 excluding hospital costs). ESC noted the DCAR included detailed costings for a muscle biopsy (such as for hospital visits and pathology tests). ESC considered the estimated cost seemed high, but also that it differed from published estimates that were even higher: the micro-costing study by Wu 2021¹⁷ reported muscle biopsy to cost \$5,839 (+\$417 for hospital costs), though disaggregated cost data were not provided. ESC considered that although the DCAR had recognised this uncertainty and included sensitivity analyses showing that using biopsy costs of \$1,576 and \$4,728 changed the ICER in adults by 97% in each direction, and in children by 28% in each direction, the cost of muscle biopsy remained uncertain. ESC considered that using the higher cost for muscle biopsies would have made the ICER more conservative. ESC also considered any difference in ongoing investigations in patients with conclusive results to be uncertain.

ESC noted that the comparator was no genetic testing, which it considered may not accurately reflect the current standard of care, which includes common gene panels and single gene tests. ESC considered that the current standard of care testing (with or without genetic testing) provides a MD diagnosis so is suitable as a comparator. ESC noted the DCAR included a scenario analysis where the comparator reflects other testing currently taking place, including testing funded by the States or by patients. This decreased the ICER for affected individual testing to \$1,553 per additional MD proband identified in adults (Table 17) but increased it to \$7,770 in children (Table 22). ESC noted that the diagnostic yield was high (31%) for this genetic testing assumed to take place under usual care. ESC noted that a substantial proportion (>70%) of muscle biopsies are avoided under current standard of care genetic testing.

ESC noted the financial impact to the MBS was \$1.2 million in 2023 (year 1) to \$1.0 million in 2027 (year 5), comprised mostly of virtual panel testing. ESC also noted that the DCAR estimated an offset cost to state and territory health budgets of \$660k-\$870k per year due to muscle biopsies avoided, though considered the extent of the cost-offset to be uncertain given the cost of a muscle biopsy is uncertain. ESC noted that sensitivity analysis in the DCAR showed that raising the fee for fetal testing (GGGG) to \$1,600 had very little effect on the financial cost of testing, increasing the net cost to the MBS in 2023 (year 1) from \$1,219,065 to \$1,224,491.

ESC noted advice from the National Pathology Accreditation Advisory Council (NPAAC), that the proposed WGS testing requires expertise and is highly specialised. It would be low volume testing

¹⁷ Wu, Y, Balasubramaniam, S, Rius, R, et al. 2021, 'Genomic sequencing for the diagnosis of childhood mitochondrial disorders: a health economic evaluation', *Eur J Hum Genet*, Jun-8.

likely to be conducted by a small number of laboratories. NPAAC further commented that external quality assurance (EQA) programs are available for specific mtDNA variant but not for whole genome sequencing.

17. Applicant comments on MSAC's Public Summary Document

The Mitochondrial Disease Medical Network sincerely thanks the MSAC and the Department of Health and Aged Care for their support throughout the assessment process of 'Whole Genome Sequencing for the diagnosis of mitochondrial disease (MD)'. Genetic testing is essential to the confirmation of a MD diagnosis, and pivotal in the advancement of patient care and management. An earlier and definitive genetic diagnosis will alleviate much of the patient stress experienced during the MD diagnostic odyssey, allow for more targeted management pathways, avoid inappropriate treatments and investigations that can be both costly and potentially harmful, inform reproductive planning, provide greater access to services and clinical trials, and improvements in quality of life for patients and carers. Additionally, the importance of involving the mitochondrial specialist cannot be understated in prenatal testing, particularly when managing maternal (mtDNA) inheritance. Due to the nature of heteroplasmy, understanding mtDNA inheritance requires experience in the illness beyond that of its genetic transmission. The Network is happy to offer any assistance required to optimise the implementation of these MBS items, with particular regards to the further refinement of item descriptors, MD specialist skills, and potential accreditation in accessing these MBS items.

While noting that nation-wide access to publicly funded WGS is a significant advancement, we believe the proposed fees for WGS should be higher and will work with the Department and MSAC to address this. Since the original submission of our application, the provision of WGS by Australian laboratories has continued to expand, now inclusive of five pathology services nationally. Whilst being increasingly adopted and recognised as the most comprehensive Next Generation Sequencing (NGS) technique, WGS intrinsically includes analysis of the mitochondrial genome, *unique and essential to MD diagnostics*, reaching beyond the nuclear analysis required to date for other illnesses. A test which otherwise if performed alone (that being mtDNA analysis), is now offered in only one laboratory nationally, greatly limiting access to the complicated two step, inferior and alternate pathway of mtDNA &/or WES analysis. Thus, the Network would also be supportive of any future discussions towards the utilisation of WGS alone in proposed MBS item AAAA.

18. Further information on MSAC

MSAC Terms of Reference and other information are available on the MSAC Website: [visit the MSAC website](#)