



Australian Government
Department of Health

Application 1675

Diagnostic genetic testing for mitochondrial disease

This application form is to be completed for new and amended requests for public funding (including but not limited to the Medicare Benefits Schedule (MBS)). It describes the detailed information that the Australian Government Department of Health requires to determine whether a proposed medical service is suitable.

Please use this template, along with the associated Application Form Guidelines to prepare your application. Please complete all questions that are applicable to the proposed service, providing relevant information only. Applications not completed in full will not be accepted.

Should you require any further assistance, departmental staff are available through the Health Technology Assessment Team (HTA Team) on the contact numbers and email below to discuss the application form, or any other component of the Medical Services Advisory Committee process.

Email: hta@health.gov.au

Website: www.msac.gov.au

PART 1 – APPLICANT DETAILS

1. Applicant details (primary and alternative contacts)

Corporation / partnership details (where relevant): Australian Mitochondrial Disease Medical Network Ltd

Corporation name: Australian Mitochondrial Disease Medical Network Ltd

ABN: 89642827138

Business trading name: Mito Medical Network

Primary contact name: REDACTED

Primary contact numbers

Business: **REDACTED**

Mobile: **REDACTED**

Email: **REDACTED**

Alternative contact name: Not applicable

2. (a) Are you a lobbyist acting on behalf of an Applicant?

- Yes
 No

(b) If yes, are you listed on the Register of Lobbyists?

- Yes
 No

PART 2 – INFORMATION ABOUT THE PROPOSED MEDICAL SERVICE

3. Application title

Diagnostic genetic testing for mitochondrial disease

4. Provide a succinct description of the medical condition relevant to the proposed service (no more than 150 words – further information will be requested at Part F of the Application Form)

Mitochondrial disease is a genetic disorder that robs the body of energy. Mitochondrial diseases are the most common heritable metabolic diseases in Australia and are caused by genetic variations in either the mitochondrial (mtDNA) or nuclear genome (nDNA). Mitochondrial diseases can present with a diverse range of phenotypic expression and clinical features at all ages, making diagnosis difficult. Although some mitochondrial diseases are inherited through a maternal pattern of inheritance (mtDNA variants), others follow autosomal dominant, recessive or X-linked traits (nDNA variants) and some occur sporadically. A family history may not always be evident, as individual family members may have different clinical manifestations to each other. These diagnostic challenges mean that there are often significant delays in diagnosis for affected persons (children and adults), leading to delays in treatment and management, use of inappropriate treatments and unknowing transmission of the disease.

PLEASE NOTE: For the purposes of this application, mitochondrial disease is referring to ‘Primary Mitochondrial Disease’ only, where the defect lies within oxidative phosphorylation (as opposed to diminished function secondary to other pathologies)

5. Provide a succinct description of the proposed medical service (no more than 150 words – further information will be requested at Part 6 of the Application Form)

Whole genome sequencing (WGS) comprehensively sequences genes (coding sequences) in both mtDNA and nDNA in a single genetic test. A similar result can be achieved by whole exome sequencing. For simplicity, we will refer to either of these approaches as the proposed investigative test and abbreviate to “WGS”. Diagnostic testing is agnostic of technology, tissue sample, and services, and hence it is not prescriptive to the methodologies/equipment and reagents involved. Diagnosis is provided by analysis of a phenotype driven curated list of known mitochondrial disease genetic variants (>350) delivered by a NATA accredited diagnostic laboratory providing compliant testing services .

WGS has emerged as the diagnostic test of choice and is being rapidly adopted worldwide, as evidenced by the publications listed in the table (see Q17, Part4).

Cascade testing of the causative single gene variant only, may be required for relatives of affected individuals for whom a diagnosis was made via WGS.

6. (a) Is this a request for MBS funding?

- Yes
 No

(b) If yes, is the medical service(s) proposed to be covered under an existing MBS item number(s) or is a new MBS item(s) being sought altogether?

- Amendment to existing MBS item(s)
 New MBS item(s)

(c) If an amendment to an existing item(s) is being sought, please list the relevant MBS item number(s) that are to be amended to include the proposed medical service:

N/A

(d) If an amendment to an existing item(s) is being sought, what is the nature of the amendment(s)?

N/A

(e) If a new item(s) is being requested, what is the nature of the change to the MBS being sought?

- i. A new item which also seeks to allow access to the MBS for a specific health practitioner group
- ii. A new item that is proposing a way of clinically delivering a service that is new to the MBS (in terms of new technology and / or population)
- iii. A new item for a specific single consultation item
- iv. A new item for a global consultation item(s)

(f) Is the proposed service seeking public funding other than the MBS?

- Yes
- No

(g) If yes, please advise:

N/A

7. What is the type of service:

- Therapeutic medical service
- Investigative medical service
- Single consultation medical service
- Global consultation medical service
- Allied health service
- Co-dependent technology
- Hybrid health technology

8. For investigative services, advise the specific purpose of performing the service (which could be one or more of the following):

- i. To be used as a screening tool in asymptomatic populations
- ii. Assists in establishing a diagnosis in symptomatic patients
- iii. Provides information about prognosis
- iv. Identifies a patient as suitable for therapy by predicting a variation in the effect of the therapy
- v. Monitors a patient over time to assess treatment response and guide subsequent treatment decisions

9. Does your service rely on another medical product to achieve or to enhance its intended effect?

- Pharmaceutical / Biological
- Prosthesis or device
- No

10. (a) If the proposed service has a pharmaceutical component to it, is it already covered under an existing Pharmaceutical Benefits Scheme (PBS) listing? Not applicable

N/A

(b) If yes, please list the relevant PBS item code(s):

N/A

(c) If no, is an application (submission) in the process of being considered by the Pharmaceutical Benefits Advisory Committee (PBAC)?

N/A

(d) If you are seeking both MBS and PBS listing, what is the trade name and generic name of the pharmaceutical?

N/A

11. (a) If the proposed service is dependent on the use of a prosthesis, is it already included on the Prostheses List?

N/A

(b) If yes, please provide the following information (where relevant):

N/A

(c) If no, is an application in the process of being considered by a Clinical Advisory Group or the Protheses List Advisory Committee (PLAC)?

Yes

No

(d) Are there any other sponsor(s) and / or manufacturer(s) that have a similar prosthesis or device component in the Australian market place which this application is relevant to?

Yes

No

(e) If yes, please provide the name(s) of the sponsor(s) and / or manufacturer(s):

N/A

12. Please identify any single and / or multi-use consumables delivered as part of the service?

N/A

PART 3 – INFORMATION ABOUT REGULATORY REQUIREMENTS

Whole exome and whole genome sequencing for clinical purposes is regulated by the National Association of Testing Authorities (NATA) and the Royal College of Pathologists Australia (RCPA).

- 13. (a) If the proposed medical service involves the use of a medical device, in-vitro diagnostic test, pharmaceutical product, radioactive tracer or any other type of therapeutic good, please provide the following details:**

Type of therapeutic good: **In-vitro diagnostic test**

Manufacturer's name: N/A

Sponsor's name: N/A

- 14. Is the medical device classified by the TGA as either a Class III or Active Implantable Medical Device (AIMD) against the TGA regulatory scheme for devices?**

N/A

- 15. (a) Is the therapeutic good to be used in the service exempt from the regulatory requirements of the *Therapeutic Goods Act 1989*?**

Yes

No

- (b) If no, has it been listed or registered or included in the Australian Register of Therapeutic Goods (ARTG) by the Therapeutic Goods Administration (TGA)?**

Yes

No

- 16. If the therapeutic good has not been listed, registered or included in the ARTG, is the therapeutic good in the process of being considered for inclusion by the TGA?**

N/A

- 17. If the therapeutic good is not in the process of being considered for listing, registration or inclusion by the TGA, is an application to the TGA being prepared?**

N/A

PART 4 – SUMMARY OF EVIDENCE

18. Provide an overview of all key journal articles or research published in the public domain related to the proposed service that is for your application (limiting these to the English language only). Please do not attach full text articles, this is just intended to be a summary.

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
1.	Invited review	Mitochondrial disease in adults: recent advances and future promise	Recent review highlighting advances in high-throughput sequencing technologies have changed the diagnosis of mitochondrial disease to using a “genetics first” approach and have identified >300 disease-causing genes.	Ng et al., 2021. Lancet Neurology. Jul;20(7):573-584 https://www.thelancet.com/journals/lanneur/article/PIIS1474-4422(21)00098-3/fulltext	July 2021
2.	Invited review	The broadening spectrum of mitochondrial disease-shifting the diagnostic paradigm	Invited review of the use of next generation sequencing techniques, that affected patients access to achieve earlier molecular diagnosis and management.	Liang et al., 2014. Biochimica et Biophysica Acta (BBA) - General Subjects, Vol:1840(4), pp1360-1367, ISSN 0304-4165 https://doi.org/10.1016/j.bbagen.2013.10.040	April 2014
3.	Invited review	New Diagnostic pathways for mitochondrial disease	Review demonstrating a minimally invasive “genetics first” approach using whole genome sequencing to “improve diagnostic yield and streamline diagnosis, leaving invasive investigations to address diagnostic challenges and functional validation of novel variants”.	Watson et al., 2020. Journal Of Translational Genetics and Genomics. https://doi:10.20517/jtgg.2020.31 .	June 2020
4.	Cohort study	The diagnostic utility of genome sequencing in a paediatric cohort with suspected mitochondrial disease.	WGS performed on 40 patients with mitochondrial disease demonstrating that diagnosis could be reached in 55% cases.	Riley et al., 2020. Genet Med 22 , 1254–1261 (2020). https://doi.org/10.1038/s41436-020-0793-6	21 April 2020

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
5.	Minireview	Current molecular diagnostic algorithm for mitochondrial disorders	Whilst molecular testing is viewed as definitive in mitochondrial disorders by most, it often still presents a challenge. The ever-expanding genotype spectrum, the two-genome complexity, and heteroplasmy, all add to the complexity of molecular testing, and obtaining a definitive molecular diagnoses in the mitochondrial patient.	Wong et al., 2010. Mol Genet Metab. 2010 Jun;100(2):111-7. http://www.americanchildneurologyuae.com/ar/files/neurological-diseases/NEUROMETABOLIC/Updated-mitochondrial-genetic-testing-algorithm-MGM-2010[1].pdf	4 March 2010
6.	Cohort Study	Genomic sequencing for the diagnosis of childhood mitochondrial disorders: a health economic evaluation	Implementing genomic sequencing in Australia for mitochondrial disease could save AU\$0.7million annually relative to traditional investigative costs, as well as providing an earlier definitive genetic diagnoses, offering large benefits to children and their families, personally. Therefore, “our findings support the prioritization of genomic sequencing for children with mitochondrial disease”.	Wu et al., 2021. Eur J Hum Genet. https://doi.org/10.1038/s41431-021-00916-8	8 June 2021
7.	Review	Diagnosis, management, and follow-up of mitochondrial disorders in childhood: a personalized medicine in the new era of genome sequence	Diagnostic mitochondrial biochemical tests may be deceptive, especially in children who display normal biochemistry, muscle histology, or enzymatic analysis. Clinical, biochemical, histological, and functional criteria, may not be enough to differentiate it from conditions causing secondary mitochondrial dysfunction, making genetic confirmation necessary.	Coelho et al., 2017. Eur J Pediatr 178, 21–32 (2) https://doi.org/10.1007/s00431-018-3292-x	7 December 2017

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
8.	Cohort Study	Whole exome sequencing of suspected mitochondrial patients in clinical practice	Mitochondrial diseases are typified by a wide range of phenotypes, caused by varying and overlapping mtDNA or nDNA variants, and vice versa. Mimicking other genetic and neuromuscular syndromes further complicates the diagnostic process, making WES the “state of the art next generation sequencing technique” necessary to identify mitochondrial diseases.	Wortmann, Saskia B. et al. 2015. Journal Of Inherited Metabolic Disease, vol 38(3) pp. 437-443. Wiley https://doi:10.1007/s10545-015-9823-y	4 March 2015
9.	Cohort Study	A Comprehensive Genomic Analysis Reveals the Genetic Landscape of Mitochondrial Respiratory Chain Complex Deficiencies	Three novel disease-causing mitochondria-related genes were identified as well as other pathogenic variants, validated by genetic and/or functional evidence. A comprehensive genomic analysis allowed for a firm genetic diagnoses in 34.5% of suspected cases, higher than the 25% using general diagnostic methods.	Kohda, Masakazu et al., 2016. PLOS Genetics, vol 12(1), p. e1005679. Public Library Of Science https://doi:10.1371/journal.pgen.1005679	7 January 2016
10.	Cohort Study	Diagnosing newborns with suspected mitochondrial disorders: an economic evaluation comparing early exome sequencing to current typical care.	Early exome sequencing whilst expensive, is an innovative diagnostic tool. Its reimbursement has been difficult to sell, but its value was shown by modelling the “clinical, health status, and cost” associated with a severe neonatal mitochondrial disease, as well as reducing the emotional toll on families awaiting diagnosis.	Crawford et al., 2021. Genet Med https://doi.org/10.1038/s41436-021-01210-0	26 May 2021

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
11.	Retrospective clinical and data analysis	Neonatal onset of mitochondrial disorders in 129 patients: clinical and laboratory characteristics and a new approach to diagnosis	To date, the gold standard for mitochondrial disease diagnosis has been muscle biopsy. However, it's invasive and has several limitations specific to neonates, in particular, difficult enzymatic study interpretations, low muscle mass, and their often-unstable clinical status. Therefore, "extensive molecular-genetic studies should be done before biopsy".	Honzik et al. 2012. J Inherit Metab Dis. 2012 Sep;35(5):749-59. https://pubmed.ncbi.nlm.nih.gov/22231385/	10 January 2012
12.	Review	Genetics of mitochondrial diseases: Identifying mutations to help diagnosis	Due to diversity, phenotypic overlap with other diseases, and absence of reliable biomarkers, the integration of WGS and WES, early in the diagnostic algorithm has become increasingly necessary. Capturing all genes, increases the mitochondrial disease diagnostic rate, accelerates novel disease gene discovery, and detects unexpected or treatable genetic diseases.	Stenton & Prokisch. 2020. Ebiomedicine, vol 56, p. 102784. Elsevier BV, https://doi:10.1016/j.ebiom.2020.102784	23 May 2020
13.	Invited Review	Mitochondrial Disease: Advances in Clinical Diagnosis, Management, Therapeutic Development, and Preventative Strategies	The importance of a genetic mitochondrial diagnosis is supported by improved standards of patient care, 'complemented by emerging therapies that target specific molecular subtypes', reproductive counselling options with now available preimplantation genetic diagnosis at the time of IVF, and mitochondrial replacement technologies for some mtDNA disorders.	Muraresku et al., 2018. Curr Genet Med Rep 6, 62–72 https://doi.org/10.1007/s40142-018-0138-9	2 May 2018

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
14.	Review	Mitochondrial disease genetics update: recent insights into the molecular diagnosis and expanding phenotype of primary mitochondrial disease	With >350 genetic mutations now recognized in mitochondrial disease, WGS/WES approaches have dramatically accelerated gene discovery, diagnostic yield, and has significantly expanded our understanding of clinical phenotypes and mitochondrial physiology, thus allowing for more precise and targeted therapeutics.	McCormick et al., 2018. Curr Opin Pediatr. Dec;30(6):714-724. https://doi:10.1097/MOP.0000000000000686	December 2018
15.	Review	Diagnosis of 'possible' mitochondrial disease: an existential crisis	When a genetic diagnosis is not obtained, the term 'possible' mitochondrial disease is often used, which may create harm through increased anxiety, a delayed correct diagnosis and inappropriate management or care. Advances in genomic testing have therefore led to other genetic disorders being appropriately diagnosed instead	Parikh et al., 2019. Journal Of Medical Genetics, vol 56, no. 3, pp. 123-130. BMJ, https://doi:10.1136/jmedgenet-2018-105800	25 January 2019
16.	Clinical Trials Review	Therapeutic Approaches to Treat Mitochondrial Diseases: "One-Size-Fits-All" and "Precision Medicine" Strategies	New strategies are emerging in mitochondrial disease management, with numerous promising preclinical and clinical results. This review examines both "one-size-fits-all" approaches and precision medicine strategies.	Bottani et al., 2020. Pharmaceutics. 12(11):1083. https://doi:10.3390/pharmaceutics12111083	11 November 2020

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
17.	Letter to the Editor	Treatable mitochondrial diseases: cofactor metabolism and beyond	NGS technologies expands our knowledge of treatable subgroups. The table recognises crucial subgroups presenting similarly to classical phenotypes (e.g. Leighs), that may suffer from a vitamin/cofactor-responsive condition. These disorders need to be urgently diagnosed via rapid genetic strategies to maximize treatment benefits and avoid any delays.	Distelmaier et al., 2016. Brain. 2017 Feb;140(2):e11. https://doi:10.1093/brain/aww303	9 December 2016
18.	Case Study	MELAS Missed for Years: Stroke-Like Lesions Are No Indication for Brain Biopsy	Late-onset mitochondrial disease may be missed for years, and stroke-like lesions may be easily misinterpreted and lead to brain biopsies. Complex and insidious presentations further delays and reduces the necessity for a proper diagnostic mitochondrial workup, whilst accruing excessive, unwarranted, and costly investigations.	Finsterer, 2019. Case Reports In Neurological Medicine, vol 2019, 2019, pp. 1-4. Hindawi Limited, https://doi:10.1155/2019/9312451	16 December 2019
19.	Cohort study	Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases	The cost-effectiveness of using 'massively parallel sequencing technologies' in the investigation of paediatric muscle disease was examined. The value of implementing these diagnostic technologies into clinical practice was re-enforced, especially those relating to Mendelian inheritance, providing further 'crucial evidence for government subsidy and equitable access'.	Schofield et al., 2017. npj Genomic Med 2, 4 https://doi.org/10.1038/s41525-017-0006-7	3 March 2017

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
20.	Review	Outcome Measures and Quality of Life in Mitochondrial Diseases	Due to its inherent complexities, such as clinical and genetic heterogeneity, unpredictable prognosis, and difficult management with no current cures, identifying appropriate outcome measures for MD has remained elusive. This article assesses current outcome and quality of life measures utilised, assessing their potential benefits and limitations.	Koene et al., 2019. Springer, Cham https://doi.org/10.1007/978-3-030-05517-2_19	4 May 2019
21.	Data base analysis	Lifetime risk of autosomal recessive mitochondrial disorders calculated from genetic databases	The 'lifetime risk of all known autosomal recessive' mitochondrial disease were calculated on the basis of genetic data available on the various data bases such as the European gnomAD. Results revealed a substantially higher cumulative prevalence, than previous estimates.	Tan et al., 2020. EBioMedicine. Vol 54:102730. https://doi:10.1016/j.ebiom.2020.102730	16 April 2020
22.	Cohort Study	Prevalence of mitochondrial 1555A-->G mutation in adults of European descent.	Involved the collection of audiologic data, and DNA samples (blood and hair-follicle) from 2856 subjects (> 49 years) in the NSW Blue Mountains area. The m.1555A>G, and m.3243A>G variants were investigated, with a combined prevalence of approximately 1 in 250, signifying their importance in ADULT sensorineural hearing loss.	Vandebona et al., 2009. N Engl J Med. 5;360(6):642-4. https://DOI:10.1056/NEJMc0806397	5 February 2009

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
23.	Cohort Study	Prevalence of nuclear and mitochondrial DNA mutations related to adult mitochondrial disease.	Evaluation of clinic data confirmed that the combined prevalence of both pathogenic mitochondrial and nuclear genome variants was approximately 1 in 4,300 ADULTS, making it “one of the commonest adult forms of inherited neurological disorders”.	Gorman et al. 2015. Ann Neurol. Vol 77(5):753-759. https://doi:10.1002/ana.24362 .	28 March 2015
24.	Retrospective study	Minimum birth prevalence of mitochondrial respiratory chain disorders in children	The minimum birth prevalence of respiratory chain disorders with onset in childhood, from ‘our’ total data set, was a predicted 5/100,000. Combining this data with a previous study on adults, predicted a minimum birth prevalence of 13.1/100 000 with onset at any age.	Skladal et al., 2003. Brain. Vol 126(Pt 8):1905-12. https://doi:10.1093/brain/awg170 .	21 May 2003
25.	Cohort Study	Psychological functioning in children suspected for mitochondrial disease: the need for care.	The psychological concerns of children with suspected MD is important to note in the early stages, to provide support, regardless of the outcome. Children without a confirmed diagnosis were just as vulnerable to negative psychological effects because they remained uncertain about where they stood in regard to care and diagnosis.	van de Loo et al., 2020. Orphanet J Rare Dis 15, 76. https://doi.org/10.1186/s13023-020-1342-8	24 March 2020
26.	Cohort Study	Quality Of Life In Adult Patients With Mitochondrial Myopathy	The objective of this study determined that a relationship existed between higher disease progression in MD, and decreasing quality of life.	Orsucci et al., 2012. Neuroepidemiology, vol 38, no. 3, 2012, pp. 194-195 https://www.karger.com/Article/FullText/337161	May 2012

	Type of study design	Title of journal article or research project	Short description of research	Website link to journal article or research	Date of publication
27.	Cohort Study	Caregiver's Burden And Quality Of Life In Mitochondrial Disease	Mothers of children with mitochondrial disease and those of intractable epilepsy were compared in indicators of caregivers burden, which were significantly higher in the MD mothers. Poorer health-related quality of life was also determined, especially in regard to role limitations, vitality, and mental health.	Kim, Kyung Ran et al., 2010. Pediatric Neurology, vol 42, no. 4, 2010, pp. 271-276. https://doi:10.1016/j.pediatrneurol.2009.11.012	1 April 2010
28.	Cohort Study	Diagnostic odyssey of patients with mitochondrial disease: Results of a survey.	The mitochondrial diagnostic odyssey is complex, burdensome, requiring numerous consults and investigations, producing often conflicting and uncertain diagnoses, reflecting clinician unfamiliarity. The replication of this study at appropriate intervals will assist in tracking future approaches to MD, in particularly the emergence and acceptance of exome testing in diagnostics.	Grier et al., 2018. Neurol Genet. 2018 Mar 26;4(2):e230. https://doi:10.1212/NXG.000000000000230 .	April 2018
29.	Study of diagnostic accuracy	Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children.	A new proposed MD criteria classification dubbed the "Nijmegen Clinical Criteria", allows for more precise definitions of clinical and metabolic indicators, with an independent scoring system inclusive of investigations, before determining the overall risk of MD.	Wolf and Smeitink, 2002. Neurology. 2002 Nov 12;59(9):1402-5. https://doi:10.1212/01.wnl.0000031795.91814.d8 .	

19. Identify yet to be published research that may have results available in the near future that could be relevant in the consideration of your application by MSAC (limiting these to the English language only). *Please do not attach full text articles, this is just intended to be a summary.*

	Type of study design	Title of research	Short description of research	Website link to research (if available)	Date
1.	Cohort Study	<i>Whole genome sequencing simplifies the diagnosis of mitochondrial disease</i>	Cohort study demonstrating that WGS simplifies the diagnosis of mitochondrial disease and identifies causative gene mutations in 54% of patients suspected to have mitochondrial disease	In submission REDACTED	In submission

PART 5 – CLINICAL ENDORSEMENT AND CONSUMER INFORMATION

20. List all appropriate professional bodies / organisations representing the group(s) of health professionals who provide the service (please attach a statement of clinical relevance from each group nominated):

Mitochondrial disease is diagnosed by expert doctors, familiar with the diagnostic clinical pathway. These include neurologists, metabolic physicians, clinical geneticists, and ophthalmologists. Professional organisations representing these doctors include:

- Australian and New Zealand Association of Neurologists (ANZAN): Most patients with mitochondrial disease are diagnosed by adult neurologists (Grier et al)ⁱ
- Royal Australasian College of Physicians (RACP): Many patients with mitochondrial disease are diagnosed by general neurologists or other consultant physicians (Grier et al)
- Human Genetics Society of Australasia (HGSA): Some patients with mitochondrial disease are diagnosed by clinical geneticists and metabolic physicians (Grier et al)
- Royal College of Ophthalmologists: Some patients with ophthalmological features/manifestations of mitochondrial disease are diagnosed by ophthalmologists

NATA approved laboratories offering WGS as diagnostic testing are able to provide diagnostic genetic testing for mitochondrial disease. Professional organisations involved in the service provision for this include:

- Royal College of Pathologists of Australasia (RCPA). It should also be noted that the previous gold standard diagnostic investigation for mitochondrial disease (histological review of a muscle biopsy), was also provided by pathologists.

21. List any professional bodies / organisations that may be impacted by this medical service (i.e. those who provide the comparator service):

Royal College of Pathologists Australia (RCPA): there will be a shift in the diagnostic paradigm from muscle biopsy to genetic testing- both services are/will be provided by pathology services.

State level Pathology: there will be a shift in the diagnostic paradigm from muscle biopsy to genetic testing- both services will be provided by pathology service.

22. List the consumer organisations relevant to the proposed medical service (please attach a letter of support for each consumer organisation nominated):

- Australian Mitochondrial Disease Medical Network (current applicants)
- Royal Australasian College of Physicians (RACP)
- Australian and New Zealand Association of Neurologists (ANZAN)
- Human Genetics Society of Australasia (HGSA)
- Royal College of Pathologists Australia (RCPA)

PLEASE NOTE: that all the above relevant 'letters of support' have been electronically forwarded onto the HTA with the first submission of this application earlier this year, drafted as either an email response or as a letter of attachment.

23. List the relevant sponsor(s) and / or manufacturer(s) who produce similar products relevant to the proposed medical service:

N/A

24. Nominate two experts who could be approached about the proposed medical service and the current clinical management of the service(s):

REDACTED

PART 6 – POPULATION (AND PRIOR TESTS), INTERVENTION, COMPARATOR, OUTCOME (PICO)

PART 6a – INFORMATION ABOUT THE PROPOSED POPULATION

25. Define the medical condition, including providing information on the natural history of the condition and a high-level summary of associated burden of disease in terms of both morbidity and mortality:

Mitochondrial diseases (MDs) are a group of rare disorders that are genetically and clinically heterogeneous. They represent the largest group of inborn errors of metabolism, having more than 350 pathogenic genetic variants ascribed either to the mitochondrial or nuclear genome.

Severe cases may present in childhood, but most cases develop across the life span. Many cases (both adult and children) are severely debilitating or fatal, with age of onset being the strongest predictor of mortality. However, survival into adulthood is often seen, and progression of symptoms in many affected organs (e.g., brain, heart, liver, etc) is often relentless, with neuromuscular symptoms and fatigability being the greatest contributors to functional disabilities. For some disorders such as MNGIE (mitochondrial neuro-gastrointestinal encephalomyopathy), therapeutic intervention with life-saving procedures such as liver or bone marrow transplantation can be curative. For others such as POLG (DNA polymerase gamma genetic illness) or MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes), preventative strategies can limit life-threatening complications such as status epilepticus (persistent and/or recurring +/- refractory seizures) or stroke-like episodes/encephalopathy, if diagnosed early enough to intervene. Early, accurate diagnosis can optimise medical management and allow for appropriate targeted therapies whilst avoiding harmful medications, which then slows the natural progression of the condition.

Accurate diagnosis can also inform appropriate medical surveillance and avoidance of at-risk lifestyle behaviours (e.g., smoking in Leber's Hereditary Optic Atrophy). Furthermore, establishing a genetic diagnosis has the potential to inform family planning for affected families.

MD is also costly to the individual, their family and to the community, because of its progressive nature and tendency to advance to multi-organ dysfunction. Those with a high variant load in their mtDNA often have severe presentations of MD early in life, while those with a lower variant load can remain in good health for much longer before showing signs of the disease in later life. Surgery, procedures, or illnesses such as bacterial or viral infections may also expedite the natural progression of MD symptoms in some patients. This broad nature of MD's disease burden means that MD sufferers tend to require several specialised physicians for various system involvement (e.g., endocrinologists for diabetic management) and are more liable to suffer deterioration in their health that requires extensive medical interventions, such as cochlear implants, further increasing healthcare costs.

To date, a handful of studies have demonstrated the effects of disease burden on mitochondrial patients, their families and/or carers. Besides the numerous symptoms patients display, they have also reported health outcomes in terms of increased fatigue, mental health problems, and a lowered quality of life in direct relationship to disease progression. A study by van de Loo et al (2020), highlighted the psychological effects within children, suggesting that those without a diagnosis are just as vulnerable to anxiety and depression due to the absence of explanation for their symptoms, as those who receive confirmation of MD. Reinforcing the need for WGS in children, to not just avoid harmful biopsies, but for clarification and in a sense 'justification' of their often complex, ill-defined symptoms.

Similarly, disease burden on carers may also go beyond the effects of symptoms alone. When mothers of children with MD, were compared to mothers of children with intractable epilepsy, they demonstrated significantly higher caregiver burden, through indicators such as increased depression and anxiety, role limitations and reduced vitality, all contributing to a lower quality of life (Kim et al, 2010).

There are few studies on the health costs of MD. Cohen et al. (2018) reported six-fold insurance costs for MD patients compared to the general population. McCormack et al. (2017) reported total hospital costs for MD patients at about US\$113M per annum. Individual Patients with MD were quoted within an Australian Senate Committee report to have benefited from Medicare subsidies of about \$4000 in a single yearⁱⁱ.

26. Specify any characteristics of patients with the medical condition, or suspected of, who are proposed to be eligible for the proposed medical service, including any details of how a patient would be investigated, managed, and referred within the Australian health care system in the lead up to being considered eligible for the service:

Whilst the general presentation of MD in neonates and children differs in many aspects to that of adults, there are also many overlapping features, along with that of the numerous phenotypes, of which many can present at any age. In summary, the adage of “any organ, any symptom, any age”, often applies to MD presentations,

Principal clinical indicators in neonates and children include;

- unexplained single/multi- organ dysfunction or fulminant failure (in particular, liver),
- CNS involvement (seizures, particularly if they are refractory, developmental delays, neuro-regression, intellectual disabilities, progressive encephalopathy or encephalomyopathy, somnolence, stroke-like events, and severe/recurrent migraines, movement disorders, dystonia),
- feeding difficulties ,
- failure to thrive
- short stature
- cardiomyopathy, cardiac arrhythmias,
- profound myopathy and/or rhabdomyolysis, ptosis, exercise intolerance
- GIT disturbances,
- profound hypoglycaemia,
- diabetes mellitus and other endocrinopathies including hypothyroidism, hypoparathyroidism, hypopituitarism, diabetes insipidus, adrenal insufficiency, hypogonadism, growth hormone deficiency, SIADH
- anaemia, pancreatic and/or bone marrow failure,
- hearing loss (in particular, neurosensory hearing loss),
- visual loss, external ophthalmoplegia, pigmentary retinopathy & optic neuropathy, cataract, corneal clouding, glaucoma
- renal impairment, renal tubular acidosis
- prenatal- intrauterine growth retardation
- hirsutism,
- severe & recurrent fevers, immunodeficiency
- family history (unexplained developmental delays, seizures, premature death, unexplained neuromuscular disorders, migraine, deafness, and diabetes, cardiomyopathy),

Similarly principal clinical indicators in adults (over 18 years of age) would include;

- CNS involvement (ataxia, neuropathy, seizures especially if refractory, progressive encephalopathy or encephalomyopathy, stroke-like events, severe/recurrent migraines, unexplained cognitive regression, dementia),
- cardiomyopathy, cardiac arrhythmias, cardiac failure
- profound myopathy (fatigue, weakness), rhabdomyolysis, worsening exercise intolerance,
- GIT disturbances, in particular pseudo-obstruction
- unexplained single/multi- organ dysfunction or fulminant failure (in particular the liver, kidneys),
- anaemia, pancreatic &/or bone marrow failure,
- hearing loss (neurosensory hearing loss especially if initially unilateral, fluctuating),
- visual loss which can be transient, acute, or progressive, chronic progressive external ophthalmoplegia, pigmentary retinopathy and/or optic neuropathy,

- family history (unexplained developmental delays, seizures, premature death, unexplained neuromuscular disorders, migraine, deafness, and diabetes),
- endocrine disturbances (diabetes, hypothyroidism), short stature, hirsutism,
- severe & recurrent fevers with slow recovery periods

Patients with MD may present with a single or with a combination of many of these clinical manifestations, at any given time during their disease course. Occasionally these patients present in crisis during acute exacerbations as inpatients.

Most affected patients undergo an initial clinical assessment and are investigated and managed by expert adult neurologists, paediatric neurologists, metabolic physicians, or clinical/metabolic geneticists after being referred to by a non-expert specialist or general practitioner. After a comprehensive clinical assessment involving a complete neuromuscular workup, hearing and visual assessments, and systems review (GIT, CVS, endocrine, haemopoietic and immune), MD experts undertake investigatory tests based on the clinical phenotype or presentation, which may include the following:

- Blood tests including comprehensive biochemical and haematological investigations,
- Metabolic analysis for biomarkers of mitochondrial dysfunction may reveal raised serum lactate and/or pyruvate, with a normal or decreased lactate/pyruvate ratio, hyperammonaemia, raised serum alanine, raised creatine kinase, presence of urinary Krebs cycle intermediates and organic acids, cerebrospinal fluid elevations of lactate, pyruvate, alanine and protein, and the emergence of new primary biomarkers such as FGF21 and GDF15.
- Neuroimaging (CT, MRI, MRS), neurophysiological studies (EEG, NCS, EMG, VEPs, SEPs)
- Systems review including cardiological (ECG, echo, holter), gastrointestinal (U/S, AXR, CT, transit studies, gastric emptying studies, endoscopies), liver, renal assessments.
- Ophthalmological assessment
- Muscle or tissue biopsies for histological, biochemical (including CoQ10), enzymological (including functional respiratory chain) analysis, histochemical stains, electron microscopy.

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. Therefore, its presentation is often confused with numerous other illnesses, especially those featuring neuromuscular symptoms, such as Multiple Sclerosis and Motor Neurone Disease, whilst its broad range of symptoms, often places it undiagnosed, under the care of varying specialities and services, such as endocrinologists for diabetes and audiologists for hearing aids. Hence MD has long been referred to as the *“great mimicker”* or *“notorious masquerader”*.

This overlapping phenotype with other illnesses, may well be reciprocated when they in turn are misdiagnosed for MD. Safeguards against misdiagnosis have long been adhered to by a clinician with mitochondrial experience, whilst journeying the diagnostic pathway, because until the recent emergence of NGS technologies, MD diagnosis was as much a *‘diagnosis of exclusion’* as it was of ‘inclusion’. The reliance of a ‘suspected diagnosis’ being determined by the intuitive clinician, evidencing the clinical and investigative indicators (and excluding alternatives), whilst seeking support from biochemical, enzymological, and histopathological analysis of both the mitochondria and respiratory chain (RC).

To date, the same diagnostic principles are still inherent in the ‘workup’ of a mitochondrial patient, to maximise the yield of the tissue biopsy, and can now similarly be practised if/when WGS is adopted.

Similarly, since mitochondrial disease is a ‘primary’ disorder, that is, as a result of a defect in the respiratory chain causing oxidative phosphorylation dysfunction, secondary causes are also excluded along the course of the diagnostic pathway.

Within the current boundaries of the ‘Medicare Benefits Schedule (MBS)’, the only subsidised genetic testing available to confirm a MD diagnosis is for children <10 years with clinical features pertaining to item numbers 73358 and 73359. In this clinical representation WGS may be applied if the presentation includes moderate developmental delay and/or intellectual disability, or dysmorphic facial appearance and >1 major structural congenital anomaly. All other MD presentations, and all presentations for

children >10years, are currently not eligible for subsidised genetic testing to confirm their diagnosis. Regardless of a genetic variant being detected (or not) in children qualifying for item numbers 73358/9, a clear and precise phenotypical classification of their MD and its pathogenic relevance, must still proceed.

Where the medical climate is amenable, and there is an indicative phenotypical presentation, an “expert mitochondrial specialist” may order a specific candidate gene test within the current Australian Healthcare system. This is, however, subject to approval from the remunerating tertiary hospital service or paid for (out of pocket) by the patient/child’s family. If the specific candidate gene test was non-confirmatory, WGS would still be required to identify any causative MD genes.

In summary, within the environment of today’s Australian Healthcare system and MBS subsidisation, a diagnosis of MD can only be **suspected** in the majority of cases, since it is solely reliant upon the combination of clinical, biochemical, enzymological, histopathological, and respiratory chain (RC) analysis, via a tissue biopsy, predominantly muscle. A process that can often take years and is thwart with hesitations when determining what level of investigations and re-investigations are necessary. Instead, a genetic **confirmation** expedites diagnosis, avoids invasive biopsies, optimises management earlier, avoids harmful and unnecessary treatments, allows cascade testing of affected relatives and family planning. However, it is only possible in some children <10 years who qualify for MBS item numbers 73358 and 73359.

27. Define and summarise the current clinical management pathway *before* patients would be eligible for the proposed medical service (supplement this summary with an easy-to-follow flowchart [as an attachment to the Application Form] depicting the current clinical management pathway up to this point):

The current clinical management pathway as depicted by ‘Flow Diagram A’ within the Appendix, demonstrates the diagnostic process for children under 16 years, suspected of MD.

Neonates present acutely and often with immediate admission to a neonatal intensive care unit with variable manifestations including encephalopathy, encephalomyopathy, severe systemic metabolic acidosis, lactic acidemia, multi-organ failure (liver, renal), hypoglycaemia, cardiomyopathy which may mimic other inborn errors of metabolism, and as such may often be diagnosed without the need for MBS listed WGS testing. Children in their early infancy may also present with sudden deteriorations, a ‘failure to thrive’ and/or motor regression of their developmental milestones. As children approach their adolescence, less acute and more chronic presentations begin to emerge, with many in their teenage years presenting similarly to an adult MD patient.

Due to the crisis presentation in neonates and earlier childhood, diagnosis of MD is often more swiftly sought, and investigations are extensive in an attempt to rapidly alter the deteriorating health of the child. As the child ages, presentations become more sub-acute or chronic. Investigations by the consulting paediatric neurologist, clinical geneticist/metabolic geneticist, or paediatrician, become more selective. Often in teenage years, diagnosis is all too frequently delayed as the symptoms become more ill-defined, complex, slowly progressive and ‘adolescent behaviour’ often obscures the presentation.

Whilst general clinical and investigative assessments are easily managed by the patient and their family, the more informative but invasive test of tissue biopsies, in particular muscle, is often ‘balked’ at by the caring physician to avoid what may be perceived as harmful, extensive, costly, and unnecessary procedures. Diagnosis of MD is often then delayed and deferred until adulthood.

A ‘strongly suspected’ MD diagnosis is therefore more often actively sought in the younger child, and unless it is phenotypically evident or clinical deterioration necessitates it, a diagnosis is often very late or deferred in an older child or adolescent.

A point to note is that, in current diagnostic practices, the clinical presentation and evidence of mitochondrial dysfunction is frequently guided by the Nijmegen scoring criteria (see table 2 in Appendix), especially in the paediatric environment. However, because it was derived nearly 20 years ago, before the emergence of current NGS technologies, one of its limitations is that tissue morphology (invasive biopsies) is included in the scoring system, and there are caps on clinical and metabolic/imaging criteria, hence this may preclude some patients from genetic testing if the score is insufficient (i.e., within probable range). In these situations, a round table discussion with ‘mitochondrial experts’, may best guide the need for WGS.

For children <10 years with developmental delay, intellectual disability or at least two congenital abnormalities in whom MBS item numbers 73358 and 73359 does apply, confirmatory genetic testing may be an option. Independent of a genetic variant being discovered, a full physical assessment is still necessary for these children, as it is for a 'highly suspected' MD child, to fully determine their clinical phenotype and spectrum of mitochondrial-related features. This is needed in order to monitor symptomatology, optimise management, better understand their prognosis, assess disease progression and the underlying pathogenic relevance. However, in children without a genetic diagnosis, a muscle biopsy (or other tissue) is often still required in order to procure a comprehensive understanding of their 'mitochondrial involvement'.

The current clinical management pathway as depicted by 'Flow Diagram B' within the Appendix, demonstrates the diagnostic process for adults (>16 years) suspected of having MD.

Similarly, to adolescents, adults more often present with less acute and more chronic disease progression. Again, diagnosis is all too frequently delayed, obscured by ill-defined, complex symptoms, which slowly progress, causing years of frustration and 'all too frequently' psychological disturbances, complicated by 'behaviour of a chronic illness'. More often than not, patients find themselves managed under numerous specialities, and attending multiple clinics for their various system involvement. It often requires a crisis event, strong clinical acumen or a renewed perspective by a caring physician, a classical phenotype evolving, or an unrelenting frustrated patient seeking an answer, for the suspicion of MD to emerge, and a diagnosis to be sought in an adult.

The diagnostic odyssey involves both general clinical and investigative assessments, similar to that of children, and are well tolerated by a patient, simply wishing to understand their symptomatology. The more informative but invasive test of tissue biopsies, in particular muscle, is often still delayed by the caring physician, in part due to the same reasons of causing harm, and being excessive, costly, and potentially unnecessary, but also in part due to the physician's awareness that it may exacerbate the patients actual condition. Hence, a 'strongly suspected' diagnosis, an evident phenotype, or a rapid deterioration, is sought before a referral is made to a mitochondrial specialist, and allowing appropriate management to commence.

- a. Unlike children, there is no option currently under the Medicare Benefit Scheduling Scheme, for adults to meet any criteria for genetic testing. Through restricted availability and access, some patients may have single candidate gene testing dependent upon the approval of the remunerating tertiary hospital service, or it may be paid for (out of pocket) by the patient themselves.
- b. With WGS, both nDNA and mtDNA variants would be identified. Given the specific characteristics of heteroplasmy and variable tissue specific thresholds, causative mtDNA variants may on occasion be undetectable in the blood of patients with MD. Should WGS fail to identify a causative nDNA or mtDNA variant, then further genetic testing such as long-range PCR in samples such as saliva, urinary epithelial cells, or muscle tissue, may still be required, but only in cases with high indexes of clinical suspicion of MD, i.e., undiagnosed patients with CPEO (chronic progressive external ophthalmoplegia) who were suspected as having single mtDNA deletion may need a needle muscle biopsy and a long-range PCR.

Rarely, biochemical tests performed on the muscle biopsy in undiagnosed cases may be required in suspected cases. We believe, the complex costing for which has been calculated in previous MSAC submissions (e.g., MSAC application 1585) also involving the examination of muscle biopsies.

PART 6b – INFORMATION ABOUT THE INTERVENTION

28. Describe the key components and clinical steps involved in delivering the proposed medical service:

Once MD is suspected after a thorough analysis of the clinical and investigatory indicators by the diagnosing neurologist (or other MD diagnostic specialist), a recommendation and request for WGS (as the proposed test) would be made. The patient would then require genetic counselling to provide informed consent to undergo WGS.

The WGS data would undergo detailed bioinformatic analysis, with variant calling in a curated list of more than 350, known to be associated with MD and the phenotype under investigation. This reference gene

list will be developed in consultation with the NATA accredited genetic service and the referring MD experts, neurologists, clinical geneticists, metabolic physicians, and genetic pathologists. Such lists already exist and guide practise within current genetic services that provide candidate gene testing. The “PanelApp Australia” is used in Australia, available at <https://panelapp.agha.umccr.org/>, that includes 277 genetic variants for MD. However, the mitochondrial panel available at “PanelApp UK”, is more comprehensive and includes over 400 mitochondrial genetic variants, and would be referred to in any re-analysis.

The proposed MBS item number for WGS would NOT replace any currently available item numbers for candidate gene testing, but would instead be ‘in addition to’. However, its application would negate the need for the majority of muscle (or other tissue) biopsies, their analysis, and all costs incurred for these procedures. A definitive diagnosis of MD would be achieved earlier by a WGS test, avoiding numerous future, repeated investigations, and potentially harmful unnecessary treatments.

In the event of a nondiagnostic or negative WGS/WES result, the need for further investigations (if any) depends on the phenotype or presentation of the patient. The one example being phenotypically evident CPEO, as mentioned in Q26, which may be due to a single mtDNA deletion, sometimes not detected on WGS/WES. If still a high level of suspicion remains for MD, then a muscle (or other tissue) biopsy may still be warranted, otherwise regular follow up and review to monitor for changes or disease progression would be recommended.

29. Does the proposed medical service include a registered trademark component with characteristics that distinguishes it from other similar health components?

No.

30. If the proposed medical service has a prosthesis or device component to it, does it involve a new approach towards managing a particular sub-group of the population with the specific medical condition?

Not applicable

31. If applicable, are there any limitations on the provision of the proposed medical service delivered to the patient (i.e. accessibility, dosage, quantity, duration or frequency):

Currently there are only a few Australian diagnostic laboratories that offer WGS diagnostic genetic testing. Victorian Clinical Genetics Services is the main service provider in Australia. It is expected that other state level diagnostic laboratories (such as SEALS - NSW Pathology) will become accredited to deliver equivalent services in the near future (i.e., 2021).

The WGS sample is agnostic to tissue chosen, and generally blood is preferred due to its convenience, accessibility and transportability. However, due to issues of heteroplasmy, other samples such as saliva or urinary epithelial cells would be used to detect mtDNA mutations.

The WGS test would only need to be performed once in the patient’s life, and the data permanently recorded for later analysis if and when required. As new disease genes or variants are discovered the gene lists will be expanded, allowing subsequent re-analysis of the initial WGS data. Ideally, provision to permit re-analysis of the WGS data at future dates when clinically indicated should be made.

32. If applicable, identify any healthcare resources or other medical services that would need to be delivered at the same time as the proposed medical service:

The volume of access to the relevant healthcare resources and medical services is partaken within the processes of the diagnostic workup, moving towards, and thereby **before** WGS, justifying the need for this service and maximising diagnostic yield. As mentioned earlier in Q25, mitochondrial disease diagnosis has been as much a ‘**diagnosis of exclusion**’ as of ‘inclusion’, reliant upon the combination of clinical and investigative indicators, with the exclusion of mimickers, before seeking support from tissue sample analysis. The proposed service simply replaces the possibility of tissue analysis evidence, with more sensitive and definitive diagnostic WGS.

Genetic counselling, to support patients undergoing diagnostic genetic testing for MD, is required prior to WGS, and best undertaken by the caring physician with mitochondrial experience. An appropriately qualified laboratory geneticist/bioinformatician would be responsible for overseeing the WGS in the laboratory and providing the clinical report that would include interpretation of the results. Specialist /expert consultation for delivery of genetic results after the test results became available, would also be needed.

33. If applicable, advise which health professionals will primarily deliver the proposed service:

Experts in MD, including neurologists (adult and paediatric), metabolic physicians, ophthalmologists and clinical/metabolic geneticists would all have the potential to request the proposed service, and to organise or offer appropriate genetic counselling in the process of ensuring an informed consent has occurred. Pathology service staff would be integral in the collection, processing, transporting and storage of the WGS samples taken.

34. If applicable, advise whether the proposed medical service could be delegated or referred to another professional for delivery:

Not applicable

35. If applicable, specify any proposed limitations on who might deliver the proposed medical service, or who might provide a referral for it:

Mitochondrial disease experts', including neurologists (adult and paediatric), metabolic physicians, ophthalmologists and clinical/metabolic geneticists who have experience in the care of MD patients would be able to request the WGS test.

36. If applicable, advise what type of training or qualifications would be required to perform the proposed service, as well as any accreditation requirements to support service delivery:

Only NATA approved pathology laboratories would be able to perform WGS for MD diagnostic testing. Neurologists, metabolic physicians, ophthalmologists and clinical/metabolic geneticists with clinical expertise and experience in MD care would be able to access the test results. Depending on the clinician's subgroup or specialty, accreditation by their relevant college (e.g., RACP, RACPA, HGSA) would be required.

37. (a) Indicate the proposed setting(s) in which the proposed medical service will be delivered (select ALL relevant settings):

- Inpatient private hospital (admitted patient)
- Inpatient public hospital (admitted patient)
- Private outpatient clinic
- Public outpatient clinic
- Emergency Department
- Private consulting rooms - GP
- Private consulting rooms – specialist
- Private consulting rooms – other health practitioner (nurse or allied health)
- Private day surgery clinic (admitted patient)
- Private day surgery clinic (non-admitted patient)
- Public day surgery clinic (admitted patient)
- Public day surgery clinic (non-admitted patient)
- Residential aged care facility
- Patient's home
- Laboratory
- Other – please specify below

(b) Where the proposed medical service is provided in more than one setting, please describe the rationale related to each:

Inpatient private hospital – while this setting would not account for delivery of WGS for many of the patient population suggested, there is the possibility that patients may be seen in this setting, for example patients undergoing elective surgery with adverse reactions to anaesthetic in private hospitals.

Inpatient public hospital – these patients may be admitted to the hospital with acute or complex medical presentations requiring investigation. This may be the first time that a genetic syndrome is suspected as the cause of their medical condition and the provision of WGS could be ordered while that patient is still admitted under public hospital care.

Outpatient clinics / consulting rooms – this is the commonest setting that provides clinical review/service for patients with mitochondrial disease. Patients are often referred for review and diagnosis to an outpatient clinic or the consulting rooms of a clinical expert. Diagnosed patients often need monitoring by a number of different specialists in the context of outpatient clinics or consulting rooms within both public and private settings.

38. Is the proposed medical service intended to be entirely rendered in Australia?

- Yes
 No – please specify below

PART 6c – INFORMATION ABOUT THE COMPARATOR(S)

39. Nominate the appropriate comparator(s) for the proposed medical service, i.e. how is the proposed population currently managed in the absence of the proposed medical service being available in the Australian health care system (including identifying health care resources that are needed to be delivered at the same time as the comparator service):

Comparator technology:

No genetic testing is veritably the current comparator.

However, the diagnosis of MD is currently informed by MBS subsidised diagnostic tests such as muscle or other tissue biopsies that are performed in suspected patients. Biopsies are not suitable comparators to WGS as they do not provide a definitive genetic (i.e., molecular) diagnosis for MD. However, muscle or tissue biopsies are often still performed, and in order to justify their invasiveness, ‘out of pocket’ expenses that result from proceeding to genetic testing become the comparator for the patient. When incurred, these expenses are currently met by affected individuals or families, or on occasion, by access through the state public health system. In the absence of a genetic diagnosis, targeted therapies and/or reproductive technologies which prevent the inheritance of the condition in future children are unable to be initiated. Also, the current muscle or tissue biopsies do not allow for cascade testing in family members. Thus, without genetic testing affected relatives are also unable to undergo informed family planning still need to undergo extensive investigation if clinically affected.

It should also be noted that muscle biopsy is usually performed under general anaesthetic during a short stay admission. Muscle or tissue biopsies will not be required if diagnostic WGS is introduced on the MBS.

It is also important to note that, in current clinical practice, clinicians may request sequential series of single gene tests, again, funded outside the MBS on either a user-pay basis or by utilising services such as state/territory health department funding.

40. Does the medical service (that has been nominated as the comparator) have an existing MBS item number(s)?

- Yes (please list all relevant MBS item numbers below)
 No

Whilst there is no relevant nor specific MBS item numbers for genetic testing in MD, as stated earlier, a very limited number of children <10 years may qualify for item numbers 73358 and 73359. This however leaves the vast majority of patients with MD not meeting the criteria for any of the other MBS subsidised genetic tests. However, if WGS is introduced, the following MBS item numbers below will be unnecessary for the diagnosis of MD:

1. Muscle biopsy 30075 (\$154)
2. Other medical services that are required to support the nominated comparator (i.e., muscle biopsy) which include:
 - Day stay surgery admission
 - Procedural (theatre) costs
 - General anaesthesia
 - Anaesthetic consultation
 - Surgeon consultation
 - Pathology Services (Histological evaluation of muscle tissue)

(Totals for which have been calculated in regard to the current NMD application 1585, we believe)

40. Define and summarise the current clinical management pathway/s that patients may follow after they receive the medical service that has been nominated as the comparator (supplement this summary with an easy-to-follow flowchart [as an attachment to the Application Form] depicting the current clinical management pathway that patients may follow from the point of receiving the comparator onwards, including health care resources):

The current clinical management pathway (applicable to both children and adults) as depicted by 'Flow Diagram C' within the Appendix, demonstrates the current clinical management pathway of a patient suspected of MD, *after* they receive a clinical evaluation +/- biopsy only to assess their diagnosis.

Referring to our response in Q38, currently there is no genetic testing available under the MBS to confirm the diagnosis of MD. Clinicians instead are reliant upon 'building a case' to evidence the diagnosis for MD, and the use of a tissue biopsy, predominantly muscle, to support probable dysfunction within the mitochondrial respiratory chain.

After a muscle biopsy is performed that is 'diagnostic' of mitochondrial disease, patients proceed to symptomatic treatment that may be general to the illness or specific to the patient's phenotype, [e.g. seizures are treated with anticonvulsants with avoidance of sodium valproate, diabetes is treated with avoidance of metformin, exercise intolerance is treated with aerobic exercise that is restricted to 70% Maximal heart rate, profound hearing loss treated with cochlea implant- see Liang et al. 2011, 'How to treat mitochondrial disease' within the *Australian Doctor*, 2011 Mar 25:27-34]. However, without a confirmed genetic diagnosis, patients are unable to be accurately informed of disease transmission when family planning, as clinicians are left to 'best guess' the most common variant applicable to the phenotypical presentation of the patient and their family history. The genetic counselling process is hindered by the same barriers. Similarly, patient management is indexed according to the level of clinical suspicion for the specific mitochondrial disease presentation in the patient, supported by investigations indicative of the diagnosis. However, limitations on implementing precision therapies remain without an identified genetic variant.

Without a 'diagnostic' muscle (or tissue) biopsy, which occurs in approximately two thirds of mitochondrial disease patients, the clinician is then reliant upon either a strong phenotypical presentation within the patient, a 'best guess' from clinical indicators and/or other investigations undertaken, family history and/or clinical representation within other relatives, disease progression assessed during follow-up consultations, the elimination of MD mimics, in discussion with other 'mitochondrial specialists' within their network, similar presenting case studies, and online review.

41 (a) Will the proposed medical service be used in addition to, or instead of, the nominated comparator(s)?

- In addition to (i.e. it is an add-on service)
 Instead of (i.e. it is a replacement or alternative)

(b) If instead of (i.e. alternative service), please outline the extent to which the current service/comparator is expected to be substituted:

Muscle biopsies will no longer be required when WGS testing confirms the diagnosis of MD and is utilised as the diagnostic service of choice. This would avoid histological, enzymological and/or biochemical analysis of muscle biopsy tissue, general anaesthetic, a surgical procedure, and a short stay admission for patients suspected of having mitochondrial disease. These patients would also avoid the risks of the procedure precipitating deterioration of the MD patient's physical state, a recognised complication after exposure to surgical procedures/ general anaesthetic.

By providing WGS as a single, comprehensive test, patients will avoid follow-on and/or repeat genetic counselling, follow-up consultations for candidate gene testing which may be offered serially, and will also allow the data retrieved from the single WSG testing to be stored and accessed later as required when new MD-associated genetic variants emerge.

42. Define and summarise how current clinical management pathways (from the point of service delivery onwards) are expected to change as a consequence of introducing the proposed medical service, including variation in health care resources (Refer to Question 39 as baseline):

Please refer to Flow Diagram D (children <16 years) and E (adults >16 years) within the Appendix for changes in the *diagnostic process* for a patient suspected of MD, and Flow Diagram F, for general changes in the *clinical management pathway*, after the introduction of WGS (applicable to both children and adults).

As discussed earlier, MDs are characterized by a broad spectrum of phenotypes, clinically non-specific presentations, and an often chronic and complex progression of symptoms, caused by varying and overlapping mtDNA or nDNA mutations. Their mimicking of other conditions including various genetic and neuromuscular syndromes, further complicates the diagnostic process, and reaffirms the need for a genetic confirmation via WGS (supported by various articles listed in Part 4, Q17 table).

Patients are currently reliant upon a 'suspicion only' diagnosis of MD, available through the combination of clinical, biochemical, enzymological, histopathological, and respiratory chain (RC) analysis, supported by the analysis of a tissue biopsy, predominantly muscle. As there is no definitive genetic diagnostic test available on the MBS for MD, it is proposed that WGS, as the 'state of the art next generation sequencing technique' be adopted here in Australia, as it is becoming increasingly essential in the confirmation, identification, and management of MD.

With the introduction of diagnostic WGS, a precise molecular diagnosis can be achieved in the majority of patients. In the cohort study of adults suspected of having mitochondrial disease, 54% of patients achieved a diagnosis compared to 10-27% receiving a diagnosis through the current pathway using muscle biopsy +/- singleton genetic testing (which is currently not MBS subsidised, and so falsely raises the diagnostic yield). Muscle biopsies will no longer be needed except in some circumstances where a genetic diagnosis is not determined via WGS, and a follow-up biopsy has been shown to be of value. This is estimated to occur in approximately 13% of patients (adjusted from our cohort), and in 'undiagnosed' patients with CPEO who were suspected of having a single mtDNA deletion. In this situation, a saliva sample, a needle biopsy (agnostic of tissue chosen) or a long-range PCR would be performed.

Currently there are >350 genetic variants recognised to be causal of MD, which may well extend beyond 400 by the completion of this application. NATA accredited diagnostic services such as the "Victorian Clinical Genetic Services", have access to comprehensive reference lists of known variants available nationally or internationally on websites such as PanelApp Australia or PanelApp UK. Otherwise, they may seek advice from alternative sites such as the international "Mitochondrial Disease Sequence Data Resource (MSeqDR) Consortium", which identifies and prioritizes specific genomic data analysis to meet the needs of the mitochondrial medical and research community world-wide.

After determination of a genetic diagnosis, patients with MD are able to undergo disease specific treatments and interventions to prevent adverse outcomes (e.g., MNGIE, LHON, MELAS), avoid medications that can cause adverse outcomes (e.g. POLG) and unnecessary treatments as well as consider informed family planning options. That is, with genetic testing, access to targeted therapies or assisted reproductive technologies (ART) to prevent disease inheritance, can be confidently provided. The precise clinical management pathway followed is dependent upon the specific MD that is identified by the genetic testing (i.e., precision medicine).

The most common general therapies available to the MD patient are those described within the mitochondrial cocktail which can vary slightly from specialist to specialist and country to country. This includes a combination of nutraceuticals, often in higher than the recommended doses but within safe thresholds, and includes CoQ10, magnesium orotate, riboflavin, thiamine, and vitamins E and C. Other nutraceuticals often recommended in addition to the cocktail, include Idebenone (in place of CoQ10), nicotinamide riboside (NAD+), vitamin A, carnitine, and creatine. These are all currently freely available 'over the counter' and not subsidised by the PBS. Due to the large variety and higher doses of nutraceuticals required within the mitochondrial cocktail, patients may often spend in excess of \$400 per month, with NAD+ alone costing approximately \$180 per month. Whilst the 'mitochondrial cocktail'

remains the primary current and general recommendation for MD management, studies are inconclusive as to the real value of it as a whole.

A genetic diagnosis would allow a patient to seek more targeted therapeutics, and nutraceuticals appropriate for treating their individual genotype, thereby improving disease management, slowed disease progression with the minimisation of metabolic crises. If a targeted nutraceutical could be delivered more effectively and cost efficiently, then greater compliance would also follow, as would greater health outcomes. A non-genetic diagnosis allows the current management strategies of costly broad spectrum 'cocktails' to continue .

Current PBS approved therapies available to the MD patient include those predominantly used for **symptomatic control** and **organ involvement**. Such examples include the current PBS listed oral hypoglycaemic agents and Insulin available to treat diabetes, with the judicious use of Metformin. The wide range of anti-epileptic medications available, are vital for the control of seizures in the majority of patients affected, again practising caution in regard to Sodium Valproate.

There are no current treatments (or cures) specifically for MDs as a whole available on the PBS, although the list below demonstrates several PBS and MBS funded targeted therapies. However, alternative funding is available for a few select targeted treatments via various subsidised schemes, such as Idebenone for LHON, and arginine and/or carnitine for use in children with a confirmed metabolic disorder such MELAS.

Targeted treatment and management strategies listed include,

- numerous available and condition-effective nutraceuticals (generally non-subsidised but may occasionally be subsidised via various schemes),
- current PBS available medication to support organ dysfunction/failure (e.g., pancreatic replacement enzymes in Pearson's disease)
- conventional but potentially harmful therapies to avoid (e.g., sodium valproate for seizures in POLG),
- available surgical and therapeutical options (e.g., allogeneic hematopoietic stem cell transplant, and liver transplant in MNGIE),
- rescue and supportive therapies,
- effective lifestyle options (e.g., ketogenic diet),
- preventative strategies (e.g., ART and PGD in respect to nDNA variants), and
- new developments offering curative interventions or improvements decreasing morbidity and mortality.

PLEASE NOTE : Table 1 in the Appendix, details a comprehensive list of available treatments linked to the known genetic variant/s of each phenotype.

PART 6d – INFORMATION ABOUT THE CLINICAL OUTCOME

43. Summarise the clinical claims for the proposed medical service against the appropriate comparator(s), in terms of consequences for health outcomes (comparative benefits and harms):

In a study using WGS to diagnose 242 patients with suspected MD (Davis et al, submitted 2021), a definitive genetic diagnosis was achieved in 130/242 patients.

This diagnosis changed management in 42/130 patients by informing precise treatment for 27/130 patients who achieved a molecular diagnosis (16 patients with Stroke-like episodes, 6 patients with LHON, 3 patients with MNGIE, 2 patients with congenital myasthenia), enabling informed avoidance of inappropriate medications in 15 patients (MIDD x8, POLG x7) and restored reproductive confidence in 30 additional families.

From a clinical perspective, an accurate assignment of a genetic variants has important prognostic and therapeutic implications for the patient. Clinicians can reach a definitive diagnosis earlier in the medical odyssey); they can provide patient-centred monitoring and therapy; and refer patients to other relevant healthcare services and clinicians as required. Also, genetic counselling services can be offered, with clinicians able to provide relevant information on prognosis, family planning and long-term management.

Providing patients with a definitive genetic diagnosis prevents recurrent reviews and often invasive, costly re-evaluations. A single WGS test will often prove to be much more cost-effective, than a repeated complex array of investigations including potentially harmful invasive tissue biopsies and successive candidate gene testing. Mitochondrial mimickers can be more confidently detected, and patients moved more quickly and decisively into appropriate management strategies that can prevent metabolic crises, improve quality of life indicators, and avoid inappropriate management that may be harmful.

Identification of the causative gene variant in a MD patient also allows for cascade testing, not just amongst those asymptomatic family members seeking family planning guidance, but more significantly in those family members with spurious symptomology that are undergoing their own complex diagnostic pathway to find a definitive diagnosis for themselves.

Precision therapies would become available to a patient with a genetically confirmed diagnosis, and are often expected to slow disease progression rather than reverse any related morbidity. Since such therapies target the underlying biochemical defect associated with a particular pathogenic variant, to reliably identify and quantify any subtle resultant changes in symptomatology and health outcomes, becomes the challenge. Clinician-observed outcomes may not necessarily reflect what the patient (or carer) perceives, whereas patient-reported outcomes best reflect quality-of-life status, any small changes detected in an extensive list of symptoms, perceived performance changes, altered social participation, and disease barriers, are so best studied when seeking potential health outcomes subsequent to precision therapeutics. In return, gene identification also allows for greater understanding of MD pathophysiology, and the emergence of an increasing number of precision therapeutics.

Other genetic disorders, such as childhood neuromuscular disorders, have benefited from next generation sequencing (NGS) technologies into the diagnostic pathway for patients, with improved cost-effectiveness (as reported by Schofield et al. 2017), better management strategies, and informed family planning options.

Overall, determining health outcomes in mitochondrial disease studies and services is challenging for many reasons inherent to the illness, and clinical trials are only as reliable and credible as the endpoints chosen to produce the meaningful data needed.

Therefore, future large-scale clinical trials to determine more accurate health outcomes in mitochondrial disease, and detailed subsequent disease progression, will require multicentre collaboration nationally and most likely internationally, to overcome as many of its inherent barriers as feasible. Then the benefits of WGS and the identification of a pathogenic variant, can be more widely determined in both diagnostics and disease management.

44. Please advise if the overall clinical claim is for:

- Superiority
 Non-inferiority

45. Below, list the key health outcomes (major and minor – prioritising major key health outcomes first) that will need to be specifically measured in assessing the clinical claim of the proposed medical service versus the comparator:

MAJOR:

Clinical Effectiveness

- confirmed diagnosis allowing for effective and appropriate MD treatment and management to commence,
- earlier confirmed diagnosis allowing for earlier effective and appropriate management which may halt or delay disease progression, preventing and/or minimising metabolic crises,
- genetic diagnosis allowing for effective and appropriate targeted key management strategies,
- earlier genetic diagnosis allowing for earlier effective and appropriate targeted key management strategies which may halt or delay disease progression, preventing and/or minimising metabolic crises,
- identification of the specific pathogenic variant allowing for the provision of effective targeted treatments within each individual syndrome,
- cessation of inappropriate, ineffective, and potentially harmful treatments,

- Improved and appropriate clinical monitoring for known disease/syndrome indicators and complications,
- accurate and informed counselling for family planning,
- avoidance of children born with MD,
- informed decision making applying to any current or future children and/or foetus at risk of MD,
- informed clinical and inheritance risk management applying to any potentially affected family members

Safety Outcomes

- avoidance of intrusive and potentially harmful investigations currently in practice to aid diagnosis, such as invasive tissue biopsies (particularly muscle) and general anaesthetics (e.g., muscle biopsies, childhood MRI),
- avoidance of inappropriate or unnecessary investigations and interventions, (mitochondrial patients are particularly vulnerable to delayed recovery and metabolic crises, as a result of procedures and/or general anaesthetics),
- avoidance of inappropriate, ineffective, and potentially harmful treatments,

MINOR:

Clinical Effectiveness

- Improved accessibility to services upon receipt of confirmed diagnosis (e.g., NDIS, Enable)
- Improved accessibility to funds upon receipt of confirmed diagnosis (e.g., superannuation, disability support pensions, carers payments, sick leave entitlements)
- Improved 'Quality of Life' (QoL) contributors and parameters,
- Positive social impacts secondary to improved and effective management strategies, and improved QoL parameters,
- Positive relationship impacts secondary to improved and effective management strategies, and improved QoL parameters,
- Positive psychological impacts from genetic counselling and/or testing and/or pre-emptive results,
- Positive psychological impacts of cascade or WGS testing in children and/or foetus, and the resultant decisions (e.g., early diagnosis, option of termination, removal of anxiety if negative),
- Availability of cascade testing to family members,
- Clinical and psychological benefits to family members of a confirmed diagnosis

Safety

- Avoidance of inappropriate and potentially harmful treatments in family members,
- Avoidance of extensive, unnecessary, and harmful investigations in family members

Cost effectiveness

- Cost-effective targeted treatments and management strategies for patient and caring family,
- Cost-effective benefits of targeted treatments for the patient rather than the more expensive 'broad spectrum mitochondrial cocktail',
- Economic impacts on work and family finances with improved accessibility to funds, services, and better management,
- Economic adverse events from receiving an identified genetic variant (e.g., health, work, and life insurance policies)
- Cost-efficient benefit of WGS over both multiple single-gene tests and combined WES (for nDNA and mtDNA),
- Cost-efficient benefit of a single genetic test, that is WGS only, means a reduction in the need for multiple follow-up consults with/without genetic counselling,
- Less economic impacts (for family and healthcare system) by avoiding children born with MD,
- Economic benefits to families and healthcare, from cascade testing, which results in reduced unnecessary investigations and treatments, earlier appropriate management, and a reduction in an inheritable condition (i.e., MD),
- In reference to cascade testing, any additional costings in performing tests, consultations (+/- genetic counselling), and family planning technologies (ART), would be offset by the economic benefits of

reduced burden of disease load, both within families and healthcare systems, and greater efficiency of care,

- Cost effectiveness of ART +/- PGD procedures against a lifetime of healthcare costs in a child or adult born with MD, for parents, families, carers and healthcare systems,
- Cost of more effective targeted treatments against quality-adjusted life years,
- Cost benefits to Australian Government healthcare as a whole

PART 7 – INFORMATION ABOUT ESTIMATED UTILISATION

46. Estimate the prevalence and/or incidence of the proposed population:

Prevalence: MD is caused by genetic mutations in either nuclear and mitochondrial DNA (nDNA and mtDNA). Although the minimum prevalence of disease associated with mtDNA mutations in the Australian community is estimated to be 1 in 250 or 120 000 Australians (Vandebona NEJM 2009), the birth prevalence of MD is reported to be 1 in 5000 children (Skadel Ann Neurol 2003) and 1 in 4300 adults (Gorman Ann Neurol; 2015) in international studies.

Incidence: There are no data documenting the incidence of mitochondrial disease in Australia. However, a study by Tan et al (2020) in Munich Germany, evaluated 249 genetic variants on their in-house database and determined that the collective lifetime risk of mitochondrial disease was on average 31.8/100,000 (or 1.6/5000) and similar in that of the global dataset, but 48.4/100,000 (or 2.4/5000) in the European gnomAD dataset. In general, their results suggested that the MD collective lifetime risk is significantly higher than previously thought, especially when compared to newborn screening tests such as PKU, calculated as 0.8/5,000.

Based on the numbers referred (70-80/year) and confirmed (approximately one third of referred patients or approximately 25 patients) for the diagnosis of MD in NSW, it is estimated that approximately 150 patients per year across Australia would have confirmed MD.

47. Estimate the number of times the proposed medical service(s) would be delivered to a patient per year:

WGS would be undertaken once per patient per lifetime during the course of a diagnostic workup. Re-analysis of the WGS data would occur in undiagnosed cases as new variants emerge over time, or as clinically indicated. This would apply to approximately 50% of cases with the potential for re-analysis no more than once every 18 months.

The re-analysis of WGS data would apply to fewer patients in the future as the diagnostic rate increases. Cascade testing may be required during the initial reporting of the WGS test to aid in the interpretation of variants, but this is included the costings by the lab. Cascade testing of family members after obtaining a positive result, would only be required once per variant per lifetime.

Currently, there is no relevant clinical indication for the isolated testing of MD in a foetus in the absence of genetic variants in the parents and/or siblings, as there's no distinct foetal presentation, subject to altering antenatal management. However, confirmation of a genetic variant in one parent (autosomal dominant), both parents (autosomal recessive), the mother's mitochondrial genome, or in a sibling, could lead to prenatal genetic testing of the foetus after informed genetic counselling.

48. How many years would the proposed medical service(s) be required for the patient?

Not applicable for WGS testing [see proposed Items A and B].

Re-analysis of WGS data from a single test would only occur in the absence of an initial genetic diagnosis [refer to proposed item C], and would be applied no more than once every 18 months for the duration of the person's illness, or until a diagnosis is confirmed.

49. Estimate the projected number of patients who will utilise the proposed medical service(s) for the first full year:

Given the backlog of patients awaiting this service in 2021, the projected number of patients seeking WGS for the diagnosis of MD is estimated to be up to 400 cases nationally, within the first full year

50. Estimate the anticipated uptake of the proposed medical service over the next three years factoring in any constraints in the health system in meeting the needs of the proposed population (such as supply and demand factors) as well as provide commentary on risk of 'leakage' to populations not targeted by the service:

Given that we estimate that 300 cases/year would be seeking diagnosis, and allowing for the backlog of 100 cases seeking this medical service already waiting, it is anticipated that 1000 WGS tests will be requested in the first 3 years.

Risk of leakage will be managed by restricting ordering of WGS testing to neurologists (adult and paediatric), metabolic physicians, ophthalmologists, and clinical geneticists with experience in the assessment, management and treatment of MD. The Australian Mitochondrial Disease Medical Network would provide support and education to doctors seeking to gain this level of expertise.

PART 8 – COST INFORMATION

51. Indicate the likely cost of providing the proposed medical service. Where possible, please provide overall cost and breakdown:

Initially, the cost incurred towards WGS testing would involve the consultation process that has already been established and embedded into the diagnostic pathway. This would include everything from the original presentation to the GP, with referral onto a general specialist (most commonly a neurologist or paediatrician) and then onto the specialised services of a ‘mitochondrial expert’, most often established within the outpatient clinic of a larger teaching hospital.

The diagnostic path would only vary after completing the preliminary round of investigations, during which the suspicion of a mitochondrial disease is either raised or repealed. The decision to then consider WGS, as opposed to the traditional tissue biopsy, is where the proposed medical service costs begin to accrue.

Pre-WGS counselling would be performed by either the ‘mitochondrial expert’, or caring physician within the setting of the hospital outpatient department, and approximates to \$100-200 for a 30-60minute consult (MBS Item number 116 or 110). Follow-up consultation to deliver the diagnosis and plan future management would then be required with similar costing.

The WGS testing is most often performed by the pathology service within the hospital setting in communication with a **NATA accredited diagnostic service** appropriate for the molecular diagnosis of mitochondrial disease. Currently there are >350 variants associated with mitochondrial disease, see reference nuclear gene list in supplementary table 1, Ryan et al (2021, page 39) as an example. However, by the termination of this application it may well be >400, at which time the diagnostic WGS costing as offered by the VCGS for >400 genes, may need to be applied. Either international or national recognised reference lists of mitochondrial pathogenic variants, such as those available in PanelApp (UK or Australia) could be sourced.

The clinical diagnostic laboratories providing such WGS services, and their costs, currently include;

Victorian Clinical Genetic Services

WGS (overall cost from receipt of patient sample, DNA sequencing, informatics analysis through to provision of clinical report, and includes cascade testing if required for variant interpretation)

- \$3000 for 101-400 genes + Inclusive of both nDNA and mtDNA analysis and
- \$4300 for >400 genes + Inclusive of both nDNA and mtDNA analysis

PLEASE NOTE: WGS includes mtDNA analysis in addition to variant calling in nDNA; this includes running the raw data through an additional mtDNA variant calling program and additional bioinformatic analysis of mtDNA. (Hence the additional costs over item numbers 73358 and 73359)

Cascade testing of single variant = approximately \$450

Re-analysis of WGS data:

- Production of a report where there are no new findings = \$350
- Production of a report including curation of new variants = \$650

SEALS-NSW PATHOLOGY

Only offering ‘Whole **Exome** Sequencing’ and so currently not a viable option for a comprehensive mitochondrial disease analysis,

- \$2000 (singleton - nDNA analysis only)
- \$2800 (triome - nDNA analysis only))

Cascade testing of single nuclear DNA variant: \$350

Re-analysis of whole exome data:

- Production of a report (irrespective of no or new variants being detected): \$420

52. Specify how long the proposed medical service typically takes to perform:

Provision of WGS requires pre-test genetic counselling for the patient and any relevant family members (such as parents), which may take 30-60 minutes of the specialist's time.

The test itself can be performed almost immediately (after the appropriate genetic counselling) by a local pathology centre with access to a NATA accredited diagnostic service. Turnaround times for the WGS are currently 3-4 months, but this may improve when the current bottleneck of bioinformatics/curation is appropriately resourced.

Delivery of the results to the patient (and/or the relevant family members such as parents) by the treating clinician would require another consult with an expected duration of 30-60 minutes if the WGS pathology report includes a genetic diagnosis and a discussion of cascade testing is required. Further genetic counselling (of approximately one hour duration) may be required for the delivery of cascade testing results.

If a genetic diagnosis is not able to be determined from the WGS testing, then follow-up consults (of indeterminable varying lengths), in discussion with the patient/family would then be required. In the event of either a VUS, novel gene or a known genetic variant of uncertain clinical significance to the patient, then repeated assessments and evaluations of uncertain time periods over undetermined time frames would need to occur.

Re-analysis of WGS data would take between 6-8 weeks, and then dependent upon its findings, follow-up as above would be required, including appropriate genetic counselling by the specialist when a diagnosis has been determined.

53. If public funding is sought through the MBS, please draft a proposed MBS item descriptor to define the population and medical service usage characteristics that would define eligibility for MBS funding.

Upon the acceptance of genetic testing for mitochondrial disease, the new additional item numbers A, B and C would need to either be incorporated into current MBS Item numbers 73361, 73362 and 73363 applicable to cascade testing, otherwise as proposed below, new item numbers D, E, F and G would need to be generated.

Item A

Characterisation via whole exome or genome sequencing and analysis, of germline variants, from a phenotypically driven gene list (accessible present in nuclear and 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) Evident mitochondrial dysfunction in children <16years, and/or
- (ii) Unexplained hypotonia, weakness, failure to thrive and a metabolic acidosis in children, and/or
- (iii) Unexplained single or multi- organ dysfunction or fulminant failure (in particular but not limited to hepatopathy, pancreatic and/or bone marrow failure) in children <10years, and/or
- (iv) Unexplained profound hypoglycaemia and/or
- (v) Refractory seizures, developmental delays, and progressive encephalopathy or encephalomyopathy in children <16years, and/or
- (vi) Cardiomyopathy and/or cardiac arrhythmias in children <16years, and/or
- (vi) Rapid hearing and/or visual loss in children <16years, and/or
- (vii) Stroke-like episodes, encephalopathy, seizures, muscle fatigue and weakness, and/or
- (viii) External ophthalmoplegia, and/or

- (ix) Hearing loss, diabetes, short stature, endocrinopathy, and/or
- (x) Family history of any of the above(c) the characterisation is performed following the performance for the patient of a service to which item 73292 applies for which the results were non-informative; and

(c) the characterisation is not performed in conjunction with a service to which items B, 73358 or 73359 applies

Applicable only once per lifetime

Fee: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

Item B

Characterisation via whole exome or genome sequencing and analysis, of germline variants, from a phenotypically driven gene list present in nuclear and 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) the request for the characterisation states that singleton testing is inappropriate; and

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

- (i) Evident mitochondrial dysfunction in children <16years, and/or
- (ii) Unexplained hypotonia, weakness, failure to thrive and a metabolic acidosis in children, and/or
- (iii) Unexplained single or multi- organ dysfunction or fulminant failure (in particular but not limited to hepatopathy, pancreatic and/or bone marrow failure) in children <10years, and/or
- (iv) Unexplained profound hypoglycaemia and/or
- (v) Refractory seizures, developmental delays, and progressive encephalopathy or encephalomyopathy in children <16years, and/or
- (vi) Cardiomyopathy and/or cardiac arrhythmias in children <16years, and/or
- (vi) Rapid hearing and/or visual loss in children <16years, and/or
- (vii) Stroke-like episodes, encephalopathy, seizures, muscle fatigue and weakness, and/or
- (viii) External ophthalmoplegia, and/or
- (ix) Hearing loss, diabetes, short stature, endocrinopathy, and/or
- (x) Family history of any of the above(c) the characterisation is performed following the performance for the patient of a service to which item 73292 applies for which the results were non-informative; and

(d) the characterisation is performed following the performance for the patient of a service to which item 73292 applies for which the results were non-informative; 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 A, 73358 or 73359 applies.

Applicable only once per lifetime

Fee: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

Item C

Re-analysis of whole exome or genome data obtained in performing a service to which item A or B 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 A or B 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: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

Item D

Detection of a single gene variant for diagnostic purposes in suspected mitochondrial disease, if:

(a) 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

(b) the patient has a biological sibling with a known monogenic mitochondrial disease; and

(c) a service to which item numbers A, B, C, 73358, 73359 or 73360 applies, has identified the causative variant for the sibling's condition; and

(d) the results of the testing performed for the sibling are made available for the purpose of providing the detection for the patient; and

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

Applicable only once per variant per lifetime

Fee: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

Item E

Detection of a single gene variant for the purpose of reproductive decision making, if:

(a) the detection is requested by a Consultant Physician or Specialist experienced in the treatment of mitochondrial disease; and

(b) the patient has a first-degree relative with a known monogenic mitochondrial disease that can be plausibly shared between them; and

(c) a service to which item numbers A, B, C, 73358, 73359 or 73360 applies has identified the causative variant for the relative; and

(d) the results of the testing performed for the relative are made available for the purpose of providing the detection for the patient; and

(e) the detection is not performed in conjunction with item numbers D, F, 73361, 73362, or 73363

Applicable only once per variant per lifetime

Fee: \$xxx.xx **Benefit:** 75% = \$xxx.xx 85% = \$xxx.xx

Item F

Detection of a single gene variant for segregation purposes in relation to a person, if:

(a) 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

(b) the patient:

(i) is a biological parent or other biological relative of the person and has a known mitochondrial disease phenotype of the person; or

(ii) is a biological parent of the person and has suspected monogenic mitochondrial disease; and

(c) the single gene variant can be plausibly shared between the patient and the person who has known or suspected monogenic mitochondrial disease; and

the biological parent or biological relative of the person and has a with the single gene variant; and

(d) a sample has not previously been tested for the patient for a service to which item A, B, 73358, or 73359 applies; and

(e) a service to which item A, B, C, 73358, 73359 or 73360 applies has identified a potentially mitochondrial disease causative variant for the person; and

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

(g) the detection is not performed in conjunction with item D, E, 73361, 73362 or 73363.

Applicable only once per variant per lifetime

Fee: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

Item G

Detection of a single gene variant for diagnostic purpose, in a sample of foetal tissue, if

(a) 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

(b) the single gene variant has been:

(i) identified in the biological mother and is of mitochondrial genome lineage; or

(ii) identified in both biological parents, 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

(c) a service to which item numbers A, B, C, 73358, 73359 or 73360 applies, has identified the causative variant for the condition of the foetus's first-degree relative; and

(d) the results of the testing performed for the foetus are made available for the purpose of providing the detection for the parent/s; and

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

Applicable only once per variant per lifetime

Fee: \$xxx.xx **Benefit:** 75% = \$xxx.xx 85% = \$xxx.xx

Item H

Characterisation of a single mitochondrial DNA deletion 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) Evident mitochondrial dysfunction in children <16years, and/or

(ii) Unexplained hypotonia, weakness, failure to thrive and a metabolic acidosis in children, and/or

(iii) Unexplained single or multi- organ dysfunction or fulminant failure (in particular but not limited to hepatopathy, pancreatic and/or bone marrow failure) in children <10years, and/or

(iv) Unexplained profound hypoglycaemia and/or

(v) Refractory seizures, developmental delays, and progressive encephalopathy or encephalomyopathy in children <16years, and/or

(vi) Cardiomyopathy and/or cardiac arrhythmias in children <16years, and/or

(vi) Rapid hearing and/or visual loss in children <16years, and/or

(vii) Stroke-like episodes, encephalopathy, seizures, muscle fatigue and weakness, and/or

(viii) External ophthalmoplegia, and/or

(ix) Hearing loss, diabetes, short stature, endocrinopathy, and/or

(x) Family history of any of the above(c) the characterisation is performed following the performance for the patient of a service to which item 73292 applies for which the results were non-informative; and

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

Applicable only once per lifetime

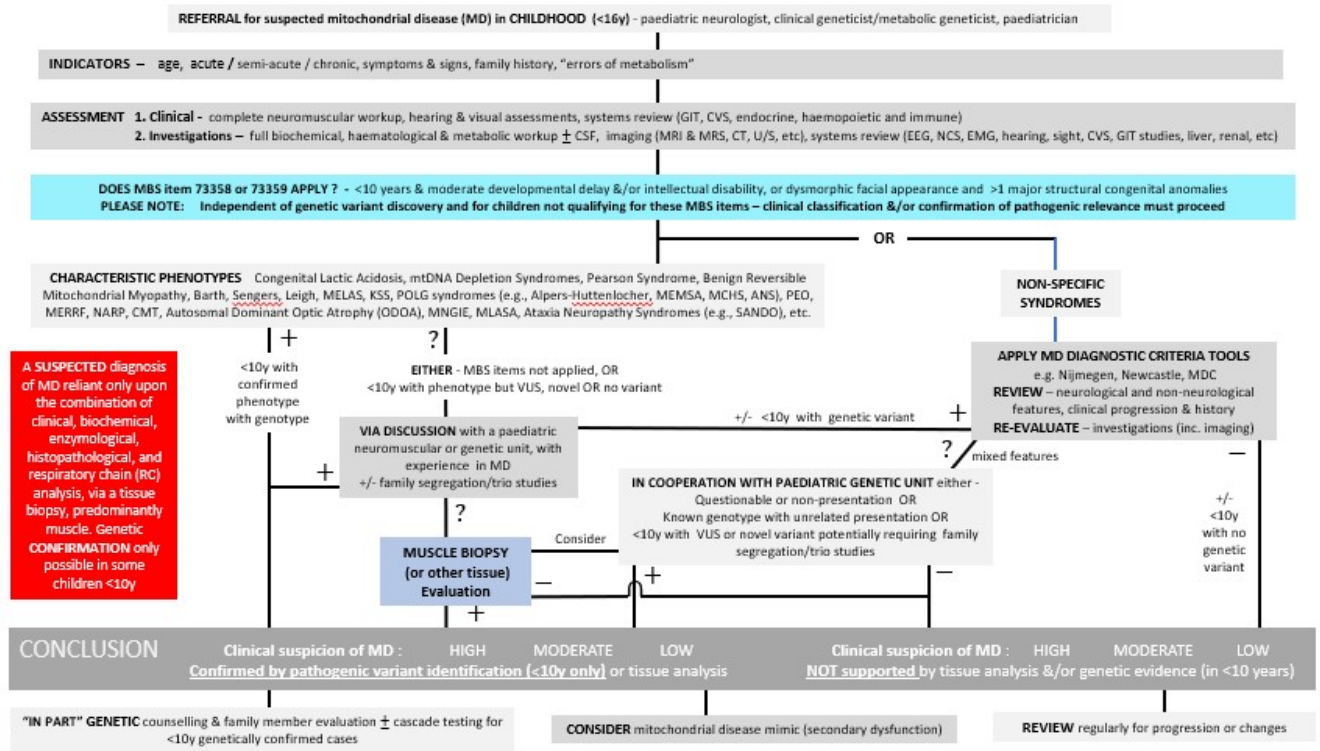
Fee: \$xxxx.xx **Benefit:** 75% = \$xxxx.xx 85% = \$xxxx.xx

ⁱ Johnston Grier, Michio Hirano, Amel Karaa, Emma Shepard, and John L.P. Thompson, (2018) Diagnostic odyssey of patients with mitochondrial: Results of a Survey. *Neurol Genet.* 2018;4(2):e230. Published 2018 Mar 26. doi:10.1212/NXG.0000000000000230

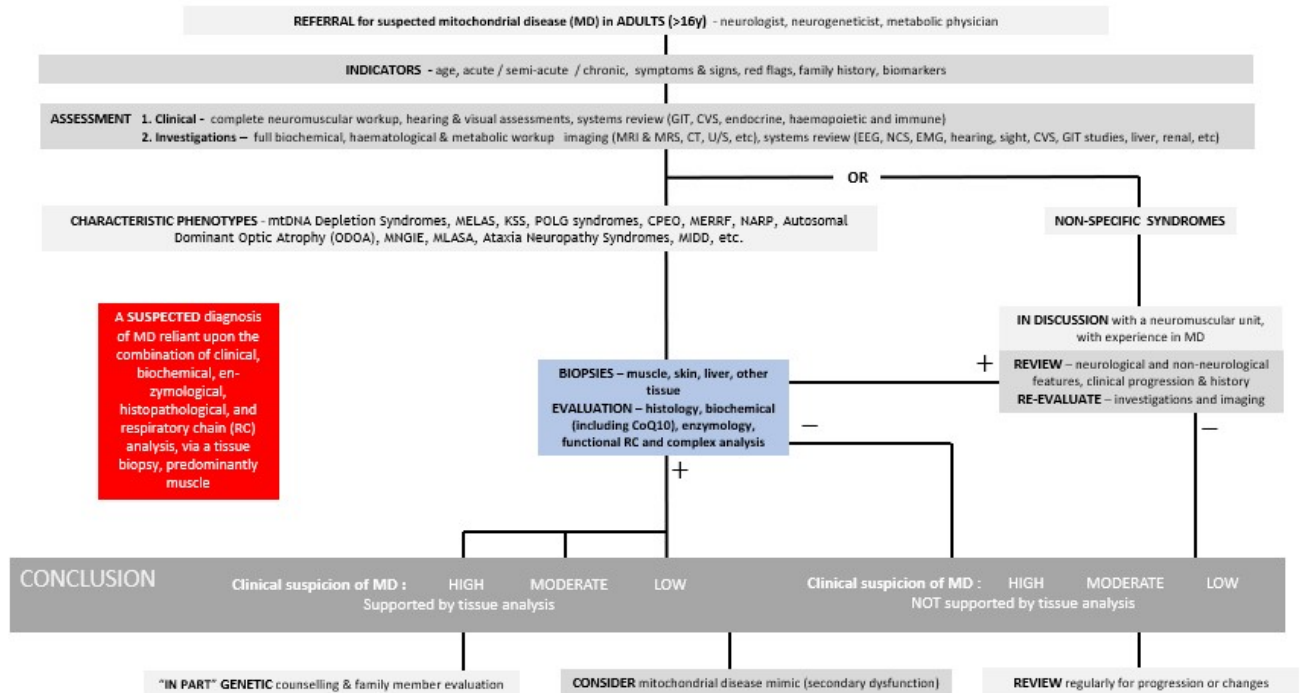
ⁱⁱ Science of mitochondrial donation and related matters. The Senate, Community Affairs References Committee. Commonwealth of Australia, June 2018, Australia.

APPENDIX

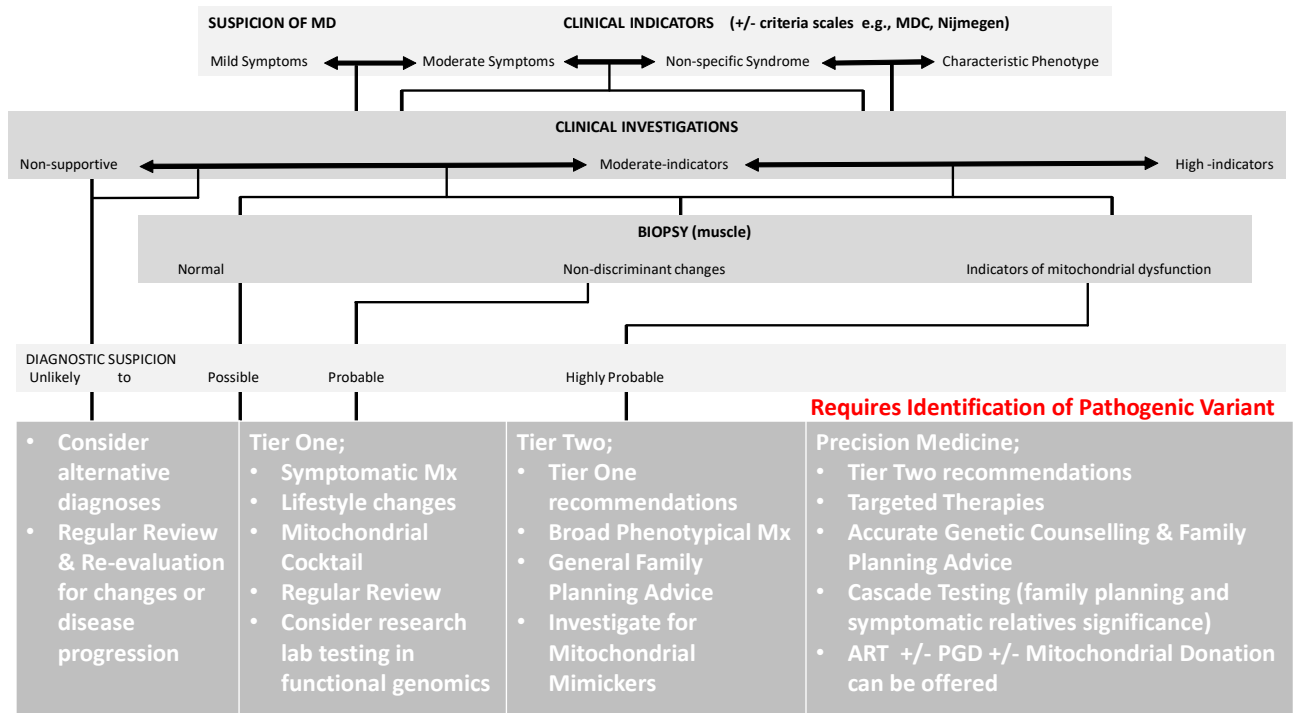
Flow Diagram A



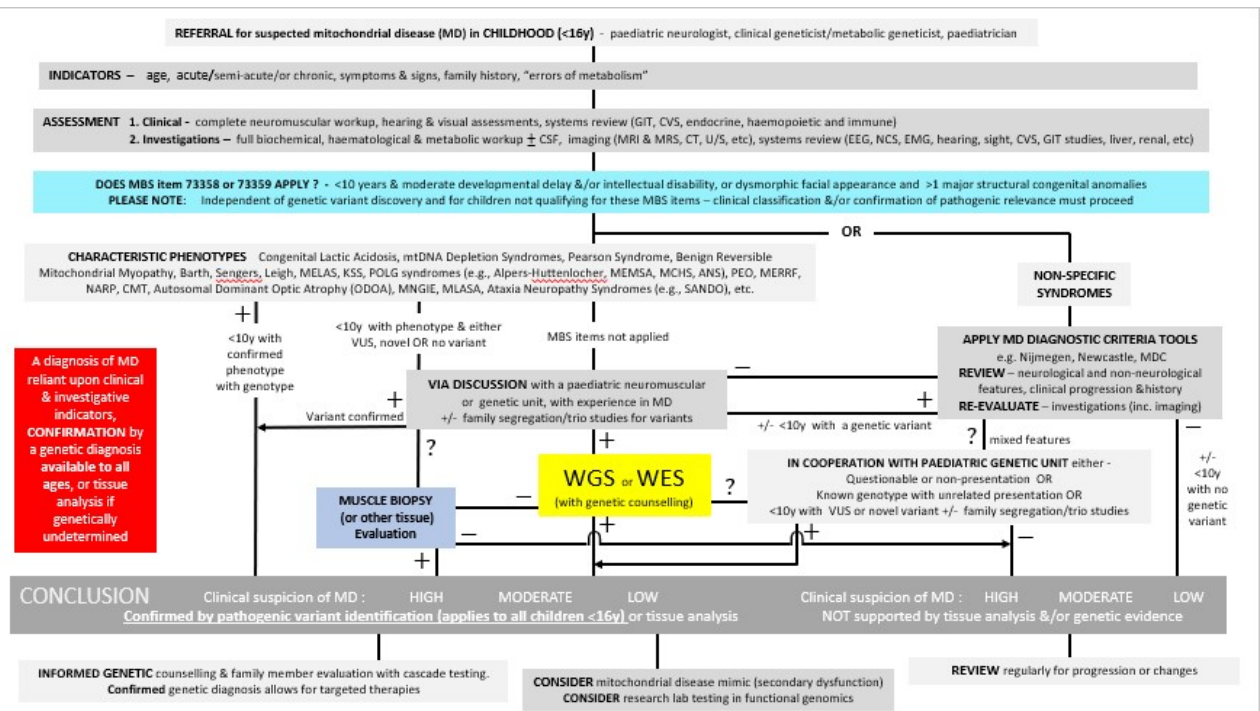
Flow Diagram B



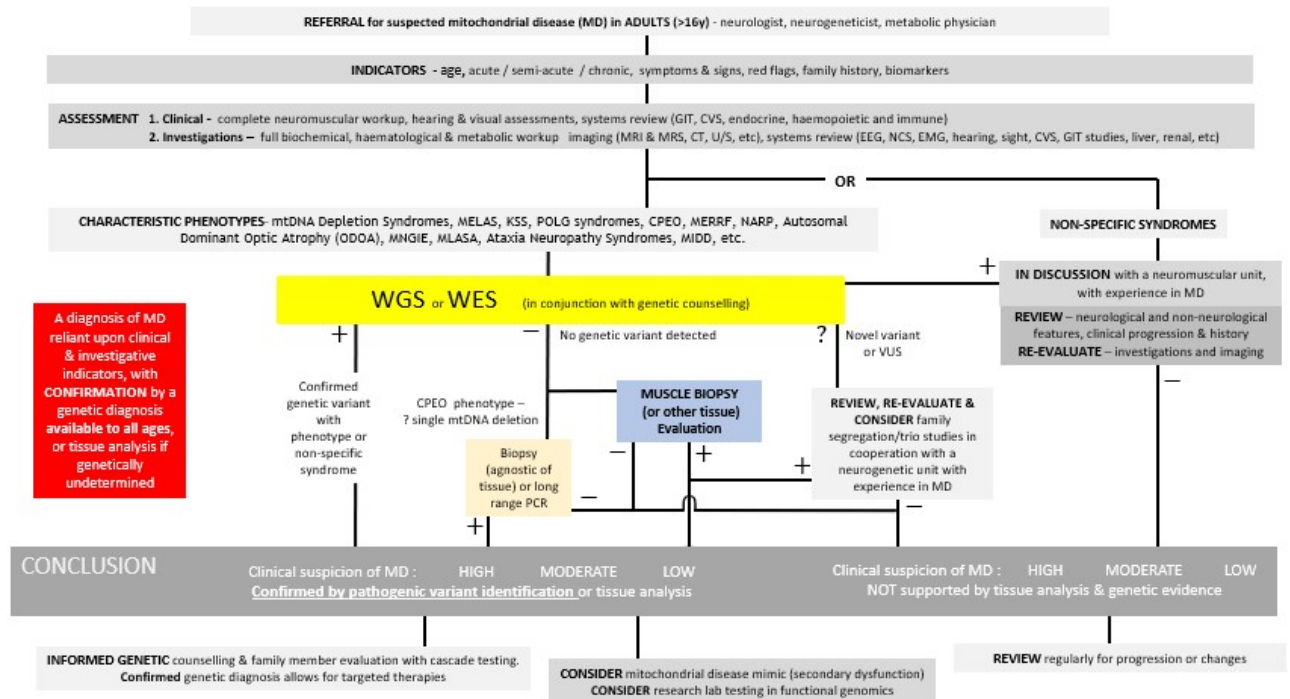
Flow Diagram C



Flow Diagram D



Flow Diagram E



Flow Diagram F

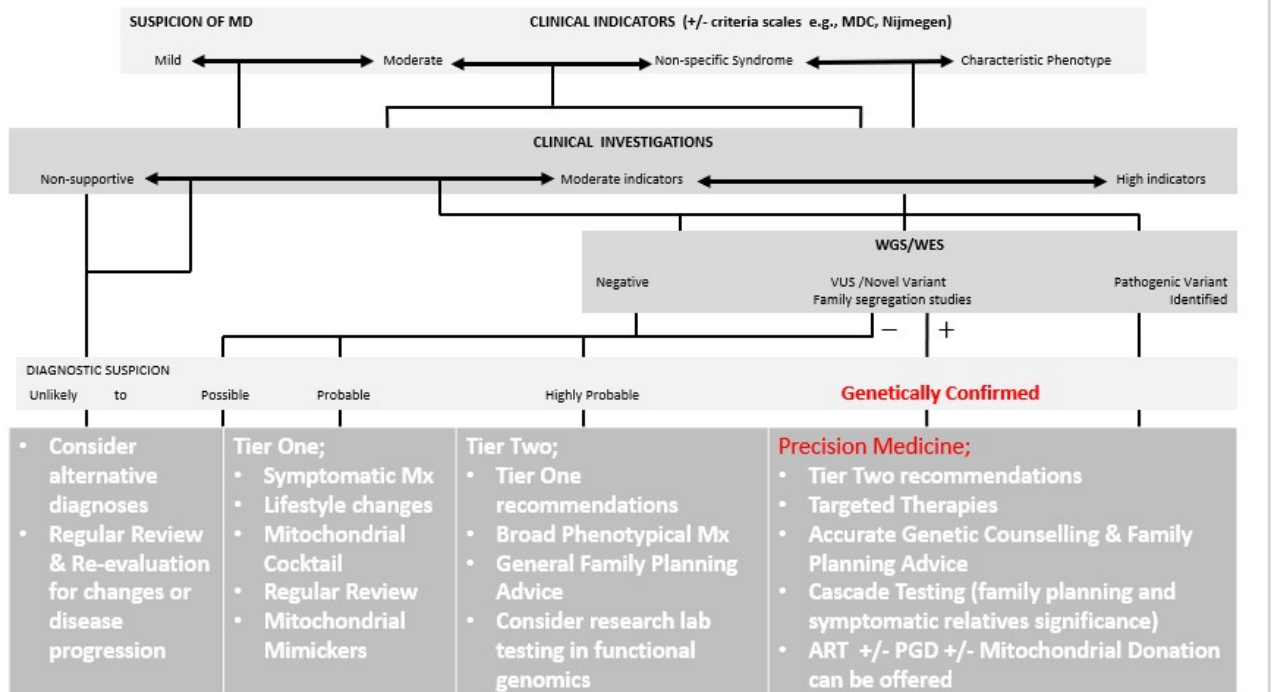


Table 1 - Targeted Therapies - reliant upon the confirmation of a genetic diagnosis
(Sourced from Bottani et al., 2020, and Distelmaier et al., 2016)

<i>PHENOTYPE</i>	<i>GENOTYPE</i>	<i>MANAGEMENT</i>
MELAS (mitochondrial encephalopathy, lactic-acidosis, stroke-like syndrome) MIDD (maternally inherited diabetes and deafness)	mtDNA: m.3243A>G nDNA: variants in MT-TL1 and MT-TK genes	Treatment of acute mitochondrial stroke-like episodes with intravenous (IV) arginine hydrochloride, and daily oral arginine to prevent strokes. Citrulline appears to be more potent, but more studies needed. KH176 - positive effects on attention performance and measures of depression and anxiety Idebenone has shown to lower fatigue severity scale scores. Avoidance of metronidazole is warranted to avoid sudden elevations in lactic acid, potentially inducing a stroke-like event. Current pre-clinical studies on potential Glutathione treatment (A3243G & A8344G variants), and clinical trials being conducted in the use of Acipimox (A3243G & single large-scale deletions), Nicotinamide Riboside and Everolimus (a rapamycin analogue). Pre-clinical studies are being undertaken in the use of resveratrol
MNGIE (Mitochondrial Neuro-Gastro-Intestinal Encephalomyopathy)	nDNA: Autosomal recessive variants in TYMP gene	Allogeneic hematopoietic stem cell transplant Liver transplantation, Erythrocyte-encapsulated thymidine phosphorylase, and gene therapy are also potential treatments. Trials commencing on Enzyme Replacement Therapy - Erythrocyte Encapsulated Thymidine Phosphorylase
Leber hereditary optic neuropathy (LHON)	mtDNA: m.3460G>A, m.11778G>A, and m.14484T>C	Idebenone can prevent further visual loss, especially in patients with discordant vision. iii Awaiting results of trials in the use of Elamipretide Intravitreal injection of AAV vector and Allotopic gene expression. Clinical studies currently active on the use of PDE5 inhibitors, Curcumin, EPI-743, and preclinical studies in NDI1 usage
Dominant Optic Atrophy	nDNA: OPA 1	Current trials involving the use of Idebenone
Thiamine transporter-2 deficiency (Biotin-thiamine-responsive basal ganglia disease)	nDNA: SLC19A3	Thiamine (20–40 mg/kg daily) and biotin (15 mg/kg daily) greatly improves patients' clinical condition preventing further episodes with metabolic decompensation, and improvement of neurological and biochemical abnormalities of this disease
Mitochondrial Complex I deficiency	nDNA: Especially ACAD9 variants (e.g., ACAD9 deficiency)	Particularly benefit from Riboflavin orally at 10-20mg/kg daily. JP4-039 is also under investigation.

Pearson's Disease	mtDNA: Specific mtDNA deletion includes deletion of the complete genes for ATPases 6 and 8, cytochrome c oxidase III, and NADH dehydrogenase 3, 4, 4L, and 5	Supportive therapy includes pancreatic enzyme replacement, blood transfusions, as well as granulocyte-colony-stimulating factor application. Clinical trials commencing (by appointment only) in respect to transplantation of MNV-BM-BLD (autologous cd34+ cells enriched with blood derived mitochondria) in paediatric patients
Mitochondrial Disorders presenting with liver failure	nDNA: DGUOK, MPV17, SUCLG1, POLG1	Supportive management includes supplementation of vitamin K, administration of fresh frozen plasma, and treatment of hypoglycaemia
	nDNA: TRMU	Can cause transient hepatic problems, which resolve spontaneously after the first year of life. So, in the absence of neurological impairment, liver transplantation may be warranted when liver failure is severe
Hearing loss	mtDNA: Especially in m.1555A>G, and m.1494C>T mutations	There is an increased susceptibility to aminoglycoside-induced ototoxicity, so caution must be adhered to in their use.
Pyruvate dehydrogenase deficiency e.g., Thiamine-responsive pyruvate dehydrogenase deficiency .	nDNA: PC pathogenic variants nDNA: PDHA1	Ketogenic diet has been shown to increase longevity and improve mental development. Recruiting for trials in Sodium Phenylbutyrate and dichloroacetate Variable but possible response to Thiamine orally at 30-40mg/kg daily
Coenzyme Q10 deficiency	nDNA: variations in PDSS1, PDSS2, COQ2, COQ4, COQ6, ADCK3, ADCK4, and COQ9	Excellent to highly variable response to oral CoQ10 orally at 10-30mg/kg daily, depending on the underlying defect
mtDNA variants	mtDNA variants	Replacement of mutant mtDNA in oocytes or single-cell embryos by mitochondrial replacement therapy to prevent their transmission (legislation changes for approval currently under review)
nDNA variants	nDNA variants	Preventive therapy through prenatal diagnosis after genetic counselling is becoming increasingly important for nDNA-related disorders.
Ethylmalonic encephalopathy	nDNA: ETHE1 variants	Metronidazole and N-acetyl cysteine given together in a cohort of patients was able to improve some of the clinical features of the disease (given on compassionate grounds). Potentially liver transplantation JP4-039 and gene therapy approaches are being investigated for potential clinical trialling
Kearns-Sayre Syndrome	mtDNA: mtDNA depletions	CoQ10 & Mitochondrial augmentation therapy trials are currently being undertaken
Leigh Syndrome	nDNA: ND1-G3697A, SUCLA2, ETHE1, ND5-G13513A, EARS2, SURF1, ND6-T14487C NDUFS4	EPI-743 improves clinical outcomes in children with genetically confirmed Leigh syndrome.

		Clinical trials being piloted in the use of Rapamycin and Everolimus (a rapamycin analogue). Preclinical studies in the use of gene therapy approaches and NAD+ precursors
MERRF	mtDNA: m.8344A > G variant in MT-TK gene, and other variants in MT-TL1, MT-TH, MT-TS1, MT-TS2, and MT-TF genes	Cytotoxic Necrotizing Factor 1 (CNF1) and the delivery of nucleic acids to the mitochondria are both currently being explored for trialling
Barth Syndrome	nDNA: TFAZZIN gene variants	Current active trialling of Elamipretide in patients
TK-2 Disease	nDNA: TK-2 variants	Molecular bypass therapy in disorders of mtDNA instability are currently being undertaken
POLG-related disorders	nDNA: POLG variants	Sodium Valproate is absolutely contraindicated in patients with <i>POLG</i> -related disease as it can precipitate liver failure and therefore the <i>POLG</i> gene should be sequenced before prescribing Sodium Valproate to patients with status epilepticus iv
Episodic muscle weakness, mimicking periodic paralysis	mtDNA: MT-ATP6 variants	Affected individuals may improve with acetazolamide treatment. Pre-clinical trials are also being undertaken for PDE5 inhibitors, Rapamycin and Resveratrol treatments.
Mitochondrial defects associated with hyperammonemia	nDNA: TMEM70 or ATP5F1D mutations	Require additional detoxification therapy with drugs like L-arginine and sodium benzoate during episodes of metabolic decompensation.
Brown-Vialetto-Van Leare syndrome/Fazio-Londe disease	nDNA: SLC52A2, SLC52A3, (SLC52A1)	Riboflavin given orally at 10-50mg/kg daily provides a generally good treatment response
Biotinidase deficiency	nDNA: BTB	Biotin given orally at 5-10mg/kg daily provides a generally good treatment response
Holocarboxylase synthetase deficiency	nDNA: HLCS	Biotin given orally at 10-20mg/kg daily with a variable but generally good response
Thiamine pyrophosphokinase deficiency	nDNA: TPK 1	Variable but possible response to Thiamine orally at 20mg/kg daily
Multiple acyl-CoA dehydrogenase deficiency	nDNA: ETFA, ETFB, ETFDH, SLC25A32, FLAD1	Good responses to Riboflavin orally at 10mg/kg daily
Molybdenum cofactor deficiency	nDNA: MOCS1, MOCS2, GPHN	Intravenous Cyclic Pyranopterin Monophosphate at doses of 80-320µg/kg daily provides a generally good response in MoCD type A patients. JP4-039 is currently also under investigation
Thioredoxin 2 deficiency	nDNA: TXN2	Minimal data available but good response reported to antioxidant treatments, such as Idebenone orally at 20/kg daily
DGUOK (Deoxyguanosine Kinase) Deficiency	nDNA: DGUOK	Liver transplantation has been used as an option in some patients. Molecular bypass therapy under investigation for the use in clinical trials

Table 2 - The Nijmegen Clinical Criteria for Mitochondrial Disease
 (Sourced and provided by The Foundation for Mitochondrial Medicine, Atlanta, USA)

Nijmegen Clinical Criteria for Mitochondrial Disease: Clinical scoring for diagnostic assessment is according to (Wolf NI, Smeitink JA. Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children. Neurology. 2002 Nov 12;59(9):1402-5.)

CLINICAL CRITERIA	SCORE
Neuromuscular manifestations (Maximum of 2 points)	
a. Progressive external ophthalmoplegia (2 points)	
b. Ptosis (1 point)	
c. Exercise intolerance (1 point)	
d. Muscle weakness (1 point)	
e. Rhabdomyolysis (1 point)	
f. Abnormal electromyogram (1 point)	
Central nervous system and other organ involvement (Maximum of 2 points)	
g. Isolated central nervous system involvement (1 point)	
h. Any other isolated organ system (1 point)	
i. Two or more organ systems (2 points)	
Metabolic and imaging studies (Maximum of 4 points)	
j. Elevated blood lactate on 3 occasions (2 points)	
k. Elevated cerebrospinal fluid lactate (2 points)	
l. Elevated blood alanine (2 points)	
m. Elevated cerebrospinal fluid alanine (2 points)	
n. Elevated urine tricarboxylic acid (Kreb) cycle intermediates (2 points)	
o. Elevated urine ethylmalonic, 3-methylglutaconic, or dicarboxylic acids (1 point)	
p. Abnormal ³¹ P-MRS (magnetic resonance spectroscopy) in muscle with reduced Phosphocreatine/P _i ratio (2 points)	
q. Abnormal T2 signal in basal ganglia on brain MRI (2 points)	
r. Decreased resting metabolic rate or abnormal exercise studies (cycle ergometry protocol) (2 points)	
Tissue morphology (Maximum of 4 points)	
s. Ragged red fibers on muscle biopsy (2 points if present, 4 points if >2%)	
t. Diffuse reduction in cytochrome c oxidase histochemical reaction or scattered COX deficient fibers(4 points)	
u. Strongly succinate dehydrogenase positive vessels by histochemistry (1 point)	
TOTAL SCORE	

Scoring for evaluation of Clinical Criteria:

Definite: 8-12 points

Probable: 5-7 points

Possible: 2-4 points

Unlikely: 1 point

The Nijmegen Biochemical Criteria are modified for incorporation of contemporary technologies. Genetic criteria for diagnosis are added.

BIOCHEMICAL CRITERIA	SCORE
1. Abnormal high resolution respirometry in muscle or fibroblasts (measurements <5% reference level) (Live (fresh tissue) assessment of Complex V function, coupling and protein leak across the mitochondrial membrane:)	
2. Abnormal OXPHOS subunit immunohistochemistry or immunofluorescence in skeletal muscle tissue sections (Qualitative assessment of OXPHOS enzyme assembly within tissue sections. Defects in OXPHOS enzyme assembly are readily recognized by this testing.)	
3. Abnormal OXPHOS enzymology (single or multiple enzyme defects) (activity measurements <5% reference level) (Testing must be performed on mitochondria isolated from fresh (not frozen) tissue to minimize risk of artifacts caused by freezing skeletal muscle prior to mitochondrial isolation).	
4. Abnormal quantitative Western Blot of Selected OXPHOS subunits from Complexes I-V (levels <5% reference level for subunit) (Western blot can detect defects that are not evident by other techniques. (2))	
5. Abnormal muscle CoQ10 level (<50% of control mean) (Allows assessment for primary defects in Coenzyme Q10 synthesis. (3))	
6. Supercomplex evaluation: Optimal OXPHOS function requires aggregation of individual OXPHOS enzymes into supercomplexes which allows efficient and rapid transport of electrons. (4-8) Supercomplexes allow efficient formation of an electrochemical (proton) gradient created by Complexes I, III, and IV that is then used by Complex V to synthesize ATP. Supercomplex formation is impaired in a variety of OXPHOS diseases. (9-23)	
a. Abnormal supercomplex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.)	
b. Abnormal monomeric OXPHOS enzyme complex formation (Score 0.5 if present only in Blue Native OR OXPHOS Clear Native Immunoblot. Score 1 if present in BOTH tests.)	
7. Abnormal in-gel OXPHOS enzyme activity. (Qualitative in-gel assessment of OXPHOS enzyme activity in intact enzymes. This is particularly important in assessment of the ATPase activity of Complex V and Complex V assembly. (11-15))	
TOTAL SCORE	

Scoring for evaluation of Biochemical Criteria

Unlikely: Criteria Score 0

Possible: Criteria - Score is ≥ 1 and < 2

Probable: Criteria- Score = 2

Highly Probable: Score > 2

GENETIC CRITERIA	SCORE
1. mtDNA depletion (mtDNA copy number <5% reference interval)	
2. Identification of confirmed pathogenic mtDNA or nuclear DNA mutation	
3. Identification of provisional pathogenic mtDNA or nuclear DNA mutation (i.e. mutation requires additional data supporting pathogenicity)	
4. No mutation identified	

Scoring for evaluation of Genetic Criteria

Definite: Criteria 1 or 2 are abnormal