Practice Guidelines for the Molecular Diagnosis of Mitochondrial Diseases.

These guidelines were prepared following the mitochondrial DNA disease best practice meeting held on February 10th 2004, and recently updated in April 2008 and ratified by the CMGS Executive on 7th July, 2008.

1.0 INTRODUCTION

1.1 Mitochondrial DNA.

Though clinically and genetically heterogeneous, mitochondrial disorders are characterised by biochemical abnormalities of the mitochondrial respiratory chain, a key component of oxidative phosphorylation [McFarland et al, 2002; DiMauro and Schon, 2003; Taylor and Turnbull, 2005]. Located in the inner mitochondrial membrane, complexes I-V of the respiratory chain comprise >80 individual polypeptides, the vast majority of which are encoded by nuclear DNA. However, some 13 essential subunits (and the necessary RNAs required for their expression) are encoded by the mitochondrial genome, the only non-chromosomal DNA in human cells [Anderson et al, 1981]. Mitochondrial DNA (mtDNA) is a small (16.5kb), closed-circular molecule of DNA that exhibits a strict maternal transmission and is present within cells in multiple copies. In addition, many of the proteins responsible for the maintenance, replication and transcription of the mitochondrial genome are nuclear encoded and as such the genetic defect in patients with mitochondrial disorders may occur in either the mitochondrial or nuclear genome [DiMauro and Schon, 2001; Shoubridge, 2001]. Emerging examples are the mtDNA depletion and multiple mtDNA deletion syndromes due to mutations in enzymes involved either in mitochondrial nucleoside metabolism or salvage.

1.2 Heteroplasmy, threshold and segregation.

The presence of many hundreds or thousands of copies of mtDNA within each cell plays an important role in the expression of a pathogenic mutation in terms of a biochemical and clinical phenotype. Homoplasmy describes the situation where all mtDNA copies are identical within a cell. In contrast to this, the majority of patients with pathogenic mtDNA mutations harbour a mixture of wild-type and mutant mtDNA within each cell, the phenomenon of heteroplasmy [Lightowlers et al, 1997]. For any heteroplasmic mutation, the proportion of mutant mtDNA molecules in a cell typically exceeds a critical threshold before a biochemical defect is apparent. At mitosis, mutant and wild type mtDNA is believed to be randomly segregated to each of the daughter cells, affecting both mitochondrial disease expression and inheritance as it will lead to different percentage levels of mutant mtDNA between tissues, whole organs and even individuals. Since higher levels of heteroplasmic, pathogenic mtDNA mutations accumulate in post-mitotic tissues such as skeletal muscle (which is often a clinically-affected tissue), the majority of diagnostic tests for mtDNA mutations are usually performed using DNA extracted from this tissue. Nevertheless, some mutations can reliably be detected in blood.

1.3 Clinical Manifestations of Mitochondrial DNA disease.

Though recognised as a clinically heterogeneous group of disorders, there are a number of well-defined mitochondrial syndromes that are characterised by their association with certain clinical features. These include chronic progressive external ophthalmoplegia (CPEO) and Kearns Sayre Syndrome (KSS) which are typified by external ophthalmoplegia, ptosis and large-scale mtDNA rearrangements (both single and multiple mtDNA deletions). Mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged-red fibres (MERRF) and Neurogenic weakness with ataxia and retinitis pigmentosa (NARP) are all associated with common point mutations within the mitochondrial genome (m.3243A>G MELAS, m.8344A>G MERRF and m.8993T>G/C NARP). Taken together alongside the three primary mtDNA mutations that cause Leber hereditary optic neuropathy (LHON), these represent the most common mitochondrial genetic defects that are routinely screened by a diagnostic laboratory.

Laboratory Diagnosis of Mitochondrial Respiratory Chain Disease.

The investigation and diagnosis of patients with mitochondrial respiratory chain disease remains a challenge, often necessitating a combination of techniques including muscle histocytochemistry, biochemical assessment of respiratory chain function and molecular genetic studies alongside clinical assessment [Taylor et al, 2004]. Any molecular genetic test for a mtDNA mutation should ideally be directed by the clinical phenotype and results of these other investigations, yet the overwhelming majority of patient samples referred to regional genetics laboratories are accompanied by scant clinical information and a generic request for mitochondrial screening. In formulating these Best Practice Guidelines, our primary aim is to address the variability that currently exists between CMGS diagnostic laboratories in terms of the most appropriate genetic assays and patient samples to help reach a definitive diagnosis. As indicated below, the recently designated National Commissioning Group (NCG) Service for Rare Mitochondrial Disorders of Adults and Children – hosted in Newcastle, Oxford and UCLH, London – now offers the opportunity for further testing (histochemical, biochemical and molecular genetics) for specific patients outside of the routine service provided in regional centres.

2.0 Molecular Genetics Investigations for Mitochondrial DNA diseases.

2.1 Service context.

A multidisciplinary approach is required for the effective diagnosis of mitochondrial diseases. Molecular Genetics services are therefore best sited in departments specialising in mitochondrial genetics and where there is good access to clinical and research expertise in the field.

In defining these guidelines a pragmatic approach has been taken in order to account for the significant variation in clinical practice in the request for molecular investigations. Whilst purists may feel that biochemical and histological investigation should precede and hence inform molecular analysis, the reality is that many referrals are received with little information. A balance must be struck between the need to provide timely reports and the need to use resources wisely by undertaking the most appropriate investigations. Where clinical information is lacking centres often choose to analyse for a minimal range of investigations and if negative request further clinical information (an example form is available to download as additional file 1) or additional samples (e.g. muscle). More specialist and thorough investigations will be warranted in some cases and these should be referred to specialist centres for further work.

In this regard, the NCG Service for Rare Mitochondrial Disorders of Adults and Children was designated from April 2007 to provide both diagnostic and clinical services for those patients with suspected mitochondrial respiratory chain disease and in whom screening of common mtDNA mutations - readily available at several CMGS-affiliated diagnostic laboratories – does not provide a diagnosis. These more complex tests including whole mtDNA genome sequencing and screening nuclear genes implicated in mtDNA maintenance disorders (e.g. *POLG*, *PEO1*, *SLC25A4*). All enquiries about the NCG service can be directed to Professor Rob Taylor (robert.taylor5@nuth.nhs.uk).

2.2 Clinical Information and Gatekeeping.

Good gatekeeping is essential in providing clinically relevant and cost -effective mtDNA services. Referrals do not always fall clearly into one of the clinically defined syndromes and referring neurologists may rely on the laboratories to undertake the most appropriate investigations and provide advice. As clinical pathways for the mitochondrial referrals are not well developed nationally it is advisable for referrals received by the genetics laboratories to be discussed with a neurologist or /geneticist with a specific interest in mitochondrial disorders. Laboratories may choose to obtain additional clinical information prior to analysis or where a minimal range of investigations is performed at the stage of reporting. Exceptions to this may include LHON and MIDD. An example of the questionnaire used by the Oxford Laboratory is included (see additional file 2).

2.3 Sample type.

Sample type is important and affects the likelihood of detecting a mutation in certain referral categories. Age of the patient must also be taken into consideration when advising on the most appropriate sample type.

The following table indicates preferred sample types for the major referral categories.

	Blood	Muscle
LHON	***	Not necessary
Pearsons	***	Not necessary
KSS/CPEO	*	****
Depletion	*	***
MELAS/MIDD	*	***
MERRF	***	***
Leighs/NARP	***	Not necessary
Deafness (1555A>G)	***	Not necessary

Notes

- mtDNA rearrangements are unlikely to be detected in blood using Southern hybridisation in adults >20years [Poulton et al 1991].
- MELAS (m.3243A>G) levels decline in blood with age and low levels may be missed in older patients [Rahman et al 2001, Hammans et al 1995].
- MERRF (m.8344A>G) levels in blood are reliable. [Larsson et al 1992].
- Blood: most laboratories standardise on blood in EDTA.
- Muscle should be snap frozen and delivered to laboratories on dry ice.
- Urine is a very good alternative for the analysis of mutations especially the m.3243A>G, as levels in urine correlate more closely with levels in muscle: 20 ml of an early morning sample is sufficient for most applications [McDonnell et al 2004].

3.0 ANALYSIS AND REPORTING

The following sections provide guidance on the analysis and reporting issues for routine referrals. Sections are categorised by clinical presentation, and where it is not unusual to have a request for a specific mutation this has also been included. A section on mutation nomenclature is also included.

3.1 Reason for referral:
Chronic Progressive External Ophthalmoplegia (CPEO)
Kearns Sayre Syndrome (KSS)
Pearson Syndrome
? mtDNA rearrangement

Mitochondrial DNA rearrangements (deletions and duplications, multiple mtDNA deletions) characterise this group of referrals. Screening for the m.3243A>G point mutation is also indicated. Muscle is the preferred tissue for the detection of rearrangements (apart from in cases of Pearson syndrome where deletions are readily detectable in blood), however laboratories often receive blood samples.

mtDNA rearrangements are not readily detectable in blood in adults and laboratories should impose a cut off age above which they will not routinely screen samples for rearrangements [McShane et al, 1991]. Currently this varies from centre to centre and ranges from 18-30 years. A negative result will not exclude a deletion (at any age).

mtDNA rearrangements are usually sporadic although familial cases have been reported. Duplications have been associated with familial cases and therefore may be associated with a

recurrence risk [Poulton et al, 1994]. Multiple mtDNA deletions can be autosomal dominant, autosomal recessive or sporadic.

Long Range PCR: Long Range PCR (LPCR) is commonly used to provide rapid and reliable exclusion of rearrangements in samples. Additional bands may represent deletions (incl. multiple mtDNA deletions) or PCR artefacts. Long range PCR is sensitive and may detect rearrangements that are at levels which are not clinically significant and may not account for the phenotype. Further investigation is always required before issuing a final report on positive results. PCR artefacts may be distinguished from genuine rearrangements by performing LPCR with several template dilutions. PCR artefacts tend to disappear with successive dilutions whereas rearrangements continue to amplify [Kajander et al, 1999]. This strategy decreases the number of referrals requiring Southern blotting and keeps reporting times down to a minimum.

***Southern Blotting*:** Southern blotting is undertaken using *Pvu*II or *Bam*HI digests of mtDNA followed by hybridisation with total mtDNA. These enzymes cut once linearising normal mtDNA and deleted mtDNA. Duplications are not detected with these enzymes as the patterns of cutting mimic normal and deleted mtDNA. If digestion is not complete circular mtDNA and circular deletion species may also be detected.

mtDNA duplications can be detected by digestion with *Sna*BI. DNA is also cut with *Bgl*IIwhich has no sites in mtDNA but reduces background. The *Sna*BI site is commonly deleted and hence deletions remain circularised whilst duplications are linearised and run above the linear mtDNA band. mtDNA deletion dimers can sometimes be mistaken for duplications as they can migrate to similar positions on the gel dependent on the size of the deletion. [Poulton et al, 1993]. *Sna*BI is also prone to star activity and this should be taken into account when analysing the data.

To further aid the distinction of deletions and duplications probing of both *Pvu*II and *Sna*BI blots with a probe isolated from the deleted region can be undertaken. mtDNA deletions will not be detected by the probe whereas mtDNA duplications will.

Normal and deletion controls (blood and muscle) should be used on each Southern blot and PCR).

Real time PCR: mtDNA rearrangements can also be detectable by real time PCR

***Multiple mtDNA deletions*:** The detection of multiple mtDNA deletions by LPCR always requires further investigation [Kajander et al, 1999]. Multiple mtDNA deletions may arise as a consequence of the ageing process and may be secondary to other pathology or they may be directly causative. Multiple mtDNA deletions may be inherited as autosomal dominant or autosomal recessive traits and may therefore be associated with a recurrence risk. As for single rearrangements, variable deletions on LPCR may also arise due to PCR artefacts.

Multiple mtDNA deletions are often accompanied by a significant quantity of normal mtDNA species and this can make the distinction between deletions due to aging and those with clinical significance difficult. Age of the patient should be taken into account when interpreting the data (though it is important to remember that patients with multiple mtDNA deletions are often elderly with later-onset of milder disease).

Reporting issues:

- In cases where blood has been received from an adult and where analysis for rearrangements is indicated a muscle sample should be requested.
- Where ageing may explain the presence of low-level variable deletions this should be mentioned on the reports [Cortopassi et al, 1992].
- Multiple mtDNA deletions may be due to mutations in the nuclear encoded genes *POLG* [Van Goethem et al, 2001; Horvath et al, 2006], *SLC25A4* (ANT1) [Kaukonen et al, 2000] or *PEO1* (Twinkle) [Spelbrink et al, 2001]. The clinician should be advised that it would be appropriate to refer these samples for analysis of these genes, all available through the NCG service.
- A recurrence risk may be associated with variable deletions due to AD/AR mutations in nuclear genes and this should be indicated on the report [Van Goethem et al, 2001; Kaukonen et al, 2000; Spelbrink et al, 2001] Single deletions are usually sporadic but they may be familial; [Chinnery et al, 2004](1:20 recurrence risk).
- Duplications may be associated with a recurrence risk [Poulton et al, 1994].

CPEO may be due to mtDNA point mutations other than 3243A>G; further analysis of muscle biopsy is helpful in these patients if negative for single deletion, multiple mtDNA deletions and 3243A>G.

mtDNA Depletion: Mitochondrial DNA depletion syndromes are recessive disorders of childhood, characterised by a quantitative loss of mtDNA copy number in affected tissues, namely muscle for myopathic phenotypes and liver for hepatocerebral phenotypes [Elpeleg, 2003]. Real-time assays to measure mtDNA copy number in these tissues, together with screening of candidate genes including deoxyguanosine kinase (*DGUOK*) and *POLG* for the hepetocerebral form/Alpers Syndrome referrals, and thymidine kinase 2 (*TK2*) for the myopathic form are available through the NCG service.

3.2 Reason for Referral:
Mitochondrial encephalomyopthy, lactic acidosis and stroke-like episodes (MELAS)
MIDD (Maternally-inherited diabetes and deafness)
CPEO (Chronic progressive external ophthalmoplegia) or analysis for m.3243A>G requested

The m.3243A>G mutation is present in approximately 80% of patients with typical MELAS presentation. Other mtDNA mutations are also associated with MELAS (e.g. m.3271T>C and m.3252A>G in the *MTTL1* gene and in other mtDNA genes), however these are rare and unless there is strong clinical diagnosis of MELAS testing for these mutations is not routinely indicated.

Referrals for MIDD, only m.3243A>G testing is indicated. For CPEO routine screening of the m.3243A>G is indicated in addition to testing for mtDNA rearrangements.

Methodology: Laboratories use a range of methodologies and technologies (fluorescent/radioactive PCR RFLP, ARMS, Real time (TaqMan), dHPLC for detecting point mutations which are reflective of standard technologies available in the laboratory. Appropriate positive and negative controls should be used at all times.

Heteroplasmy: Caution must be used in using information on level of mutant to predict severity and progression of disease and for diagnostic reporting, recording precise levels of tissue heteroplasmy in the absence of full supporting information is

not advised. A discussion with the referring clinician around the significance of the mutation load in a given tissue may be indicated. Accurate prediction of phenotype based on test results is not possible. Therefore, whilst it is considered good practice to note levels of heteroplasmy and use appropriate methodologies which can accurately determine the amount of m.3243A>G mutation in a DNA sample, it is sufficient to report the mutation as being present. Laboratories may wish to accurately record levels of heteroplasmy for their own studies.

Suggested methodologies:

- Comparison by eye where restriction digests used, by comparison with an accurately measured heteroplasmic control.
- Last PCR cycle labelling followed by quantification (fluorescent /radioactive)
- Real time PCR
- Pyrosequencing

RFLP analysis (Ethidium Bromide) can detect heteroplasmy at levels of approximately 5%. Levels of the m.3243A>G mutation decline in blood with age and may drop below 5% in adults presenting with mild symptoms and who may be older.

3.3 Reason for Referral:
Myoclonic epilepsy and ragged red fibres (MERRF) or m.8344A>G

Common practice is analysis of the m.8344A>G mutation only for these referrals. Analysis of the m.8356T>C mutation may be worthwhile but only in cases where there is a very strong indication of a diagnosis of MERRF. Routine sequencing of *MTTK* gene is not indicated.

Methodology: PCR RFLP is commonly used to detect this mutation. Some laboratories may employ other techniques.

Reporting issues: Genotype phenotype correlations may also be more secure, however reporting heteroplasmy levels remains as for the m.3243A>G mutation.

3.4 Reason for Referral:
Maternally inherited Leigh Syndrome (MILS) Neurogenic weakness, ataxia and retinitis pigmentosa (NARP) or m.8993T>G/C mutation

MILS accounts for a small proportion of patients with the clinical presentation of Leigh's syndrome. The majority of cases of Leigh's syndrome are due to autosomal recessive [Tiranti et al,1998] or X-linked disorders of mitochondrial energy generation, involving mutations in nuclear encoded subunits of respiratory chain complex I [Loeffen et al, 2000] and IV [Tiranti et al, 1998 or pyruvate dehydrogenase (E1 alpha subunit [Brown et al, 1992].

Mutation in the *MTATP6* gene at position m.8993 accounts for greater than 50% of patients presenting with NARP. The m.8993T>G transversion is most common although some patients have been described with the m.8993T>C transition. Approximately 20% of cases with Leigh disease carry the above mutations [Rahman et al, 1996]

Other pathogenic mutations include m.9176T>C in *MTATP6*, m.3243A>G in *MTLL1*, m.8344A>G in *MTTK*, m.14459G>A in *MTND6* and m.13513G>A in *MTND5* , which account for a small proportion of cases. ***However, for routine referrals for MILS or NARP, only the m.8993T>G/C mutation should be investigated.***

Methodology

Testing of the m.8993T>G/C is often undertaken by RFLP analysis. A polymorphism exists at a frequency of 10% at position 8994 which can lead to false negative if present. Routine testing for this polymorphism is advised.

Investigations for SURF1/COX10/SCO2/SCO1/COX15:

Investigations for mutations in nuclear genes is only indicated in cases with proven cytochrome *c* oxidase-deficient Leigh Syndrome. Enzyme activity can be measured in fibroblasts or muscle. Contact Dr Garry Brown, Oxford University (garry.brown@bioch.ox.ac.uk) for this service which is offered as part of the NCG arrangement

Investigations for Pyruvate Dehydrogenase Deficiency: Contact Dr Garry Brown, Department of Biochemistry, Oxford University (garry.brown@bioch.ox.ac.uk) for this service which is offered as part of the NCG arrangement

3.5 Reason forReferral:
Leber Hereditary Optic Neuropathy (LHON)

Investigation for the m.3460G>A *MTND1*, m.11778G>A *MTND4* and m.14484T>C *MTND6* mutations are indicated for all LHON referrals. Secondary mutations should not be investigated as their significance is unknown. Presymptomatic testing for LHON should be undertaken cautiously and homoplasmy/heteroplasmy should be stated on the report.

False positive/negative results have been reported for LHON mutations where restriction digest methods have been used. All positives should be confirmed by alternative methods (e.g. direct sequencing) to rule out false positive results. Given the prevalence of LHON mutations in the population, the screening of these mutations is not covered by the NCG agreement and all testing remains within regional CMGS-affiliated molecular diagnostic laboratories.

3.6 Reason For Referral:
Prenatal diagnosis

Synopsis of ENMC consensus on recurrence risks and prenatal diagnosis of mitochondrial diseases [Poulton and Turnbull, 2000]:

CVS is now available for autosomal disorders in which the causative mutations have been identified, including cytochrome *c* oxidase-deficient Leigh syndrome and AdPEO (autosomal dominant PEO). Genetic counselling and prenatal diagnosis for women known or suspected to carry an mtDNA mutation must be done in conjunction with a centre with experience in this area. Prenatal diagnosis and counselling of mtDNA diseases is difficult [Poulton and Marchington, 2000]. The individual oocytes from women at risk of transmitting heteroplasmic mtDNA disease may contain markedly different levels of mutant mtDNA. Furthermore, the amount of mutant mtDNA may vary between different tissues, and may vary with time.

Current Options:

1. Counselling based on blood/tissue/oocyte levels of mutant mtDNA in the mother.

2. Oocyte donation. With this option, there is no risk of transmission of the disease, but oocytes should not be harvested from maternal relatives.
3. CVS – limited information is available. Such evidence that exists suggests that the mutant load in all extra-embryonic and embryonic tissues is similar. Analysis should be done on the biopsy, not on cultured cells. It should be noted that interpretation of the results might be difficult, particularly where levels of mtDNA mutant lie in the intermediate range.

mtDNA Rearrangements: For women with KSS due to mtDNA rearrangements, particularly those in whom rearrangements are detectable in blood (>5%), we recommend CVS and Southern blotting (PCR analysis may be misleading). For women with CPEO and healthy women (in whom no mutant mtDNA is detectable deletion in blood) with a single child with mtDNA rearrangements, the risk of another affected child is probably very low: CVS analysis is an option.

NARP or MILS – m.8993T>G and m.8993T>C: There is a relationship between mutant load in the mother and the risk of an affected offspring. Both mutations fulfil the criteria above for prenatal diagnosis and therefore CVS is likely to be informative. For symptomatic women and those with levels of mutant above 50%, oocyte donation or pre-implantation genetic diagnosis should be seriously considered.

MERRF – m.8344A>G: There is a relationship between mtDNA mutant load in the mother and the risk of an affected offspring. Severe disease is rare in offspring of mothers with a load of <40% mutant mtDNA in blood. CVS should be offered to mothers with levels of >40%. Oocyte donation or pre-implantation genetic diagnosis should also be considered in mothers with high mutant load.

MELAS – m.3243A>G: This is the most common and most problematic heteroplasmic mtDNA disorder because severity is not clearly related to mutant load[1].

m.1555A>G, m.11778G>A, m.3460G>A and m.14484T>C mutations:
As there is currently no way of predicting the clinical phenotype of patients with these mutations, prenatal diagnosis is not appropriate.

Private mtDNA point mutations: In this group of patients there is no good data available from which risk can be calculated. Sampling of several tissues in the mother, including oocyte sampling, may help.

3.7 Reason For Referral:
Other mitochondrial mutations/phenotypes

Deafness: m.3243A>G and m.1555A>G in *MTRNR1* gene. Other mutations have been described, particularly in the *MTTS1* ($tRNA^{Ser(UCN)}$) gene. However, these occur rarely and routine screening is not recommended.

mtDNA sequencing: Sequencing of the entire mitochondrial genome is now considered an important part of the diagnostic strategy to determine whether an underlying mitochondrial genetic aetiology is causative in some patients. This may be indicated for patients whose investigations in affected tissue reveal evidence of a mitochondrial respiratory chain defect, but no evidence of mtDNA rearrangements or common mtDNA point mutations. This service requires DNA extracted from affected tissue (e.g. muscle) to search for novel/rare pathogenic mutations.

3.8 Reason For Referral:
Nuclear Encoded mutations (summary)

The following tests are being developed as part of the NCG service – please contact the administrator for further information as required.

Complex I deficiency: Isolated Complex I deficiency is the most frequently observed biochemical defect in respiratory chain disorders. An increasing number of paediatric patients have recurrent mtDNA mutations, so sequencing of the mitochondrial genome is a worthwhile line of investigation to either establish the presence of a causative mtDNA mutation or exclude maternally-transmitted mtDNA disease. Comprising >45 subunits, there are a considerable number of nuclear structural and assembly genes which may be considered as candidates.

Complex II deficiency: The screening of nuclear-encoded complex II genes is appropriate in patients with isolaged complex II deficiency.

Complex IV deficiency: Screening of *SURF1*, *COX10*, *SCO2*, *SCO1* and *COX15* genes are appropriate in children with isolated complex IV deficiency, presenting as Leigh Syndrome.

Mutations in genes underlying multiple mtDNA deletions:
As indicated above, screening of the entire coding regions of the ***POLG***, *SLC25A4* (ANT1) and *PEO1* (Twinkle) genes should be considered in these patients.

Mutations in genes underlying mtDNA depletion:
As previously indicated, real-time PCR assays to measure mtDNA copy number, together with sequencing of candidate nuclear genes include deoxyguanosine kinase (*DGUOK*) and *POLG* for the hepetocerebral form/Alpers Syndrome referrals, and thymidine kinase 2 (*TK2*) for the myopathic form are available if requested.

MNGIE: Mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder leading to mtDNA deletions and depletion, caused by mutations in the thymidine phosphorylase (*TP*) gene. Diagnosis is made by direct measurement of TP enzyme activity or measuring elevated plasma levels. This service is offered by Dr Lynette Fairbanks, of the Purine Research Laboratory at Guy's and St Thomas' Hospital, London (lynette.fairbanks@gstt.nhs.uk).

3.9 Mutation Nomenclature

mtDNA nucleotides are conventionally numbered sequentially with reference to a whole mitochondrial genome reference sequence, and are prefixed by "m." (as above), and this nomenclature system should be followed when reporting results. The reference sequence should also be quoted on reports, and we suggest using GenBank AC_000021.2 (GI:115315570) or J01415.2 (GI:113200490). These sequences are identical and are revised versions of the Cambridge Reference Sequence. Historical nucleotide numbers (from the original Cambridge Reference Sequence)

are maintained by indicating the absence of a nucleotide at m.3107 as 'N'. Further general guidance on mutation nomenclature is available from the Human Genome Variation Society (www.hgvs.org).

For nuclear genes, HGVS nomenclature guidelines should be followed (www.hgvs.org).

4.0 OTHER RECOMMENDATIONS FROM THE BEST PRACTICE MEETING:

Nomenclature

It was recommended that the phrase **"Mitochondrial Respiratory Chain Disease" could** be used as an all encompassing term for mitochondrial disease.

5.0 REFERENCES

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