Mitochondrion 32 (2017) 42–49

Contents lists available at ScienceDirect

Mitochondrion

journal homepage: www.elsevier.com/locate/mito

# Mitochondrial oxidative phosphorylation disorders in children: Phenotypic, genotypic and biochemical correlations in 85 patients from South India

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ARTICLE INFO

*Article history:*
Received 23 July 2016
Received in revised form 12 October 2016
Accepted 3 November 2016
Available online 5 November 2016

*Keywords:*
Oxidative phosphorylation disorders
mtDNA
*POLG*1
*SURF*

ABSTRACT

Mitochondrial oxidative phosphorylation (OXPHOS) disorders account for a variety of neuromuscular disorders in children. In this study mitochondrial respiratory chain enzymes were assayed in muscle tissue in a large cohort of children with varied neuromuscular presentations from June 2011 to December 2013. The biochemical enzyme deficiencies were correlated with the phenotypes, magnetic resonance imaging, histopathology and genetic findings to reach a final diagnosis. There were 85 children (mean age: 6.9 ± 4.7 years, M:F:2:1) with respiratory chain enzyme deficiency which included: isolated complex I ($n = 50$, 60%), multiple complexes ($n = 24$, 27%), complex IV ($n = 8$, 9%) and complex III deficiencies ($n = 3$, 4%). The most common neurological findings were ataxia (59%), hypotonia (59%) and involuntary movements (49%). A known mitochondrial syndrome was diagnosed in 27 (29%) and non-syndromic presentations in 57 (71%). Genetic analysis included complete sequencing of mitochondrial genome, *SURF1*, *POLG1&2*. It revealed variations in mitochondrial DNA ($n = 8$), *SURF1* ($n = 5$), and *POLG1* ($n = 3$). This study, the first of its kind from India, highlights the wide range of clinical and imaging phenotypes and genetic heterogeneity in children with mitochondrial oxidative phosphorylation disorders.



## 1. Introduction

Mitochondrial oxidative phosphorylation (OXPHOS) disorders are one of the most common inborn errors of metabolism with a prevalence of 1/5000 (Haas et al., 2007). The current strategy for diagnosis and classification of mitochondrial disorders includes comprehensive and meticulous analysis of clinical features, family history, and results of biochemical, histopathological, magnetic resonance imaging (MRI) and molecular diagnostic studies. OXPHOS disorders in children pose diagnostic challenge due to various factors. The known 'syndromic' presentations in adults allow diagnosis by analysis of the commonly associated genetic variations (Thorburn and Smeitink, 2001). Histological evidence of ragged red fibers (RRF) or cytochrome *c* oxidase (COX) deficiency in muscle biopsy also aids diagnosis in adults. On the other hand the diagnosis of OXPHOS disorders is often difficult in children due to non-specific clinical features and inconclusive histopathological

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http://dx.doi.org/10.1016/j.mito.2016.11.002
1567-7249/

findings (Thorburn et al., 2004). Further, the genetic diagnosis in children is more challenging than in adults (Thorburn and Smeitink, 2001). This is because variations in mitochondrial DNA are detected in only 20% in children (Lebon et al., 2003). Moreover, number of variations in nuclear DNA associated with respiratory chain disorders is being increasingly recognized. This limits the utility of mitochondrial genomic analysis (Thorburn, 2004). In recent times, next generation sequencing has improved the genetic diagnosis in children with OXPHOS disorders, but the technology yields several unknown genetic variations and the data generated requires extensive bio-informatics analysis. Quantifying the deficiency of one or more of the respiratory chain enzymes becomes important for directing as well as interpreting the unknown genetic variations (Thorburn et al., 2004).

Studies have demonstrated that the genetic etiology of several diseases may be unique to Indian subcontinent (Metspalu et al., 2011; Thangaraj et al., 2008). This is also applicable to mitochondrial OXPHOS disorders where there is a dual genetic control. Mitochondrial OXPHOS disorders in Indian population remain largely unexplored. So far, a single study from India emphasized the presence of novel mitochondrial variations (Wani et al., 2007). Isolated case reports highlight novel mitochondrial variants associated with established mitochondrial syndromes in patients from India (Vanniarajan et al., 2006). In this study, the respiratory chain complex assays and genetic analyses were undertaken in a large cohort of children with a clinical diagnosis of mitochondrial OXPHOS disorders in order to define the phenotypic spectrum, nature of respiratory chain enzyme deficiency and genetic etiology.

## 2. Patients and methods

This prospective analytical study was carried out between June 2011 and December 2013. The institute ethics committee approved the study and parents of all patients gave informed consent for inclusion in the study. Patients were recruited into the study when they had a clinically complete respiratory chain encephalomyopathy or a mitochondrial cytopathy as defined by Bernier et al. (Bernier et al., 2002). All patients underwent a comprehensive laboratory evaluation tailored to the clinical presentation. Details of the demographic characteristics, general physical and neurological examination were noted in a predesigned proforma.

### 2.1. Biochemical analysis

Enzyme analysis of four complexes of mitochondrial respiratory chain (I–IV) and citrate synthase was done in muscle homogenates according to the protocol by Kirby et al. (Kirby et al., 2007). Respiratory chain enzyme deficiency was defined as residual activity of <30% of one or more of the respiratory chain complexes.(Bernier et al., 2002) The deficiency was considered isolated if other respiratory chain complexes had citrate synthase ratios that were not clearly deficient (i.e., >25%) and had residual activities at least two-fold higher than the deficient complex (Swalwell et al., 2011). Residual enzyme activity of <30% of two or more complexes was defined as multiple complex deficiencies.

### 2.2. Genetic analysis

#### 2.2.1. Analysis of complete mitochondrial genome

DNA was extracted from blood and muscle using standard protocol (Thangaraj et al., 2002). Complete mitochondrial genome was sequenced as described previously (Rieder et al., 1998). Amplicons were electrophoresed using 2% agarose gel and cycle sequencing reaction was carried out using BigDye Terminator ready reaction kit (Applied Biosystems, Foster City, USA). Extended products were precipitated with ethanol and dissolved in Hi-Di formamide, followed by analysis in an ABI 3730 automated DNA analyzer (Applied Biosystems, Foster City, USA). The mitochondrial DNA sequences obtained were edited and aligned with revised Cambridge reference sequence (*rCRS*) (NC_012920) using Sequence Analysis and AutoAssembler tools. All mismatched nucleotides along with their positions were noted, compared with 300 ethnically matched controls and searched in the human mitochondrial genome databases such as Mitomap (http://www.mitomap.org) and mtDB (http://www.genpat.uu.se/mtDB) and HmtDB (http://www.hmtdb.uniba.it:8080/hmdb) for their significance. The pathogenicity of the structural gene variations were confirmed by Polyphen-2 (http://genetics.bwh.harvard.edu/pph2) and tRNA variations observed were further checked by Mamit-tRNA (http://mamit-trna.u-strasbg.fr/) to look for the conserved nature of the loci of the variants and their position on the tRNA structure.

#### 2.2.2. Analysis of nuclear genes

The entire coding regions, including intron-exon boundaries of *POLG1* (Van Goethem et al., 2001), *POLG2* (Young et al., 2011) and *SURF1* (Tiranti et al., 1998) was sequenced as described earlier. The variations in the nuclear genes were analyzed after eliminating the polymorphisms as listed in www.Ensembl.org. For *POLG1*, we also compared the observed variations with the Human DNA Polymerase Gamma Mutation Database (http://tools.niehs.nih.gov/polg/). Further, the functional implications of these variations were predicted using polyphen-2 software.

## 3. Results

A total of 109 children with a clinical diagnosis of mitochondrial oxidative phosphorylation disorders were evaluated over a period of two and half years. Eighty-five (78%) children had muscle respiratory chain enzyme activity of <30% of one or more of the complexes and were included in the final analysis. Of these 85 children, 75 patients had a <20% activity in the muscle and 10 had <30%. The mean age at presentation was 6.9 ± 4.6 years (age range: 9 months–17 years, M:F-2:1) and mean age at onset 4.4 ± 3.9 years (range: 3 months–16 years). Of these 27 (32%), had onset in infancy. Thirty-nine patients were born to consanguineous parents (first cousin's marriage: 21, maternal uncle-niece marriage: 17 and distant consanguinity: 1). Family history was positive in 29 (34%) patients.

The commonest neurological findings were ataxia ($n = 50$, 59%), hypotonia ($n = 50$, 59%) and involuntary movements ($n = 42$, 49%). Developmental delay was noted in 35 (40%). The neurological symptoms and signs are summarized in Table 1. Systemic features included failure to thrive ($n = 42$, 49%), gastrointestinal symptoms ($n = 21$, 25%), hypertrophic cardiomyopathy ($n = 2$, 2%) and high output cardiac failure ($n = 1$, 1%).

### 3.1. Phenotypes

A known mitochondrial syndrome was diagnosed in 27 (29%) and included Leigh and Leigh like syndrome ($n = 19$), mitochondrial encephalopathy with lactic acidosis and stroke like episodes (MELAS, $n = 3$), chronic progressive external ophthalmoplegia (CPEO, $n = 4$) and juvenile Alper's syndrome ($n = 1$). The key clinical features in the non-syndromic group were epilepsy ($n = 13$), extrapyramidal disorder ($n = 11$), ataxia ($n = 10$) and myopathy ($n = 5$).

### 3.2. Magnetic resonance imaging findings

MRI brain was abnormal in 72/85 patients. The abnormal findings were classified based on the anatomical structures affected: signal changes in basal ganglia ($n = 37$), brainstem ($n = 26$), white matter