**Palatal Tremor Revisited: Disorder with nosological diversity & etiological heterogeneity**

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**Supplementary material: Methodology of genetic testing and investigations for patients with mitochondrial disorders**

**Phenotypic characterization:**

Patients were recruited as part of a larger study on phenotype-genotype correlations in mitochondrial disorders based on the clinical criteria of Bernier et al11 viz clinically complete respiratory chain encephalomyopathy or a mitochondrial cytopathy defined as fulfilling all three of the following conditions

1. Unexplained combination of multisystemic symptoms that is essentially pathognomonic for a RC disorder. Symptoms must include at least three of the organ system presentations namely neurologic, muscular, cardiac, renal, nutritional, hepatic, endocrine, hematologic, otologic, ophthalmologic, dermatologic, or dysmorphic.
2. A progressive clinical course with episodes of exacerbation (e.g., following intercurrent illnesses) or a family history that is strongly indicative of a mtDNA mutation (at least one maternal relative other than the proband whose presentation predicts a probable or definite RC disorder).
3. Other possible metabolic or nonmetabolic disorders have been excluded by appropriate testing.

All patients underwent a comprehensive set of investigations tailored to the clinical presentation. These included hematological studies, fasting and post prandial blood sugars, fasting lipid profile, renal, hepatic and thyroid function tests, electrolytes, calcium, phosphorus, creatine kinase, lactate dehydrogenase, serum ammonia and lactate, copper, ceruloplasmin, uric acid, homocysteine, vitamin B12, and vitamin E levels, 24 hour urinary copper, acyl carnitine and amino acid profile, urine organic acids, and limited lysosomal enzyme studies (serum aryl sulfatase A & B, hexosaminidase total A & B) among others. Genetic testing for Frataxin, spinocerebellar ataxia 1, 2 and 3, peripheral smear examination for vacuolated lymphocytes, bone marrow examination, axillary skin biopsy to look for inclusions, antinuclear antibody testing, rheumatoid factor levels, angiotensin converting enzyme, and antiphospholipid antibodies were also carried out whenever indicated. Patients also underwent nerve conduction studies, evoked potential studies and electroencephalography as part of the diagnostic evaluation.

**Histopathological, biochemical and molecular studies:**

*Histopathology:* Formalin-fixed and cryosections of muscle were analysed using haematoxylin-eosin and special stains viz modified Gomori Trichrome (MGT), Nicotinamide adenine dinucleotide- tetrazolium reductase (NADH-Tr), Succinic dehydrogenase (SDH), Adenosine triphosphatase (ATPase) at pH 9.5, 4.6 and 4.3 and Cytochrome oxidase c (COX)-SDH.

*Respiratory chain complex assay:* This was carried out using the technique of spectrophotometry as described previously.12

*Genetic testing:* This included complete mitochondrial DNA sequencing for point mutations12 and long range PCR of muscle DNA for large scale rearrangements in mitochondrial DNA. Sanger sequencing of *POLG1* and *SURF1* was done using standard protocols.12

*Clinical exome sequencing:* This was out-sourced to another laboratory. Targeted sequencing of a panel of 6440 genes using the Illumina sequencing platform (mean coverage >80 to 100x) was performed. They included all the nuclear genes implicated in mitochondrial disorders. The sequences obtained were aligned to the human reference genome (GRCh37/hg19) using BWA program and analysed using Picard and GATK-Lite toolkit to identify variants. Gene annotation of the variants was performed using VEP program against the Ensembl release 84 human gene model. Relevant variations were annotated using the published variants in the literature. Common variants were filtered based on the allele frequency in 1000 genome phase 3, ExAC, EVS, dbSNP141, 1000 Japanese Genome and the internal Indian population database. Pathogenicity of the identified variants was ascribed using online tools viz Polyphen2, Sorting Intolerant From Tolerant (SIFT), Mutation Taster 2, Mutation Assessor, and LRT.