EFFECTIVENESS OF THE WHOLE MITOCHONDRIAL DNA SEQUENCING IN PATIENTS WITH SUSPECTED MITOCHONDRIAL DISORDER

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Diagnosing of the mitochondrial diseases (MD) is a challenge due to the extremely non-specific clinical picture and complex genetics, where disease arises due to the mutations in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). Most of the clinical scoring schemes used in diagnosis include information obtained from muscular biopsy (U2), which is invasive procedure with the tissue analysis in the specialized centers and therefore is mostly restricted to the patients with high clinical suspicion to MD. Recent consensus statement recommends massively parallel sequencing or next generation sequencing (NGS) of mtDNA as the first-line testing for comprehensive analysis (3). We introduced the whole mtDNA sequencing from the blood as the first diagnostic step in the patients with suspected MD and analyzed its application in clinical practice.

AIM
To evaluate the effectiveness of the whole mtDNA sequencing from DNA extracted from blood in patients with suspected mitochondrial disorder.

MATERIALS AND METHODS
Clinical data of the patients were obtained from the sample submission forms fulfilled by referring physicians. DNA was extracted from blood and sequenced by Sanger or NGS and compared to the reference sequence NC_012920. Clinical interpretation of the results included the suggestions for the follow-up investigations in case no pathogenic changes were present and/or variants with unknown significance (VUS) were detected. Diagnostic algorithm applied in the study is given on figure 1. Follow-up investigations included pathomorphological, biochemical and genetic studies performed on muscle biopsy in most patients.

RESULTS
Pathogenetic changes leading to the diagnosis were present in 4 patients - 3.5% of our cohort (table 1). In 4 patients with normal mtDNA sequencing results further studies were recommended due to the high clinical suspicion of MD. From this group mtDNA depletion syndrome was subsequently confirmed in one 43 years old woman with exercise intolerance and polyneuropathy. In 14 patients functional studies were necessary to establish the clinical significance of the variant.

Table 1. Clinical and molecular characterization of patients diagnosed by mtDNA sequencing

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age</th>
<th>Reason for referral</th>
<th>mtDNA change</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34 y</td>
<td>Hearing loss + diabetes</td>
<td>Heteroplasmic m. 3243A&gt;G in MTEL1</td>
<td>MDDS</td>
</tr>
<tr>
<td>2</td>
<td>22 y</td>
<td>Hypertrophic cardiomyopathy, lactate</td>
<td>Heteroplasmic m. 3261G&gt;T in MTEL1</td>
<td>MELAS/MERRF phenotype</td>
</tr>
<tr>
<td>3</td>
<td>8 n</td>
<td>Hypertrophic cardiomyopathy, lactate</td>
<td>Heteroplasmic m. 23012T&gt;G in MTEL1</td>
<td>Fatal infantile hypertrophic cardiomyopathy</td>
</tr>
<tr>
<td>4</td>
<td>35 y</td>
<td>Myopathy, cognitive dysfunction, myoclonic epilepsy, lactate</td>
<td>Heteroplasmic m. 6544A&gt;G in MTCO1</td>
<td>MERRF syndrome</td>
</tr>
</tbody>
</table>

** VUS - variant of uncertain significance all results not known.

VUS DETECTED IN THE STUDY
Heteroplasmic m.15161T>C in MTCYB
- Detected in male aged 43 referred due to hearing loss, cognitive dysfunction, diabetes, sight problems and WPW syndrome.
- The mutations in this gene have been previously described in patients with isolated mitochondrial myopathy and exercise intolerance, rarely with multisystem disorders, as MELAS overlap syndrome (4).
- Biochemical and genetic studies from muscle biopsy confirmed the pathogenicity of this mutation.
- Clinically MIDD syndrome was diagnosed in the patient.

Homoplasmic m. 7444G>A in MTCO1
- Was detected in 4 patients in our cohort: 3 of them presented with myopathy as the single symptom and 1 of them presented with exercise intolerance and polyneuropathy.
- This variant has been previously described in MD in combination with other mutations therefore is considered clinically relevant in combination with other modifying variants (5).
- It is listed in ClinVar database as the pathogenic variant related to LHON and/or deafness.
- Biochemical studies from muscle tissue performed in 2 patients revealed normal activity of respiratory chain complexes I-IV.
- As functional studies revealed no impact on the function of complex IV homoplasmic m.7444G>A in MTCO1 was not considered clinically relevant in our patients.

m. 15526A>G in MTCYB
- This variant is classified as likely pathogenic in ClinVar database related to familial breast cancer.
- We detected this variant in majority of patients (11/115) in our cohort.
- No history of breast cancer was reported in the patients.
- Therefore we can assume that this is common mtDNA variant in our population and probably not disease-related.

REFERENCES
Parish S et al 2010 Diagnosis and management of mitochondrial disease; a consensus statement from the Mitochondrial Medicine Society Genetics in Medicine 17, 689–701
Mücker-Rehage et al 2012 A novel MT-CO1 m.6498C>A variation associated with the m.7444G>A mutation in the mitochondrial COI/tRNA(Ser(UCA)) genes in a patient with hearing impairment, typical Leigh and congenital visual loss. Biochemical and Biophysical Research Communications 420(2): p.99-106

CONCLUSIONS
- mtDNA sequencing from the blood is useful in screening of MD, as it can be applied in case minimal clinical symptomatology.
- It is helpful in establishing familial recurrence risk in this group of disorders with high genetic risk.
- Rare pathogenic variants can be identified by this analysis.
- Functional characterization of rare sequence variants is necessary for improving the clinical interpretation of the results.

ABBREVIATIONS
MDDS - Mitochondrial Diabetic Diathesis and Deafness
MELAS - mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF - myoclonic epilepsy with ragged red fibers
MIDD - Mitochondrial Disease with Intellectual Deficiency
MIDD syndrome - Mitochondrial Disease with Intellectual Deficiency
MTCO1 - mitochondrial complex I
MTCYB - mitochondrial complex IV
WPW syndrome - Wolf–Parkinson–White syndrome
VUS - variant of uncertain significance