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 (1,2), which is invasive procedure requiring 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.

COHORT

- Total 115 patients were analyzed in the period 2014-2017.
- 19 patients (16%) were children (8 months – 18 years).
- Mean age of adult patients was 42 years (19-81 years).

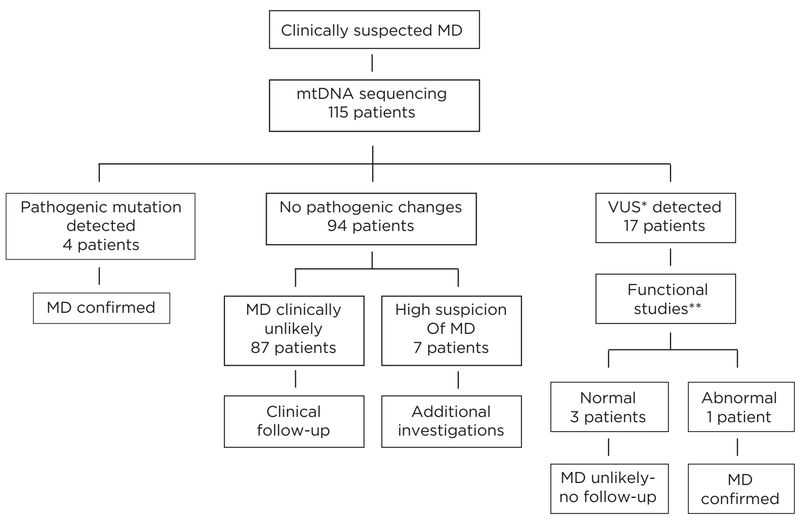
42% of patients had ≥3 clinical symptoms pointing towards MD.

- Most common reasons for referral were: myopathy (30 patients), migraine (28 patients), cognitive dysfunction (26 patients), exercise intolerance (24 patients), epilepsy (23 patients), hearing loss (18 patients), stroke-like episodes (13 patients).
- High lactate was reported in 9 patients.
- 17 patients were referred due to the complicated family history suggestive to MD for exclusion of the conditions with high recurrence risk.

MOLECULAR ANALYSIS

DNA was extracted from blood with Qiagen QIAamp DNA Mini Kit and DNA quality was controlled with spectrophotometer Nanodrop.
Extracted DNA was amplified as 4 amplicons with Thermo Scientific Long PCR Kit.
Amplification products were controlled with gel-electrophoresis.
Sequencing was performed either by Sanger method on Applied Biosystems 3730xl DNA Analyzer or by NGS method with Illumina NexteraXT kit on Illumina MiSeq. Used reference sequence was NC_012920.

Figure 1. Diagnostic algorithm applied in the study



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

RESULTS

Pathogenic 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 cognitive dysfunction, ataxia and ophtalmoplegia.
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

Pts	Age	Reason for referral	mtDNA change	Diagnosis
1	34 y	Hearing loss + diabe-tes	Heteroplasmic m. 3243A>G in *MTTL1*	MIDD
2	22 y	Hearing loss, mild cognitive dysfunction, WPW syndrome*	Heteroplasmic m. 3291T>C in *MTTL1*	MELAS/MERRF phenotype
3	8 m	Hypertrophic cardiomyopathy, lactate ↑, 3-MGAuria**	Homoplasmic m. 3303C>T in *MTTL1*	fatal infantile hypertrophic cardiomyopathy
4	35 y	Myopathy, cognitive dysfunction, myoclonic epilepsy, lactate ↑	Heteroplasmic m. 8344A>G *MTTK*	MERRF syndrome

* * WPW syndrome- Wolff-Parkinson-White syndrome
** 3-MGAuria – urinary excretion of 3-methylglutaconic acid

VUS DETECTED IN THE STUDY

Heteroplasmic m.15161T>C in *MTCYB*

- Detected in male aged 43 referred due to hearing loss, cognitive deficiency, 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 *MTCO1* was not considered clinically relevant in our patients.

m. 15326A>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 (111/115) 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.

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.

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ABBREVIATIONS

MIDD – maternally inherited diabetes and deafness
MELAS - mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
MERRF - myoclonic epilepsy with ragged-red fibers