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Effectiveness of whole exome sequencing in unsolved patients with a clinical suspicion of a mitochondrial disorder in Estonia

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ABSTRACT

Objective: Reaching a genetic diagnosis of mitochondrial disorders (MDs) is challenging due to their broad phenotypic and genotypic heterogeneity. However, there is growing evidence that the use of whole exome sequencing (WES) for diagnosing patients with a clinical suspicion of an MD is effective (39–60%). We aimed to study the effectiveness of WES in clinical practice in Estonia, in patients with an unsolved, but suspected MD. We also show our first results of mtDNA analysis obtained from standard WES reads.

Methods: Retrospective cases were selected from a database of 181 patients whose fibroblast cell cultures had been stored from 2003 to 2013. Prospective cases were selected during the period of 2014–2016 from patients referred to a clinical geneticist in whom an MD was suspected. We scored each patient according to the mitochondrial disease criteria (MDC) (Morava et al., 2006) after re-evaluation of their clinical data, and then performed WES analysis.

Results: A total of 28 patients were selected to the study group. A disease-causing variant was found in 16 patients (57%) using WES. An MD was diagnosed in four patients (14%), with variants in the *SLC25A4, POLG, SPATA5,* and *NDUFB11* genes. Other variants found were associated with a neuromuscular disease (*SMN1, MYH2,* and *LMNA* genes), neurodegenerative disorder (*TSPOAP1, CACNA1A*, *ALS2, and SCN2A* genes), multisystemic disease (*EPG5, NKX1–2, ATRX,* and *ABCC6* genes), and one in an isolated cardiomyopathy causing gene (*MYBPC3*). The mtDNA point mutation was found in the *MT-ATP6* gene of one patient upon mtDNA analysis.

Conclusions: The diagnostic yield of WES in our cohort was 57%, proving to be a very good effectiveness. However, MDs were found in only 14% of the patients. We suggest WES analysis as a first-tier method in clinical genetic practice for children with any multisystem, neurological, and/or neuromuscular problem, as nuclear DNA variants are more common in children with MDs; a large number of patients harbor disease-causing variants in genes other than the mitochondria-related ones, and the clinical presentation might not always point towards an MD. We have also successfully conducted analysis of mtDNA from standard WES reads, providing further evidence that this method could be routinely used in the future.

1. Introduction

Mitochondrial disorders (MDs) are one of the most frequent genetic disorders, with a prevalence of ~12.5 per 100,000 in adults [1] and ~4.7 per 100,000 in children [2]. In Estonia, the live-birth prevalence for MDs (estimated during 2003–2009) was 1/20,764 live births [3]. However, diagnosing MDs is a very challenging task due to their extremely broad phenotypic and genotypic heterogeneity. They arise due to a primary mitochondrial dysfunction and can affect various functional systems; most often the central nervous system, neuromuscular system, and heart and liver, causing isolated or multisystemic syndromes. The underlying genetic cause can be a mitochondrial DNA

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(mtDNA) point mutation, single large-scale mtDNA deletion, or a variant in a nuclear gene, resulting in defective mtDNA maintenance, transcription, protein translation, or a defective ancillary process. Of the ~1300 proteins in the mitoproteome encoded by nuclear DNA (nDNA), mutations have been reported in > 250 genes [4]. Therefore, different scoring systems to evaluate the probability of MDs have been developed [5–9]. These scoring systems take into account the clinical features, biochemical changes, brain imaging anomalies, as well as pathomorphological findings in muscle, and enzymatic changes in muscle or fibroblasts.

Nowadays, as next generation sequencing technologies become more widely available for use in routine clinical practice, a vast number of studies have been carried out world-wide to establish the effectiveness of whole exome sequencing (WES) for the diagnosis of different groups of inherited diseases. Studies on the effectiveness of WES in MDs have shown a diagnostic yield of 39–60% [10,11]. In addition, these studies have shown that many patients suspected of having an MD, actually have a different disorder, usually neurological, which have great overlap in the phenotypes. Therefore, Morava and Brown, 2015 [12] have proposed a new diagnostic algorithm – sequencing mtDNA from blood and performing WES analysis, before taking a muscle/skin biopsy.

Our study aimed to evaluate the diagnostic effectiveness of WES in clinical practice, in Estonia for patients with an unsolved metabolic disease and a clinical suspicion of a mitochondrial disorder. We discuss these patients' phenotype, results of metabolic, enzymatic, and imaging studies, the pathomorphology of their muscle, and genotype. We also show our first results of mtDNA variant calling and interpretation from standard WES reads.

2. Methods

This study was approved by the Research Ethics Committee of the University of Tartu (approval date 17/11/2014 and number 242/M-10). Informed consent was obtained from all patients and/or their parents in the study group, except from those who were deceased.

2.1. Patient selection

The retrospective study group was selected from a database of 181 patients whose fibroblast cell cultures had been stored in the Department of Clinical Genetics, Tartu University Hospital, during the period of January 2003 – December 2013. These 181 patients had been consulted by a clinical geneticist and had a definite diagnosis or a suspicion of a metabolic disease. The fibroblasts were cultured from a skin biopsy to carry out necessary enzyme analyses, or for future diagnostic purposes. At the same time in most of the cases the DNA sample was also stored.To our study group, we included only those patients whose physician had a strong suspicion of a MD, but previous genetic investigations (targeted mtDNA and single nuclear gene analysis) resulted in no findings. WES analysis was not performed on any of these patients because it was not available in Estonia prior to 2013.

The prospective study group was selected during January 2014 – March 2016. We included patients who were referred to a clinical geneticist in the Department of Clinical Genetics, Tartu University Hospital, had an onset of the disease in childhood, and the clinical geneticist had a clinical suspicion of an MD.

Next, we re-examined all the medical history charts of each included patient to obtain their clinical signs and symptoms, results of metabolic and imaging studies, and other medical investigations, including muscle biopsy. The retrospective patients who were alive were invited for a follow-up genetic consultation. From the patients who were deceased, we had formerly taken a blood sample for DNA extraction and their clinical history was well described. We scored each patient according to the mitochondrial disease criteria (MDC) developed by Wolf and Smeitink, [8] and Morava et al. [9]. WES analysis was performed on each patient.

2.2. Whole exome sequencing

The DNA of all the patients was extracted from blood lymphocytes. The WES analysis was carried out using different methods due to availability and cost-effectiveness at various time points. The WES enrichment kits used were SureSelect XT Human All Exon v5 (Patients 2, 3, 5, 6, 8, 10, 11, 12, 17, 18, and 19) or v4 (Patient 1) enrichment kit (Agilent Technologies, Santa Clara, CA), Nextera Rapid Capture Exome 37 Mb kit (Illumina Inc., San Diego, CA, USA) (Patients 4, 7, 9, 14, 15, 16, 20, 21, 22, 23, 24, 25, 26, 27, and 28), and the TruSeq Exome Enrichment Kit 62 Mb (Illumina Inc., San Diego, CA, USA) (Patient 13). All patients were sequenced as proband only, except Patients 7 and 28, in whom offspring-parent trios were sequenced, and Patient 13 who had two affected sibs that were sequenced. After the sequencing, the reads were aligned to hg19 reference genome using Burrows-Wheeler Aligner (BWA) [13]. Further data processing, variant calling, and annotation was performed following Genome Analysis Toolkit (GATK) best practice guidelines [14] using Picard, GATK [15,16], Annovar [17], and SnpSift [18]. All reported variants were confirmed, and familial segregation analysis was done using Sanger sequencing.

3. mtDNA analysis from standard WES reads

Every patients' reads (except patient 1) generated during standard WES (as described in the section 2.2), but mapped to mtDNA, were specifically analyzed to detect mtDNA variants, and to assess the feasibility of investigating mtDNA from standard WES reads. In order to study mtDNA, SAMtools [19] were used to extract reads mapped to chrM. These reads were aligned to the b37 reference genome (GRCh37 including rCRS mitochondrial sequence) using BWA MEM [20], and subsequently sorted and marked for duplicates by Picard. The variants were called by combining SAMtools [19] mpileup with VarScan [21], and then annotated using Annovar [17]. The analysis of guided variants along with haplogroup calling was guided by Mitomap and Mitomaster [22]. The mtDNA coverage was calculated using the GATK DepthOfCoverage tool.

4. Results

Out of the 181 patients in the fibroblast database, we selected 21 patients for the retrospective group, of which four patients were excluded due to consent withdrawal, loss of clinical symptoms, no contacts with the family and/or no stored DNA. The prospective group consisted of 11 patients, who were all included. In total, we had 28 patients that we performed WES analysis on.

The main clinical, metabolic, imaging, and myopathological characteristics of all the patients are described in the Supplementary data (Table S1). The onset of disease ranged from prenatal to seven years of age, with onset at birth or early infancy in the majority of patients. The current ages ranged from 1 y to 29 y. Nine patients had deceased between the ages of 3 m to 26 y. Motor developmental delay was present in 16, and intellectual disability in 11 patients. Developmental regression was seen in three patients. Muscle weakness was evident in 16 patients. Eleven patients had muscular hypotonia and decreased/absent deep tendon reflexes (DTRs), whereas 14 patients presented with spasticity and/or elevated DTRs/positive Babinski sign. Other neurological manifestations, such as dystonia, choreoathetosis, ataxia, tremor, nystagmus, and strabismus occurred in 18 patients. Epilepsy was diagnosed in nine patients. The heart, vision, and gastrointestinal system were involved in seven patients, the skeletal system in five, liver in four, hearing in three, thyroid in two, and the peripheral nerves in one patient. Lactate in serum and/or cerebrospinal fluid was elevated in 13 patients, and tricarboxylic acid (TCA) cycle intermediates in urine were elevated in eight patients. Brain atrophy was detected in ten