Analyzing the mitochondrial genome of over 2500 patients with retinal disease

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Purpose

Mitochondrial diseases are a heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain. The circular 16.5-kb mitochondrial genome (mtDNA) contains 37 genes, which are essential for normal mitochondrial function. Mitochondrial dysfunction caused by several disease-causing variants in the mtDNA has been associated with retinal disease and vision loss (**Figure 1**). *MT-TL1* m.3243A>G, affecting the mitochondrial tRNA for leucine (UUR), is the most common pathogenic variant in the mitochondrial genome being a molecular cause in up to 11.5% of individuals with mitochondrial disease (PMID 24375076). *MT-TL1* m.3243A>G is present in approximately 80% of individuals with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS – GeneReviews) but is also associated with isolated ophthalmologic phenotypes (PMID: 23806424, 23355809). The goal of this study was to evaluate mtDNA variants in >2500 patients with inherited retinal disease (IRD).

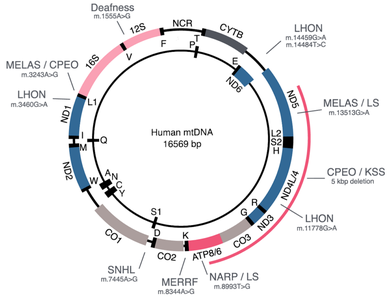

Figure 1. Schematic representation of the mitochondrial genome including the ten most common mtDNA disease-causing variants. Seven of them are associated with vision loss. Illustration based on PMID: 27023847.

Methods

We developed a highly sensitive and clinically validated mtDNA assay based on hybridization-based capture of mtDNA and next-generation sequencing (NGS) that is able to detect very low heteroplasmy levels of SNVs, INDELs and deletions. The mean read depth for the mtDNA was 18,224x, and 100% of base pairs were covered with a sequencing depth of at least 1000x. Sensitivity to detect SNVs and INDELs with over 10% heteroplasmy was 100%. For SNVs with 5-10% and <5% heteroplasmy levels the sensitivity was 93.3% and 88.9%, respectively. Sensitivity to detect large 500bp – 5,000kb deletions at 10% heteroplasmy is 99% The mtDNA assay was included in diagnostic NGS-based panel testing of 2597 IRD patients.

Variant	Gene	AA change	Count	Phenotype	MITOMAP associated disease
m.1606G>A	MT-TV		1	Macular dystrophy and hearing deficit.	MM / EXIT; AMDF
m.3243A>G	MT-TL1		11	Most often described as macular/ cone dystrophy	DM/ DMDF / SNHL / FSGS / Cardiac + multi-organ dysfunction, MELAS / LS, MM/CPEO
m.3271T>C	MT-TL1		1	Optic atrophy.	MELAS / DM
m.5703G>A	MT-TN		1	Suspicion of CPEO and/or MM.	CPEO / MM
m.8993T>G	MT-ATP6	Leu156Arg	1	Rod-cone dystrophy, neurological disease, developmental delay, cognitive impairment, and possible dystonia.	NARP / LS / other
m.11778G>A	MT-ND4	Arg340His	2	Optic atrophy / cone dystrophy.	LHON / Progressive Dystonia
m.12148T>C	MT-TH		1	Suspicion of pattern dystrophy. Hearing deficit, neurological disease, kidney anomaly, and cognitive impairment.	Developmental delay, optic atrophy, cataract, hearing loss, myopathy
m.14484T>C	MT-ND6	Met64Val	2	Optic neuropathy / *retinitis pigmentosa.	LHON
m.14596A>T	MT-ND6	Ile26Met	1	Optic neuropathy with intermittent tremor in the hands.	LHON

Table 1. Diagnostic mtDNA SNVs identified among 2597 IRD patients.

Abbreviations **AMDF** ataxia, myoclonus and deafness; **CPEO** chronic progressive external ophthalmoplegia; **DM** diabetes mellitus; **DMDF** DM + deafness; **EXIT** Exercise intolerance; **FSGS** Focal segmental glomerulosclerosis; **LS** Leigh syndrome; **MELAS** mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; **MM** mitochondrial myopathy; **NARP** neurogenic muscle weakness, ataxia and retinitis pigmentosa. ***Reference*** **MITOMAP**: http: //www.mitomap.org. * additional molecular diagnosis in a nuclear gene.

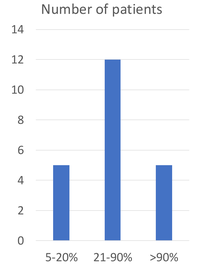

Figure 2. Heteroplasmy levels of mtDNA variants in the analyzed blood/saliva samples from 22 patients with a diagnostic pathogenic/likely pathogenic mtDNA variant.

Results

A diagnostic (pathogenic/likely pathogenic) mtDNA variant was identified in 22 patients, contributing to a diagnostic yield of 0.85%. The diagnostic variants included 21 SNVs, of which 16 were heteroplasmic (>7%) and five were homoplasmic (**Table 1 and Figure 2).** The homoplasmic variants were associated mainly with Leber hereditary optic neuropathy. In addition, one heteroplasmic large deletion was identified in a patient with syndromic retinitis pigmentosa (**Figure 3**). Diagnostic SNV variants were identified in all together 8 mtDNA genes: *MT-ND1, MT-ND4, MT-ND6, MT-ATP6, MT-TN, MT-TH, MT-TL1*, and *MT-TV*. The most common variant was *MT-TL1* m.3243A>G, identified in 11 individuals in whom the retinal disease was most often described as macular/cone dystrophy (**Figure 4**). Additionally, we reported a mtDNA VUS, likely to contribute to the patient's diagnosis, in 7 cases.

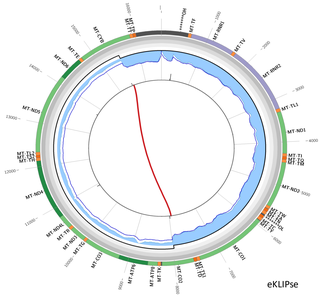

Figure 3. An eKLIPse circos plot visualizing a 54% heteroplasmic 7.5 kb mitochondrial deletion identified in a patient referred to genetic testing with the RD panel. The deletion is encompassing 17 mitochondrial genes.

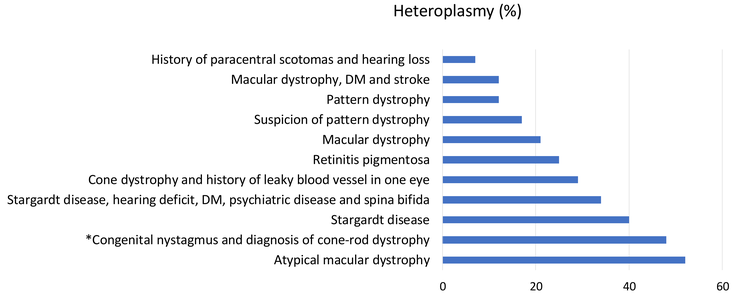

Figure 4. Phenotypes and heteroplasmy levels of 11 patients with the *MT-TL1* m.3243 variant.
* The patient had an additional molecular diagnosis in a nuclear gene.

Conclusions

- We have developed a highly sensitive and clinically validated mtDNA assay for mitochondrial diseases
- SNV diagnoses from retinal dystrophy patients were made in eight mtDNA genes in total
- The most frequently implicated variant was the retinal disease-associated *MT-TL1* m.3243A>G, always detected in a heteroplasmic state
- Adding mtDNA analysis to routine genetic diagnostic process of IRD patients can increase the diagnostic yield by >0.85%
- Analyzing the full mtDNA is beneficial as several different variants in mtDNA have been associated with both syndromic and non-syndromic IRD

Conflict of interest statement: All authors are employed by Blueprint Genetics.