CeGaT GmbH | Paul-Ehrlich-Str. 23 | D-72076 Tübingen | Germany

Dr. Jane Doe
Paul-Ehrlich-Str. 23
72076 TÜBINGEN
GERMANY

Patient	XXX, XX (*DD.MM.YYYY)
Sex	Male
Patient-ID	#
Sample receipt	xxx
Material	EDTA blood
Report date	xxx

Genetic analysis report – XXX, XX (*DD.MM.YYYY)

Indication	Mitochondrial cytopathy; peripheral neuropathy, ataxia, muscle weakness, increased CSF IgG, MRI brain normal
Order	Panel Diagnostics: Mitochondrial DNA (mtDNA) (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a heteroplasmatic pathogenic variant in gene *MT-ATP6*, which is causative for your patient's disease.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants which are likely to be causative for your patient's disease.

Gene	Variant	Zygosity	Heredity	MAF (%)	*in silico* Prediction	Classification
MT-ATP6	**m.9035T>C; p.Leu170Pro**	**heteropl. (0.94) 586/625 reads**	**mitochondrial**	**-**	**-**	**pathogenic**

Information for table interpretation:
AD: Describes a trait or disorder requiring only one copy of a gene variant at a particular locus in order to express an observable phenotype. Single heterozygous gene variants can only be causative for the phenotype if the disorder follows the **autosomal dominant** mode of inheritance.
AR: Describes a trait or disorder requiring the presence of two copies of a gene variant at a particular locus in order to express an observable phenotype. In **autosomal recessive** disorders, a single heterozygous variant in one gene cannot by itself be causative for the observable phenotype.
XL: X-linked mode of inheritance
mitochondrial: gene encoded in the mitochondrial DNA. Information on degree of heteroplasmy (heteropl. or homopl.), frequency and read count (variant/total reads).
MAF: The **minor allele frequency** describes the least frequent allele at a specific locus in a given population. The less frequently a variant occurs, the more likely it is pathogenic; however, the prevalence and mode of inheritance of any particular disease must be considered. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).
***in silico* Prediction:** The ACMG (American College of Medical Genetics) guidelines recommend using prediction programs to assess the possible pathogenicity of a variant. Each program calculates its predictions based upon different criteria, and the correspondence

between a prediction and the actual functional effect of a variant is variable. **These predictions may therefore not serve as the sole basis for the evaluation of pathogenicity.**
Classification: Refers to the possible pathogenicity of a variant, but does not necessarily provide clear evidence of clinical significance. Variants are evaluated based upon current data and specific criteria, and are then classified as follows: pathogenic, likely pathogenic, and uncertain significance. **All variants for which clinical relevance cannot be conclusively confirmed or excluded are referred as variants of unknown clinical significance.**

INTERPRETATION

***MT-ATP6*, m.9035T>C; p.Leu170Pro (heteroplasmic), ENST00000361899, rs1603222000:**

OMIM / Reference	Phenotype	Heredity
*516060	Mitochondrial diseases	mitochondrial

The ***MT-ATP6*** gene encodes a subunit of the ATP synthase (complex V). Pathogenic variants in the *MT-ATP6* gene consequently lead to complex V deficiency, which in its most severe consequence can cause Leigh syndrome (OMIM #256000). Patients with Leigh syndrome present the first symptoms typically within the first months or years of life, often following viral infection. Progression is episodic and may result in early death. Patients develop neurological symptoms including developmental delay and regression, hypotonia, movement disorders, cerebellar ataxia, peripheral neuropathy, and ophthalmological abnormalities. Multisystemic presentations including cardiac, hepatic, gastrointestinal and renal tubular dysfunction have been reported (Lake et al., 2016, PMID: 26506407). Pathogenic variants in *MT-ATP6* may also result in NARP syndrome, which is characterized by proximal neurogenic muscle weakness with sensory neuropathy, ataxia, and pigmentary retinopathy (GeneReviews, Thorburn et al., updated 2017, PMID: 20301352). In addition, a missense variant has recently been described as causative of an early-onset MLASA-like (myopathy, lactic acidosis, and sideroblastic anemia) phenotype (including Burrage et al., 2014, PMID: 25037980). Further, pathogenic alterations in *MT-ATP6* have been described as causative for spastic paraplegia (Hedera et al., updated 2020, PMID: 20301682, GeneReviews) and adult-onset spinocerebellar ataxia syndrome (Pfeffer et al., 2012, PMID: 22577227).

In your patient, the mitochondrial missense variant **m.9035T>C; p.Leu170Pro** was detected in a heteroplasmatic state (94%) in gene *MT-ATP6*. This variant has already been described several times in the literature in patients with cerebellar ataxia (i. a. Sikorska et al., 2009, PMID: 19626676; Pfeffer et al., 2012, PMID: 22577227; Ganetzky et al., 2019, PMID: 30763462; Ng et al., 2019, PMID: 31187502). In addition to ataxia, some patients had neuropathy, learning disability or retinitis pigmentosa. The onset of disease in the patients described was usually in late childhood. However, Pfeffer and colleagues reported two families with late-onset ataxia, which was clinically indistinguishable from classic spinocerebellar ataxia (2012, PMID: 22577227). In addition, functional studies have demonstrated decreased ATP hydrolysis for the variant (Sikorska et al., 2009, PMID: 19626676). In the ClinVar database, this alteration is listed twice as pathogenic and once as likely pathogenic (Variation ID: 690280).

Based upon the available data, we conclude that the pathogenic *MT-ATP6* variant is causative for your patient's disease.

Further the degree of heteroplasmy of mitochondrial variants can vary remarkably between different tissues (Wallace & Chalkia 2013; PMID: 24186072). Therefore, it is possible that disease causing variants, deletions and duplications are not detectable in the mtDNA from leucocytes, but present in other tissues. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

Mitochondrial variants are passed on only from a mother to her children. The mother of an affected individual with a pathogenic mitochondrial variant is very often also a (heteroplasmic) carrier of the pathogenic variant and may be symptomatic in a variable degree (El-Hattab et al., updated 2018, PMID: 20301411). The risk for the patient's siblings depends on the genetic status of their mother. If the mother of your patient is a carrier, all siblings of the patient are at risk of also carrying the variant and may or may not manifest symptoms.

As mitochondrial variants are passed on from a mother to her children, affected males do not pass on the variant to their children.

RECOMMENDATION

We recommend analyzing the mother of your patient regarding the variant identified in gene *MT-ATP6*. The level of heteroplasmy can be effectively determined using SNaPshot® analysis. Please contact us if you wish to perform this analysis.

According to § 10 of the German Genetic Diagnostics Legislation, appropriate genetic counseling should be offered with all diagnostic genetic testing. Following the identification of the molecular cause of a genetic disease, genetic counseling has to be offered.

Medical report written by: Scientist 1

Proofread by: Scientist 2

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup Dr. med. Friedmar Kreuz, M.A.	Dr. rer. nat. Heinz-Dieter Gabriel PD Dr. biol. hum. Christiane Maier Dr. rer. nat. Christian Wilhelm Dr. rer. nat. Martin Ritthaler
Consultant for Human Genetics	Diagnostics

ADDITIONAL INFORMATION

Requested Regions

MT-ATP6, ***MT-ATP8***, ***MT-CO1***, ***MT-CO2***, ***MT-CO3***, ***MT-CYB***, ***MT-ND1***, ***MT-ND2***, ***MT-ND3***, ***MT-ND4***, ***MT-ND4L***, ***MT-ND5***, ***MT-ND6***, ***MT-RNR1***, ***MT-RNR2***, ***MT-TA***, ***MT-TC***, ***MT-TD***, ***MT-TE***, ***MT-TF***, ***MT-TG***, ***MT-TH***, ***MT-TI***, ***MT-TK***, ***MT-TL1***, ***MT-TL2***, ***MT-TM***, ***MT-TN***, ***MT-TP***, ***MT-TQ***, ***MT-TR***, ***MT-TS1***, ***MT-TS2***, ***MT-TT***, ***MT-TV***, ***MT-TW***, ***MT-TY*** (Mitochondrial DNA (mtDNA))

Methods

Sequencing: The mitochondrial DNA was enriched using in solution hybridization technology and was sequenced using the Illumina HiSeq/NovaSeq system. At least one rare variant is resequenced using conventional Sanger sequencing, providing a second, independent confirmation.

Computational Analysis: Illumina bcl2fastq2 was used to demultiplex sequencing reads. Adapter removal was performed with Skewer. The trimmed reads were mapped to the human reference genome (hg19) using the Burrows Wheeler Aligner. Reads mapping to more than one location with identical mapping score were discarded. Read duplicates that likely result from PCR amplification were removed. The remaining high-quality sequences were used to determine sequence variants (single nucleotide changes and small insertions/deletions). The variants were annotated based on several internal as well as external databases.

Diagnostic data analysis: Variants were classified and reported based on ACMG/ACGS-2020v4.01 guidelines (Richards et al., 2015, PMID: 25741868, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to MITOMAP) are evaluated up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. MITOMAP) and an in-house database. If an acceptable sequencing-depth per base is not achieved by high-throughput sequencing, our quality guidelines demand local re-sequencing using classical Sanger-technology.

In this case, > 99.9% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base. **The evaluation of variants is dependent on available clinical information at the time of analysis.** The medical report contains all variants not classified as benign or likely benign according to current literature. Synonymous variants in mitochondrially encoded genes are classified as benign. *In silico* prediction of variants listed in the chart above is calculated on the basis of the output of the programs Mutation Taster, fathmm, Mutation Assessor, SIFT, fathmm-MKL coding, LRT, and PROVEAN according to the following criteria: 100% consensus = pathogenic/benign, ≥ 75% consensus = mostly pathogenic/benign, consensus < 75% or no prediction possible = inconsistent.

Variants are named according to the HGVS recommendations.

The sample fulfilled our quality criteria upon arrival and during/after each processing step in the laboratory.

The procedure described above was developed and validated in-house (Laboratory developed test; LDT).

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