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

Dr. Jane Doe
Paul-Ehrlich-Str. 23
D-72076 Tübingen

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

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

Indication Suspected mitochondrial disorder; lactic acidosis, epilepsy with stroke-like episodes, encephalopathy, hearing loss, cognitive impairment, ataxia

Order Single exome analysis

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *MT-TS1*, which is causative for a mitochondrial disease in your patient.**
- 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 (%)	Classification
MT-TS1	**m.7466dup**	**heteropl. (43%) 164/379 reads**	**mitochondrial**	**0.03**	**pathogenic**

Information for the interpretation of this table can be found in section *Additional Information*.

Recommendation

Testing of asymptomatic family members regarding the variant m.7466dup; identified in gene *MT-TS1* may only be performed following genetic counseling.

Genetic Relevance

Your patient is a carrier of a pathogenic variant in the mitochondrial gene *MT-TS1*. This may be of relevance for at-risk family members. Mitochondrial variants are generally passed on from a mother to her children. The probability of a child inheriting a mitochondrial variant from their mother is dependent on the degree of heteroplasmy in the mother's germline, which is not possible to evaluate.

Clinical Information and Variant Interpretation

MT-TS1, m.7466dup (heteropl. (43%) 164/379 reads), ENST00000387416

OMIM / Reference	Phenotype	Heredity
*590080	Mitochondrial disease	mitochondrial

The gene ***MT-TS1*** encodes the mitochondrial tRNA for serine. Pathogenic variants in *MT-TS1* result in a spectrum of phenotypes ranging from nonsyndromic hearing loss to variable MERRF/MELAS syndrome (OMIM *590080; MITOMAP). Individuals affected with MERRF typically have myoclonus, myopathy, ataxia, hearing loss, seizures, and intellectual impairment (e. g. Jaksch et al., 1998, PMID: 9832034; Cardaioli et al., 2005, PMID: 16368237), although the severity of the disease is very variable. MELAS syndrome has also a very variable expressivity and clinical presentation (OMIM #540000). The main symptom is the eponymous stroke-like episodes. These are typically accompanied by epileptic seizures, visual disturbances, paresis, migraines and hearing loss. Other symptoms include axonal/demyelinating polyneuropathy, myopathy, cardiomyopathy, short stature and diabetes mellitus (OMIM #540000). Palmoplantar keratoderma with deafness as well as exercise intolerance have also been reported in some individuals with pathogenic *MT-TS1* variants (OMIM *590080).

MT-TS1, m.7466dup; (heteropl. (43%) 164/379 reads), ClinVar ID: 42226 (also known as m.7471dup)

ACMG/ACGS Criterion	Points	Description
PS3 (supporting)	+1	Functional studies support a damaging effect. Toompuu et al., 1999, PMID: 10545608
PS4 (very strong)	+8	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Tiranti et al., 1995, PMID: 7581383; Ensink et al., 1998, PMID: 9708714; Hutchin et al., 2001, PMID: 11378827; Cardaioli et al., 2006, PMID: 16368237
PP1 (moderate)	+2	Cosegregation of the variant with disease in multiple affected family members on the maternal side. Tiranti et al., 1995, PMID: 7581383; Ensink et al., 1998, PMID: 9708714
ACMG/ACGS Classification: pathogenic	+11	B (≤ -7) | LB (-6 – -1) | VUS (Ice Cold) (0) | VUS (Cold) (1) | VUS (Cool) (2) | VUS (Tepid) (3) | VUS (Warm) (4) | VUS (Hot) (5) | LP (6 – 9) | **P (≥ 10)**

Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,

Dr. med. Dr. rer. nat. Saskia Biskup

Consultant for Human Genetics

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Additional Information

Requested Regions

The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

AARS2, ***ABCB7***, ***ACAD9***, ***ACO2***, ***AFG2A***, ***AFG3L2***, ***AGK***, ***AIFM1***, ***ANO10***, ***APTX***, ***ATAD3A***, ***ATP5F1A***, ***ATP5F1D***, ***ATP5F1E***, ***ATP5MK***, ***ATPAF2***, ***BCS1L***, ***BOLA3***, ***BTD***, ***C1QBP***, ***C2orf69***, ***CA5A***, ***CARS2***, ***CLPB***, ***CLPP***, ***COA3***, ***COA5***, ***COA6***, ***COA7***, ***COA8***, ***COQ2***, ***COQ4***, ***COQ5***, ***COQ6***, ***COQ7***, ***COQ8A***, ***COQ8B***, ***COQ9***, ***COX10***, ***COX14***, ***COX15***, ***COX16***, ***COX20***, ***COX4I1***, ***COX5A***, ***COX6A1***, ***COX6A2***, ***COX6B1***, ***COX7B***, ***COX8A***, ***CYC1***, ***DARS2***, ***DGUOK***, ***DLAT***, ***DLD***, ***DNA2***, ***DNAJC19***, ***DNAJC30***, ***DNM1L***, ***EARS2***, ***ECHS1***, ***ELAC2***, ***ETFDH***, ***ETHE1***, ***FARS2***, ***FASTKD2***, ***FBXL4***, ***FDX2***, ***FDXR***, ***FH***, ***FLAD1***, ***FOXRED1***, ***GARS1***, ***GATB***, ***GATC***, ***GFER***, ***GFM1***, ***GFM2***, ***GLRX5***, ***GTPBP3***, ***HARS2***, ***HCCS***, ***HIBCH***, ***HLCS***, ***HSD17B10***, ***HSPD1***, ***HTRA2***, ***IARS2***, ***IBA57***, ***ISCA1***, ***ISCA2***, ***ISCU***, ***KARS1***, ***KIF5A***, ***LARS2***, ***LIAS***, ***LIPT1***, ***LIPT2***, ***LONP1***, ***LRPPRC***, ***LYRM4***, ***LYRM7***, ***MARS2***, ***MDH2***, ***MECR***, ***MFF***, ***MGME1***, ***MICOS13***, ***MICU1***, ***MIEF2***, ***MIPEP***, ***MPC1***, ***MPV17***, ***MRM2***, ***MRPL12***, ***MRPL3***, ***MRPL44***, ***MRPS14***, ***MRPS16***, ***MRPS2***, ***MRPS22***, ***MRPS23***, ***MRPS25***, ***MRPS28***, ***MRPS34***, ***MRPS7***, ***MSTO1***, ***MTFMT***, ***MTO1***, ***MTRFR***, ***NADK2***, ***NARS2***, ***NAXD***, ***NAXE***, ***NDUFA1***, ***NDUFA10***, ***NDUFA11***, ***NDUFA12***, ***NDUFA13***, ***NDUFA2***, ***NDUFA4***, ***NDUFA6***, ***NDUFA8***, ***NDUFA9***, ***NDUFAF1***, ***NDUFAF2***, ***NDUFAF3***, ***NDUFAF4***, ***NDUFAF5***, ***NDUFAF6***, ***NDUFAF8***, ***NDUFB10***, ***NDUFB11***, ***NDUFB3***, ***NDUFB8***, ***NDUFB9***, ***NDUFC2***, ***NDUFS1***, ***NDUFS2***, ***NDUFS3***, ***NDUFS4***, ***NDUFS6***, ***NDUFS7***, ***NDUFS8***, ***NDUFV1***, ***NDUFV2***, ***NFS1***, ***NFU1***, ***NSUN3***, ***NUBPL***, ***OPA1***, ***OPA3***, ***PARS2***, ***PC***, ***PDHA1***, ***PDHB***, ***PDHX***, ***PDP1***, ***PDSS1***, ***PDSS2***, ***PET100***, ***PET117***, ***PMPCA***, ***PMPCB***, ***PNPLA8***, ***PNPT1***, ***POLG***, ***POLG2***, ***PPA2***, ***PTCD3***, ***PUS1***, ***QRSL1***, ***RARS2***, ***RMND1***, ***RNASEH1***, ***RRM2B***, ***RTN4IP1***, ***SARS2***, ***SCO1***, ***SCO2***, ***SDHA***, ***SDHAF1***, ***SDHB***, ***SDHD***, ***SERAC1***, ***SFXN4***, ***SLC19A2***, ***SLC19A3***, ***SLC25A1***, ***SLC25A10***, ***SLC25A12***, ***SLC25A19***, ***SLC25A21***, ***SLC25A22***, ***SLC25A26***, ***SLC25A3***, ***SLC25A38***, ***SLC25A4***, ***SLC25A42***, ***SLC25A46***, ***SPG7***, ***SSBP1***, ***SUCLA2***, ***SUCLG1***, ***SURF1***, ***TACO1***, ***TAFAZZIN***, ***TARS2***, ***TFAM***, ***TIMM22***, ***TIMM50***, ***TIMM8A***, ***TIMMDC1***, ***TK2***, ***TMEM126B***, ***TMEM70***, ***TOP3A***, ***TPK1***, ***TRIT1***, ***TRMT10C***, ***TRMT5***, ***TRMU***, ***TRNT1***, ***TSFM***, ***TTC19***, ***TUFM***, ***TWNK***, ***TXN2***, ***TYMP***, ***UQCC2***, ***UQCC3***, ***UQCRB***, ***UQCRC2***, ***UQCRFS1***, ***UQCRQ***, ***VARS2***, ***WARS2***, ***YARS2*** (Nuclear-encoded Mitochondrial Disorders)

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 Genome (mtDNA))

General Remarks

Additional variants may be present within regions which were not analyzed (e.g. introns, promoter and enhancer regions and long repeats). A lower specificity enrichment and/or inaccurate variant calling cannot be ruled out for homologous regions with multiple genomic copies. The occurrence of low frequency somatic mosaicism cannot be reliably assessed using a pipeline optimized for germline variant detection, and may therefore remain undetected. Moreover, detection of large deletions and duplications is not guaranteed by next-generation high-throughput sequencing. 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. The classification of variants may change in the future due to new evidence or improvements in scientific understanding.

Information for the interpretation of the tables

Heredity: AD – autosomal dominant, AR – autosomal recessive, XL – X-linked, mito – mitochondrial

MAF: The ***minor allele frequency*** describes the least frequent allele at a specific locus in a given population. For mitochondrial variants, the population frequency (MAF column) is based on the homoplasmic frequency within a reference population (gnomAD).

Classification: Variant classification is based on ACMG, ACGS-2020v4.01, and ClinGen SVI WG guidelines (Richards et al., 2015, PMID: 25741868; Ellard et al., 2020, Association for Clinical Genomic Science; https://clinicalgenome.org/working-groups/sequence-variant-interpretation/). The weighting of criteria and their modification follows the current ACGS guidelines in the strength levels *very strong* (+ 8), *strong* (+/- 4), *moderate* (+/- 2), and *supporting* (+/- 1). According to the respective category (pathogenic or benign) and criterion strength, positive or negative points are assigned as mentioned above (Tavtigian et al., 2020, PMID: 32720330). Variants of uncertain significance (VUS) are subcategorized into *hot*, *warm*, *tepid*, *cool*, *cold*, and *ice cold* VUS according to their likelihood of reaching a pathogenic classification in the future. Posterior probability decreases from 90% to 10% in this order (Ellard et al., 2020, Association for Clinical Genomic Science). If a variant reaches the classification pathogenic, after checking of all benign criteria, not

necessarily all other applicable criteria are listed.

The chromosomal positions of variants listed in the report refer to the human reference genome hg19.

Methods

Sequencing: Protein-coding regions, flanking intronic regions and additional disease-relevant non-coding regions of the nuclear encoded genes, as well as the mitochondrial DNA were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq 6000/NovaSeq X Plus system.

NGS based CNV-Calling: Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth (only applicable for nuclear encoded genes). Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as inter-sample variation. *CNV calling* was performed by computing the sample's normalized coverage profile and its deviation from the expected coverage. Genomic regions are called as variant if they deviate significantly from the expected coverage. Copy number variants are named according to current ISCN guidelines. NGS based CNV-Calling will not detect copy number neutral structural variants such as balanced translocations, inversions, uniparental heterodisomy or low-level mosaicism. Aberrations within the pseudoautosomal region (PAR) cannot be detected with high accuracy. The integration site of duplications cannot be determined by NGS based CNV-Calling.

Please note that next generation sequencing based detection of copy number variations has lower sensitivity/specificity than a direct quantification method, e.g. MLPA. Copy-neutral structural aberrations cannot be detected using this method (e.g. balanced translocations and balanced inversions). The absence of reported CNVs therefore does not ultimately guarantee the absence of CNVs.

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, Ellard et al., 2020, Association for Clinical Genomic Science).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) of the nuclear encoded genes and in the mitochondrial DNA with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD and MITOMAP) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Minor allele frequencies are taken from public databases (e.g. gnomAD, 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. Candidate CNV calls are evaluated manually. Potentially pathogenic findings are validated with a second method, like MLPA, on a case-by-case basis.

Variants identified through single exome analysis were evaluated with reference to the indicated phenotype. Therefore, single heterozygous variants in genes associated with autosomal recessive inheritance may not have been reported.

In this case, 97.97% 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 predictions were performed using the programs MetaLR (Dong et al., 2015, PMID: 25552646), PrimateAI (Sundaram et al., 2018, PMID: 30038395), and SpliceAI (Jaganathan et al., 2019, PMID: 30661751). This prediction can be complemented with additional *in silico* predictions in individual cases.

Variants are named according to the HGVS recommendations without any information regarding the cis or trans configuration.

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).

Communication, dissemination and usage of this report for scientific purposes is only permitted in accordance with the German Genetic Diagnostics Legislation.