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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	Suspected GSD or FAOD, exercise intolerance/fatigue
Order	Panel Diagnostics: Glycogen Storage Disease and Fatty acid oxidation disorders (whole exome enrichment)

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Homozygous detection of a pathogenic variant in gene *PYGM*, which confirms your clinical diagnosis of a glycogen storage disease.**
- Detection of a pathogenic variant in gene *ACADM*, which alone cannot be 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
PYGM	**c.148C>T; p.Arg50***	**homo.**	**AR**	**0.27**	**-**	**pathogenic**
ACADM	c.985A>G; p.Lys329Glu	het.	AR	0.63	-	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

PYGM, c.148C>T; p.Arg50* (homo.), NM_005609.4, rs116987552

OMIM / Reference	Phenotype	Heredity
232600	McArdle disease (GSD5)	AR

The gene ***PYGM*** encodes a muscle specific enzyme which plays an important role in the catabolism of glycogen to glucose-1-phosphate during glycogenolysis. This process is important for supplying muscles with energy during periods of increased activity. Pathogenic variants in this gene are associated with McArdle disease, a glycogen storage disease of muscle characterized by exercise intolerance manifested by rapid fatigue, myalgia, and cramps in exercising muscles. Massive elevation of creatine kinase and rhabdomyolysis with myoglobinuria after exercise is noted in around 50% of patients, potentially leading to acute kidney failure. Patients with McArdle disease typically show elevated serum creatine kinase levels and may have fixed proximal muscle weakness (Martín et al., updated 2019, PMID: 20301518). Onset usually occurs in childhood with a classical clinical presentation, but some patients may have very moderate forms with a later onset (Orphanet, ORPHA368).

The homozygous variant **c.148C>T; p.Arg50*** we detected in gene *PYGM* creates a premature stop codon, which will result in nonsense-mediated decay of the mRNA transcript (Nogales-Gadea et al., 2008, PMID: 17994553). This variant is the most common pathogenic *PYGM*-variant identified in homozygous or compound heterozygous state in 50 % of caucasian McArdle disease cases (e. g. Nogales-Gadea et al., 2008, PMID: 17994553; Gurgel-Giannetti et al., 2013, PMID: 23653251; s. HGMD Professional; ClinVar Variation ID: 2298).

Our sequencing data did not show evidence for a deletion within or including the gene *PYGM*. Therefore, the loss of one allele which would mimic homozygosity is unlikely.

Based upon the available data, we conclude that the detection of the homozygous pathogenic *PYGM* variant confirms your clinical diagnosis of glycogen storage disease and is causative for McArdle disease in your patient.

ACADM, c.985A>G; p.Lys329Glu (het.), NM_000016.6, rs77931234

OMIM / Reference	Phenotype	Heredity
201450	Acyl-CoA dehydrogenase, medium chain, deficiency of (ACADMD)	AR

ACADM encodes the medium-chain specific (C4 to C12 straight chain) acyl-Coenzyme A dehydrogenase. The homotetramer enzyme catalyzes the initial step of the mitochondrial fatty acid beta-oxidation pathway. Defects in this gene cause medium-chain acyl-CoA dehydrogenase deficiency, an autosomal recessive

inherited disease characterized by acute episodes of hepatic dysfunction, fasting hypoglycemia, and encephalopathy, which can result in infantile death. The episodes are usually triggered by infections or prolonged periods of fasting within the first two years of life (Merritt and Chang, updated 2019, PMID: 20301597, GeneReviews).

The heterozygous missense variant **c.985A>G; p.Lys329Glu** in your patient's *ACADM* gene is a prevalent causative variant, that has already been detected in multiple patients in homozygous and compound heterozygous state (e. g. Matsubara et al., 1990, PMID: 2393404; s. HGMD Professional; ClinVar Variation ID: 3586; Merritt und Chang, updated 2019, PMID: 20301597, GeneReviews). It could be proven in functional studies, that this variant affects proper polypeptide folding, oligomer assembly and stability (i.a. Bross et al., 1995, PMID: 7730333; Maier et al., 2009, PMID: 19224950).

***ACADM*-associated disorders follow an autosomal recessive inheritance pattern. Thus, the detection of a single heterozygous variant is not sufficient to explain the patient's clinical findings. A second alteration in the examined regions of *ACADM* could not be detected in your patient and our sequencing data do not indicate deletions or duplications within or including this gene.**

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. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

Your patient is homozygous for a pathogenic variant in gene *PYGM*. This may be of relevance for future family planning and at-risk family members. One altered *PYGM* allele will be passed on to each of your patient's children, who will be heterozygous carriers.

Additionally, your patient is heterozygous for a pathogenic variant in gene *ACADM*. This may also be of relevance for future family planning and at-risk family members.

Individual variants have a 50% probability of being passed on to each respective offspring.

RECOMMENDATION

Carrier testing of your patient's mother regarding the identified variants in genes *PYGM* and *ACADM* in your patient may be performed to determine her carrier status.

Testing of adult asymptomatic family members regarding the variants c.148C>T; p.Arg50* identified in gene *PYGM* and c.985A>G; p.Lys329Glu identified in gene *ACADM* may only be performed following genetic counseling.

We recommend further clinical evaluation/management/treatment/other specifics according to the current guidelines for Glycogen Storage Disease Type V (Martin et al., updated 2019, PMID: 20301518, GeneReviews).

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,

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ADDITIONAL INFORMATION

Requested Regions

AGL, ***ALDOA***, ***ENO3***, ***FBP1***, ***G6PC1***, ***GAA***, ***GBE1***, ***GYG1***, ***GYS1***, ***GYS2***, ***LAMP2***, ***LDHA***, ***PFKM***, ***PGAM2***, ***PHKA1***, ***PHKA2***, ***PHKB***, ***PHKG2***, ***PRKAG2***, ***PYGL***, ***PYGM***, ***SLC2A2***, ***SLC37A4*** (Glycogen Storage Disease)

ACADM, ***ACADS***, ***ACADSB***, ***ACADVL***, ***CPT1A***, ***CPT2***, ***ETFA***, ***ETFB***, ***ETFDH***, ***HADH***, ***HADHA***, ***HMGCL***, ***HMGCS2***, ***SLC22A5***, ***SLC25A20*** (Fatty acid oxidation disorders)

Methods

Sequencing: Protein-coding regions, as well as flanking intronic regions and additional disease-relevant non-coding regions, were enriched using in-solution hybridization technology, and were sequenced using the Illumina NovaSeq6000 system. In order to provide a second, independent validation, one or more rare variant(s) identified within the NGS data were additionally sequenced using conventional Sanger sequencing.

NGS based CNV-Calling: (only applicable for nuclear encoded genes) Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high-quality reads using an internally developed method based on sequencing coverage depth. 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.

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 can not 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, https://www.acgs.uk.com/quality/best-practice-guidelines/).

Only variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% are evaluated. Known disease-causing variants (according to HGMD) 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) 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.

In this case, 99.75% 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. SpliceAI was utilized to evaluate the consequence of variants on splicing (thresholds: 0.8-1 "splice effect", 0.6-0.8 "possible splice effect", <0.6 "no splice effect"; Jaganathan et al., 2019, PMID: 30661751). The prediction of a splice effect for missense variants is only indicated if a threshold of 0.8 is reached. 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).