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Final Report

Date: 17.08.2016

Patient name:
Your ref.: **-**
DOB (dd.mm.yyyy):
Sample collection date (dd.mm.yyyy):
Order received (dd.mm.yyyy):
Sex:
Patient no.:
Sample type:
Order no.:

Request for CentoICU platinum panel

Clinical information: male patient who is the fourth child of first degree cousins. He was hospitalized at age 45 days due to severe pallor, jaundice and respiratory distress. He is suffering from severe jaundice, hepatomegaly, coagulopathy and hypoalbuminemia. In addition elevation of tyrosine and methionine was shown, but his laboratory data did not support the diagnosis of tyrosinemia type 1. He has 2 deceased siblings; one at age of 3 days with meconium aspiration and respiratory distress and the second child was female with the same clinical picture including severe hyperbilirubinemia and liver dysfunction.

Clinically relevant variants with significant phenotype overlapping with your patient

Gene (transcript)	Nucleotide (protein)	Zygosity	In silico parameters*	MAF**	Variant classification***	Disorder (OMIM#, inheritance)
PKLR (NM_000298.5)	c.1648G>A (p.Asp550Asn)	Hom.	3/4 damaging Highly conserved	na	Likely pathogenic (Class 2)	Pyruvate kinase deficiency (266200, AR)
MTHFR (NM_005957.4)	c.973C>T (p.Arg325Cys)	Hom.	4/4 damaging Highly conserved	0.000025	Likely pathogenic (Class 2)	Homocystinuria due to MTHFR deficiency (236250, AR)

*: number of applied in silico prediction programs that are pathogenic, benign, or not applicable/not conclusive (NA/NC) as well as if the position is conserved (both GERP++ and PhyloP have positive values) or not.
**: minor allele frequency (MAF) of Exome Aggregation Consortium database (ExAC), Exome Sequencing Project (ESP), or 1000Genome project (1000G)).
***: variant classification based on CentoMD® and ACMG recommendations (see additional information below for details on the classification). Further information can be found in the interpretation, disclaimer and methods section.

A genetic diagnosis of hemolytic anemia due to red cell pyruvate kinase for the proband is likely confirmed. Moreover, genetically he is also likely diagnosed with homocystinuria due to MTHFR deficiency.

Interpretation

We detected a previously unreported homozygous variant in the *PKLR* gene, c.1648G>A (p.Asp550Asn). It is located in a highly conserved nucleotide and amino acid position (Alamut v.2.7.1). To date, this variant is not described in the Exome Aggregation Consortium, Exome Sequencing Project or the 1000 Genomes Browser. This is the first time we detect this variant and it is so far not listed in CentoMD®. An amino acid change at this position has already been described as disease-causing for pyruvate kinase deficiency by Kugler et al., 2000 c.1649A>T (p.Asp550Val), detected in one patient with severe anemias where hemolysis was not compensated (HGMD Professional 2016.2 - PMID: 10679942). Given that we consider this variant to be likely pathogenic (class 2) according to recommendations of Centogene and ACMG (please, see additional information below).
Pathogenic variants in the *PKLR* gene are causative for pyruvate kinase (PK) deficiency which is inherited in an autosomal recessive manner. Hemolytic anemia due to red cell pyruvate kinase (PK) deficiency is a metabolic disorder characterized by a variable degree of chronic nonspherocytic hemolytic anemia. Clinically, PK-deficient patients suffer from a highly variable degree of chronic hemolysis, ranging from severe neonatal jaundice and

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fatal anemia at birth, severe transfusion-dependent chronic hemolysis, moderate hemolysis with exacerbation during infection, to a fully compensated hemolysis without apparent anemia. Chronic icterus, gallstones and splenomegaly are common findings (Orphanet; ORPHA766).

We also detected a homozygous variant in the *MTHFR* gene, c.973C>T (p.Arg325Cys). It has been previously described as disease-causing for homocystinuria by Goyette et al., 1995 (HGMD Professional 2016.2 - PMID: 7726158). They found this variant in a homozygous state in the first patient reported with *MTHFR* deficiency, an Italian American male who presented at age 16 years with muscle weakness, abnormal gait, and flinging movements of the upper extremities (Mudd et al. 1972). *MTHFR* residual activity was 20% of control values. This is the first time we detect this variant and it is so far not listed in CentoMD®. It is classified as likely pathogenic (class 2) according to the recommendations of Centogene and ACMG (please, see additional information below).

Methylenetetrahydrofolate reductase deficiency is a common inborn error of folate metabolism. The phenotypic spectrum ranges from severe neurologic deterioration and early death to asymptomatic adults. In the classic form, both thermostable and thermolabile enzyme variants have been identified. Summarized symptomps includes: microcephaly, developmental delay, seizures and homocystinemia (OMIM: 236250).

Based on the obtained result a genetic diagnosis of pyruvate kinase deficiency for the proband is likely confirmed. He is also homozygous for a likely pathogenic variant in the *MTHFR* gene that needs to be considered for future pregnancies. We recommend parental carrier testing to confirm homozygosity of the detected variants. Genetic counselling is also recommended.

Best regards,

Chief Operating Officer *Human Geneticist*	*Medical Director, Director* *Medical Reporting*	*Clinical scientist*

Statistics

95,84% of the target base pairs were covered at least 20x. The average cover was: 181.

Methods

The list of the genes included in the CentoICU panel can be found in our webpage https://www.centogene.com/centogene/centogene-test-catalogue-detail.php?test=NGS&ID=20025&search=Panel&disease=CentoICU_platinum. The coding sequences were amplified and sequenced (Illumina). Raw sequence data analysis, including base calling, demultiplexing, alignment to the hg19 human reference genome (Genome Reference Consortium GRCh37) and variant calling was performed using validated in-house software. Variant annotation is performed with our in-house Bioinformatics pipeline with a final assessment by clinical scientists and/or human geneticists. Variant reporting is restricted to pathogenic and likely pathogenic variants or variants of uncertain significance with complete In silico pathogenicity prediction or found together with a pathogenic or likely pathogenic variant in AR diseases. The analysis does not include copy number variations (CNV) or large deletion/duplications.

Additional information

This test was developed and its performance validated by Centogene AG. The US Food and Drug Administration (FDA) has determined that clearance or approval of this method is not necessary and thus neither have been obtained. This test has been developed for clinical purposes. All test results are reviewed, interpreted and reported by our scientific and medical experts.
Of note:
- unless reported or predicted to cause disease, alterations found deep in the intron or variants that do not result in an amino acid substitution are typically not reported by Centogene. These and common polymorphisms identified for this patient are available upon request.
- test results have to be always interpreted in the context of clinical findings, family history, and other laboratory data. Misinterpretation of

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results may occur if the information provided is inaccurate or incomplete. Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered.
- we cannot exclude allele drop off. Polymorphic/normal genomic variation in the patient sample may interfere with mutation detection.

Centogene Mendelian variant classification
(established gene disease association)
Class 1 – Pathogenic
Class 2 – Likely pathogenic
Class 3 – Variant of uncertain significance (VUS)
Class 4 – Likely benign
Class 5 – Benign
Class 6 – Disease-associated variant

The classification of variants can change over the time. Please feel free to contact Centogene (testing@centogene.com) in the future to determine if there have been any changes in classification of any reported variants.