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Dr. Jane Doe
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GERMANY

Index	Fetus of XX XXX
Patient-ID	#
Mother	XXX, XX (*DD.MM.YYYY)
Patient-ID	#
Father	XXX, XX (*DD.MM.YYYY)
Patient-ID	#
Sample receipt	xxx
Material	Fetal DNA from amniotic fluid
External ID	#
Report date	xxx

Genetic analysis report – Fetus of XX, XXX

Indication Multiple brain anomalies, short and narrow corpus callosum, ventriculomegaly (13mm/5mm asymmetry), enlarged cisterna magna (11mm), vermis might be short, suspected Tubulinopathy;
Previous genetic diagnostic: Array-CGH normal

Order Trio exome analysis

Dear Dr. Doe,

Thank you for your request for molecular genetic analysis.

RESULTS

- **Detection of a pathogenic variant in a mosaic state in gene *PPP2R1A*, which is consistent with the brain anomalies of the fetus.**
- Due to the quality of the source material, the evaluation of copy number variants was not possible.
- The NGS data indicate no maternal DNA contamination of the fetal sample.

Gene	Variant	Zygosity			Heredity	MAF (%)	*in silico* Prediction	Classification
		Index	Mother	Father				
PPP2R1A	**c.548G>A; p.Arg183Gln**	**mosaic** (15%, 29 of 194 reads)	**-**	**-**	**AD**	**-**	**-**	**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

***PPP2R1A*, c.548G>A; p.Arg183Gln (mosaic, 15%, 29 of 194 reads), NM_014225.6, rs1057519947**

OMIM / Reference	Phenotype	Heredity
616362	Autosomal dominant mental retardation 36 (MRD36)	AD

The ***PPP2R1A*** gene encodes a subunit of phosphatase 2A (PP2A), which is one of the major serine-threonine phosphatases of the cell (Calin et. al., 2000, PMID: 10713707). PP2A is involved in the regulation of fundamental cellular processes, such as cell proliferation, DNA repair, apoptosis, and other signal transduction pathways (http://atlasgeneticsoncology.org). For this reason, it also appears to be related to various cancers (Akaike et. al., 2018, PMID: 29861864, Peyvandi et. al., 2018, PMID: 29755572, Calin et. al., 2000, PMID: 10713707). Pathogenic missense variants in *PPP2R1A* occur primarily *de novo* and have been described in patients as causative of mental retardation with absent speech, hypotonia, facial dysmorphia, ventriculomegaly, complete or partial corpus callosum agenesis, and vermis anomalies (Houge et. al., 2015, PMID: 26168268, Fitzgerald et. al, 2015, PMID: 25533962, Suppl. Table 4, Patient IDs: 258589, 259358. 263907; Wallace et al., 2019, PMID: 31687265; Lenaerts et al., 2020, PMID: 33106617).

In the analyzed DNA of the fetus we have identified the missense variant **c.548G>A; p.Arg183Gln** in gene ***PPP2R1A*** in a mosaic state. The variant is present in 15% of the covering sequencing reads (29 of 194) and therefore presumably present in 30% of the analyzed amniotic fluid cells in a heterozygous state. It was not detected in DNA extracted from leukocytes from either parent, and therefore most likely arose *de novo*. The same missense variant has been reported previously in the literature in a *de novo* state in a newborn patient with ventriculomegaly, hypoplastic corpus callosum and dysplastic vermis (Wallace et al., 2019, PMID: 31687265) and a functional defect of the variant has been demonstrated earlier (Haesen et al., 2016, PMID: 27485451). Additionally, we have identified the same missense variant in our in-house patient cohort in a *de novo* state in a fetus presenting with ventriculomegaly. Another missense variant at the same amino acid position has also been described in a patient with ventriculomegaly (p.Arg183Trp in Lenaerts et al., 2020, PMID: 33106617).

Based upon the available data, we classify the detected variant in *PPP2R1A* as pathogenic and consistent with the brain anomalies of the fetus. As we have detected the variant in a mosaic state in the analyzed DNA, we cannot fully assess a potential effect or frequency in different tissues.

Variants in regions not analyzed (e.g. introns, untranslated regions (UTRs), promotors, or enhancers), in regions with repeat expansions, and copy number variants cannot be reliably detected, and therefore their potential involvement in disease cannot be excluded. Furthermore, mosaic variants that occur at a low frequency in the sampled tissue cannot be reliably detected, and therefore, likewise cannot be excluded. Although unlikely, it is also possible that the classification of variants may change in the future due to improvements in scientific understanding.

GENETIC RELEVANCE

The variant in gene *PPP2R1A* is present in mosaic state. Somatic variants are present in different tissues at variable frequencies. The prediction of the possible effects of such a variant on different tissue types cannot therefore be determined. Somatic variants can be passed on to offspring if germline cells are also affected. It is difficult to estimate the risk for passing on a somatic mosaic variant to potential offspring as it depends on the fraction of affected germline cells.

These results have to be communicated by a human geneticist or by a genetic counselor according to the German Genetic Diagnostics Legislation. If you have any further questions please do not hesitate to contact us.

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.

Consultant for Human Genetics

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Diagnostics

ADDITIONAL INFORMATION

Analyzed Regions

Trio whole exome analysis was performed for the fetus and parents.

Additionally, we have analyzed the following regions of the fetus using a panel based approach:

PPP2R1A

Reported variants are limited to pathogenic and likely pathogenic variants associated with the clinical phenotype of the fetus, according to current scientific understanding.

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.

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% 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. X-chromosomal variants that are listed in public databases equal to or greater than 50 times in a hemizygous state and are not disease-causing variants according to HGMD are excluded from analysis. 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.

Trio analysis: Variants found in the fetus and in the parents were compared and filtered for the following cases (if applicable): *de novo* in the fetus, the fetus is compound heterozygous, the fetus is homozygous and the parents are heterozygous, the fetus is hemizygous and the mother is heterozygous for variants on the X-chromosome.

Variants identified through 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.

96.67%, 96.87%, and 96.92% of the targeted regions were covered by a minimum of 30 high-quality sequencing reads per base for the index, mother, and father, respectively.

The evaluation of variants is dependent on available clinical information at the time of analysis. The medical report contains all variants not classified as uncertain, 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).

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

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