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Dr. Jane Doe
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Index ID #	Fetus of XX, XXX
Mother ID #	XXX, XX (*DD.MM.YYYY)
Father ID #	XXX, XX (*DD.MM.YYYY)
Sample receipt	xxx
Material	DNA
External ID	#
Report date	xxx
Report-ID	R#

Genetic Report – Fetus of XX, XXX

Indication Fetal cystic hygroma

Order Trio exome analysis

Result: Report with Significant Findings

- **Detection of a likely pathogenic variant in gene *KRAS* in the analyzed fetal tissue, which is consistent with Noonan syndrome in the fetus.**
- Based upon current scientific knowledge, we did not identify any reportable copy number variants, which are likely to be causative for the fetus's phenotype.
- The NGS data indicate no maternal DNA contamination of the fetal sample.

Gene	Variant	Zygosity			Heredity	MAF (%)	Classification
		Index	Mother	Father			
KRAS	**c.149C>T; p.Thr50Ile** **chr12:25380309 G>A (hg19)**	**het.**	**-**	**-**	**AD**	**0.05**	**likely pathogenic**

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

Recommendation

We recommend further clinical evaluation, and, as circumstances require, postpartum management of manifestations and regular surveillance according to the current guidelines for *KRAS*-associated Noonan syndrome (Roberts, updated 2022, PMID: 20301303, GeneReviews).

Genetic Relevance

The fetus is heterozygous for a likely pathogenic variant in gene *KRAS* that likely arose *de novo*.

The possibility of parental germline mosaicism has to be taken into consideration as the variant in gene *KRAS* has not been detected in DNA extracted from leukocytes from the parents of your patient. The likelihood of further offspring of the parents inheriting this variant is difficult to determine due to a lack of scientific data, however the probability of reoccurrence for parents with a child carrying the likely pathogenic variant is statistically increased (Human Genetics: From Molecules to Medicine - Schaaf, Zschocke & Potocki 2011).

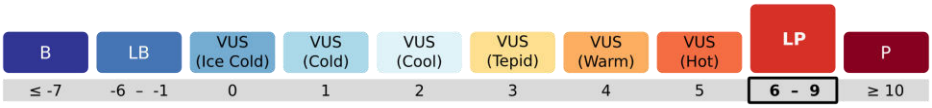
Clinical Information and Variant Interpretation

KRAS, NM_004985.5

OMIM / Reference	Phenotype	Heredity
615278	Cardiofaciocutaneous syndrome 2 (CFC2)	AD
609942	Noonan syndrome 3 (NS3)	AD
607785	Juvenile myelomonocytic leukemia (JMML) caused by somatic or germline mutations in KRAS	AD
614470	RAS-associated autoimmune leukoproliferative disorder (RALD)	AD
600268	Oculoectodermal syndrome, somatic	AD
163200	Schimmelpenning-Feuerstein-Mims syndrom, somatic	AD

The gene ***KRAS*** encodes a GTPase which acts as an intracellular signaling molecule. The protein plays an important role in the Ras-MAPK signaling transduction cascade for many downstream cellular processes, particularly proliferation, differentiation, and inducing transcriptional silencing of tumor suppressor genes. This gene can therefore promote oncogenic effects. Patients with germline mutations in *KRAS* have a broad and variable phenotypic presentation, including a characteristic facial appearance, heart abnormalities, and mild to moderate mental retardation, amongst other more variable features (Zenker et al., 2007, PMID: 17056636).

KRAS, c.149C>T; p.Thr50Ile (het.), ClinVar ID: 984504

ACMG/ACGS Criterion	Points	Description
PS2	+4	The variant has already been detected *de novo* in a patient with the disease, and no family history. Strength level of call is based on factors such as disease specificity and number of previously reported *de novo* findings for this variant.
PM2	+2	This variant is listed in the gnomAD global population dataset with very low frequency.
PP2	+1	Fewer than expected missense variants are present within gene *KRAS* in the general population, which suggests poor tolerance for missense variation.
ACMG/ACGS Classification: likely pathogenic	+7	

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: XXX

Proofread by: XXX

Validated by: XXX

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With kind regards,

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

Consultant for Human Genetics

Additional Information

Analyzed Regions

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

Reported variants are limited to pathogenic and likely pathogenic variants associated with the clinical phenotype of the fetus, according to current scientific understanding. The following differential diagnoses were also taken into account in the evaluation of our sequencing data: Increased prenatal nuchal translucency; Mitochondrial Genome (mtDNA)

General Remarks

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. 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, 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 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 balanced rearrangements, uniparental disomy, or low-level mosaicism. Aberrations on the Y chromosome and in 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, 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 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.

95.96%, 97.6%, and 97.53% 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 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.

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