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Dr. Richard Roe
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Patient	Doe, Jane
ID #	Female (*DD.MM.YYYY)
Sample receipt	xxx
Material	EDTA blood
External ID	#
Report date	xxx
Report-ID	R#

Genetic Report – Doe, Jane (*DD.MM.YYYY)

Indication Neonatal exanthema, persistent signs of inflammation, sterile meningitis, unilateral arm paralysis, generalized lymphadenopathy, anemia, joint inflammation; previous exome diagnostics in external laboratory negative

Order Molecular genetic diagnostics: Autoinflammation (DIG: Deep ImmunoGenetics enrichment)

Result: Report with Significant Findings

- **Detection of a likely pathogenic variant in gene *NLRP3* in a mosaic state, which is consistent with CINCA syndrome or NOMID in your patient.**

Gene	Variant	Zygosity	Heredity	MAF (%)	Classification
NLRP3	**c.925G>C; p.Gly309Arg** **chr1:247587670 G>C (hg19)**	**mosaic** **(80 of 1341 reads; ~6%)**	**AD, somatic**	**-**	**likely pathogenic**

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

Recommendation

We recommend further clinical management according to the current guidelines for cryopyrin-associated periodic syndromes (Romano et al., 2021, PMID: 35623638).

Genetic Relevance

A *de novo* origin of the detected mosaic variant in gene *NLRP3* can be assumed.

Mosaic variants in the *NLRP3* gene associated with childhood-onset disease arise in hematopoietic stem cells or in earlier stages of embryonic development (Horebeek et al., 2019, PMID: 31668909). It is therefore possible that the variant is also present other tissues, as well as germ cells. In germline mosaics, the inheritance risk for offspring depends on the proportion of germ cells affected by the alteration and is therefore difficult to estimate (Basiswissen Humangenetik - Schaaf, Zschocke 2018).

Clinical Information and Variant Interpretation

***NLRP3*, NM_004895.5**

OMIM / Reference	Phenotype	Heredity
191900	Muckle-Wells syndrome (MWS)	AD
120100	Familial cold-induced autoinflammatory syndrome 1 (FCAS1) / familial cold urticaria (FCU)	AD
607115	**Chronic infantile neurological cutaneous and articular syndrome (CINCA)/ Neonatal-onset multisystem inflammatory disease (NOMID)**	**AD, somatic**
617772	Deafness, autosomal dominant 34, with or without inflammation (DFNA34)	AD
148200	Keratoendotheliitis fugax hereditaria (KEFH)	AD

The ***NLRP3*** gene encodes the protein cryopyrin, which is involved in the innate immune system's response to injury, toxins, or invasion by microorganisms. Pathogenic gain-of-function variants in *NLRP3* cause autosomal dominantly inherited cryopyrin-associated periodic syndromes (CAPS) like Muckle-Wells syndrome (MWS), familial cold-induced autoinflammatory syndrome 1 (FCAS1 and CINCA syndrome (CINCA, also known as NOMID) (Welzel and Kuemmerle-Deschner, 2021, PMID: 33401496). FCAS is the mildest form of CAPS characterized by recurrent episodes of urticaria-like skin rash triggered by exposure to cold and associated with low-grade fever, general malaise, eye redness and arthralgia/myalgia (ORPHA: 47045). The Muckle-Wells syndrome is an intermediate form of CAPS that, in addition to the former symptoms, presents more severely with chronic symptoms, potentially life-threatening secondary amyloidosis, and hearing loss (ORPHA: 575). CINCA/NOMID syndrome is characterized by neonatal onset of systemic inflammation, urticarial skin rash and arthritis/arthralgia resulting in severe arthropathy and central nervous system involvement (including chronic aseptic meningitis, which leads to brain atrophy, intellectual disability and sensorineural hearing loss if left untreated; ORPHA: 1451). Heterozygous variants in *NLRP3* are also associated with autosomal dominant deafness with or without inflammation (DNFA34) and Keratitis fugax hereditaria (KEFH). Reduced penetrance and a variable phenotype are known to be associated with pathogenic *NLRP3* variants (Schuh et al., 2015, PMID: 26020059).

***NLRP3*, c.925G>C; p.Gly309Arg (mosaic), ClinVar ID: 393082**

ACMG/ACGS Criterion	Points	Description
PS2 (moderate)	+2	The variant has been identified in a *de novo* state in a patient with the disease, and no family history. The strength level of this call is based on disease specificity, the number of reported *de novo* findings for this variant, and confirmed parental relationships.
PM1	+2	The variant is located within a critical region of the gene *NLRP3*.
PM2	+2	This variant is absent from the gnomAD global population dataset.
PM5	+2	The variant results in the substitution of an amino acid residue, for which a different amino acid substitution p.Gly309Ser has already been described as pathogenic. Saito et al., 2008, PMID: 18063752
ACMG/ACGS Classification: likely pathogenic	+8	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.

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Validated by: XXX

With kind regards,

Dr. med. Berit Kerner

Consultant for Human Genetics

Additional Information

Requested Regions

ADA2, ***AP3B1***, ***BACH2***, ***CARD11***, ***CARD14***, ***CDC42***, ***CEBPE***, ***COPA***, ***CTLA4***, ***GATA2***, ***HAVCR2***, ***HCK***, ***IFIH1***, ***IL6ST***, ***JAK1***, ***LYN***, ***LYST***, ***NCKAP1L***, ***NFKB1***, ***NLRC4***, ***NLRP1***, ***NLRP12***, ***NLRP3***, ***NOD2***, ***OAS1***, ***PDGFRA***, ***PDGFRB***, ***PIK3CG***, ***PLCG1***, ***PLCG2***, ***POMP***, ***PRF1***, ***PSMA3***, ***PSMB10***, ***PSMB4***, ***PSMB8***, ***PSMB9***, ***PSMG2***, ***PSTPIP1***, ***RAB27A***, ***RC3H1***, ***RELA***, ***RIPK1***, ***SLC7A7***, ***SOCS1***, ***STAT1***, ***STAT2***, ***STAT4***, ***STAT6***, ***STING1***, ***STX11***, ***STXBP2***, ***SYK***, ***TLR8***, ***TNFAIP3***, ***TNFRSF1A***, ***TREX1***, ***UBA1***, ***UNC13D***, ***XIAP*** (Autoinflammation)

The following differential diagnoses were also taken into account in the evaluation of our sequencing data: Lymphoproliferation and autoimmunity

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 very low frequency somatic mosaicism (esp. < 1%) cannot be reliably assessed. 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/). If applicable, the following approach is used. 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.

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).

All sequence variants (SNVs/Small Indels) in the coding region and the flanking intronic regions (±8 bp) with a minor allele frequency (MAF) < 1.5% and a novel allele frequency (NAF) of ≥5% are evaluated. NAF is the frequency with which the altered allele occurs in the sequencing data (aka VAF, variant allele fraction). Known hotspot variants may also be reported up to a NAF of ≥ 1%. Known disease-causing variants (according to HGMD) are evaluated in up to ±30 bp of flanking regions and up to 5% MAF. Possible exceptions include risk factors and hypomorphic alleles. Minor allele frequencies are taken from public databases (e.g. gnomAD) and an in-house database.

In this case, the mean coverage was 1055 reads and 99.41% of the targeted regions were covered by a minimum of 300 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).

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