

Patient ID #	XXX, XX Male (*DD.MM.YYYY)
Sample receipt	xxx
Material	EDTA blood
Report date	xxx
Report-ID	R#

Genetic Report – XXX, XX (*DD.MM.YYYY)

Indication	Suspected familial Mediterranean fever, periodic fever with pain in lower limb
Order	Panel Diagnostics: Periodic fever syndromes with/without urticaria (whole exome enrichment)

Result: Report with Significant Findings

- **Detection of a pathogenic variant in gene *MEFV*, which is causative for familial Mediterranean fever in your patient.**
- 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	Zygoty	Heredity	MAF (%)	Classification
<i>MEFV</i>	c.2080A>G; p.Met694Val chr16:3293407 T>C (hg19)	het.	AD, AR	0.1	pathogenic

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

Recommendation

We recommend clinical management and treatment according to the current guidelines for familial Mediterranean fever. A colchicine therapy is considered for acute fever episodes as well as amyloidosis prevention, which can be a severe long-term complication with FMF (e. g. Shohat, updated 2016, PMID: 20301405, GeneReviews).

It is possible to investigate further affected family members regarding the variant identified in gene *MEFV*.

Genetic Relevance

Your patient is heterozygous for a pathogenic variant in gene *MEFV*. This may be of relevance for family planning and at-risk family members.

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

Clinical Information and Variant Interpretation

MEFV, NM_000243.3

OMIM / Reference	Phenotype	Heredity
249100	Autosomal recessive familial Mediterranean fever, classical	AR
134610	Autosomal dominant familial Mediterranean fever	AD
608068	Neutrophilic dermatosis, acute febrile	AD

The **MEFV** gene codes for pyrin, which acts as an important mediator in inflammatory processes. Pathogenic variants in the **MEFV** gene can cause familial Mediterranean fever (FMF), an autoinflammatory disease that is common in specific populations and which is characterized by recurrent episodes of fever and serositis with pain in the abdomen, thorax, joints and muscles (ORPHA:342). An autosomal recessive mode of inheritance is assumed for the FMF, but in approximately 20-25% of individuals with FMF only a single pathogenic change in the **MEFV** gene can be detected. An autosomal dominant inheritance is described on an apodictic basis only for a few pathogenic changes in **MEFV** (p.Thr577Asn, p.Tyr688*, p.Met694del and p.Met694Val), yet a reduced penetrance or variable expressivity must be assumed for most of these variants due to their high allele frequency. These epidemiological observations indicate that other factors such as an additional genetic predisposition and gene-dosage effect, epigenetic mechanisms or environmental influences are involved in the pathogenesis of FMF (for instance Gao et al., 2016, PMID: 27482109; Gangemi et al., 2018, PMID: 29393966; Jamilloux et al., 2018, PMID: 29040788; Magnotti et al., 2019, PMID: 31589380; Boursier et al., 2019, PMID: 30928144). Pyrin gain-of-function variants have been proposed to be the cause of familial Mediterranean fever (GeneReviews "Familial Mediterranean Fever", Shohat, updated 2016, PMID: 20301405). Additionally, autosomal dominant neutrophilic dermatitis is associated with pathogenic variants in the gene, with patients showing features of severe acne, sterile skin abscesses, pyoderma gangrenosum, and neutrophilic small vessel vasculitis (Masters et al., 2016, PMID: 27030597; Moghaddas et al., 2017, PMID: 28835462).

MEFV, c.2080A>G; p.Met694Val (het.), ClinVar ID: 2538

ACMG/ACGS Criterion	Points	Description
PS3 (moderate)	+2	Functional studies support the pathogenicity of this variant. Chae et al., 2006, PMID: 16785446; Sugiyama et al., 2014, PMID: 24318677
PS4	+4	The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls. Yilmaz et al., 2001, PMID: 11464248; Talaat et al., 2012, PMID: 23206577; Neocleous et al., 2015, PMID: 25393764
PM1	+2	The variant is located within a critical region of the gene MEFV .
PM5	+2	The variant results in the change of an amino acid residue, for which different amino acid changes (p.Met694Ile, p.Met694Lys) have already been described as pathogenic. Bernot et al., 1998, PMID: 9668175; Mansour et al., 2001, PMID: 11175300; Yesilada et al., 2012, PMID: 23031807
PP1	+1	Cosegregation of the variant with disease in multiple affected family members.
PP4	+1	The associated disease is consistent with specific symptoms in the patient.
BP4	-1	The variant was given a benign prediction by in silico tools.

ACMG/ACGS Classification:	Points	Legend
Pathogenic	+11	<div style="display: flex; justify-content: space-between; align-items: center;"> <div style="text-align: center;"> B <small>≤ -7</small> </div> <div style="text-align: center;"> LB <small>-6 - -1</small> </div> <div style="text-align: center;"> VUS (Ice Cold) <small>0</small> </div> <div style="text-align: center;"> VUS (Cold) <small>1</small> </div> <div style="text-align: center;"> VUS (Cool) <small>2</small> </div> <div style="text-align: center;"> VUS (Tepid) <small>3</small> </div> <div style="text-align: center;"> VUS (Warm) <small>4</small> </div> <div style="text-align: center;"> VUS (Hot) <small>5</small> </div> <div style="text-align: center;"> LP <small>6 - 9</small> </div> <div style="text-align: center;"> P <small>≥ 10</small> </div> </div>

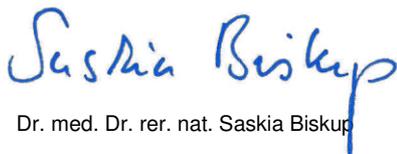
Genetic counseling should be offered with all diagnostic genetic testing, especially following the identification of the molecular cause of a genetic disease.

Medical report written by: XXX

Proofread by: XXX

Validated by: XXX

With kind regards,



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

Consultant for Human Genetics

Additional Information

Requested Regions The whole exome of the individual above was targeted and sequenced. Analysis was restricted to the following gene regions:

F12, HTR1A, MEFV, MVK, NLRC4, NLRP12, NLRP3, NTRK1, OTULIN, PLCG2, RIPK1, SLC29A3, TNFRSF1A, WDR1 (Periodic fever syndromes with/without urticaria)

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 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. 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) $< 1.5\%$ 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, 98.01% 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* 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.