Patient ID / MRN	Patient Name		Birth Date	Gender	Age
	LAST, FIRST		dd-0m-yyyy	Female	
Accession Number	Account Name		Ordering Physician		
XX-XX-XXX-XXXXX	Clinic / Hospital		LAST, FIRST		
Sample Type	Collected	Received	Reported		
Blood	DD Mmm YYYY 00:00	DD Mmm YYYY	DD Mmm YYYY		
Test		Test Indication			
1724372 - MEFV Full-gene Sequencing (based on Familial hemophagocytic lymphohistiocytosis (NGS) panel		Periodic fever, suspicion of FMF			

MEFV Full-gene Sequencing (based on Familial hemophagocytic lymphohistiocytosis (NGS) panel)

RESULT

Pathogenic Variant is Detected in Homozygous State
MEFV: Chr16(GRCh37):g.3293407; NM_000243.2(MEFV):c.2080A>G; p.Met694Val; exon 10/10; coverage 112X

No additional reportable variants were detected.

INTERPRETATION

MEFV: Chr16(GRCh37):g.3293407; NM_000243.2(MEFV):c.2080A>G; p.Met694Val

Normal gene/protein function: The MEFV gene encodes a protein, also known as pyrin or marenostrin, which is an important modulator of innate immunity.

Mutation effect: Mutations in MEFV are associated with Mediterranean fever, a hereditary periodic fever syndrome. The variant c.2080A>G; p.Met694Val in MEFV gene is one of the most common pathogenic variants that cause Familial Mediterranean fever (FMF). FMF predominantly affects populations living in the Mediterranean region, especially North African Jews, Armenians, Turks, and Arabs (PMID: 20301405; GeneReviews). FMF is typically inherited in an autosomal recessive manner; however, it has been observed that a substantial number of patients with clinical FMF possess only a single heterozygous pathogenic variant (PMID: 19479870, 34549050, 27150194). Heterozygotes typically have a later age of onset (mean age 18 years) and milder disease (manifest mainly by fever and abdominal symptoms) than persons with biallelic pathogenic variants. In a study, most of the heterozygotes had an incomplete abdominal attack (abdominal pain without frank peritonitis) as the major criterion of the disease; in most individuals, the response to colchicine therapy was either complete or partial (PMID: 20301405, 19479870). Persons who are homozygous for the pathogenic variant p.Met694Val have an earlier age of onset and higher frequencies of arthritis and arthralgia than persons who are homozygous or compound heterozygous for other pathogenic variants. Individuals with the variant p.Met694Val, particularly homozygous individuals, are at increased risk for amyloidosis and have a decreased response to colchicine (PMID: 20301405; GeneReviews). Functional studies (in-vitro and in-vivo) have demonstrated that this variant leads to the loss of suppression of IL-8 secretion and decreased binding of PKN1 and 14-3-3 protein to murine pyrin (PMID: 27270401, 24318677).

This result is consistent with the diagnosis of Familial Mediterranean fever in this individual. This result should be interpreted in the context of clinical findings, family history, and other laboratory testing. Consultation with a genetics professional is recommended for the interpretation of this result and to determine whether reproductive risk assessment and familial testing may be of benefit to this family. Genetic testing for family members is available by ordering Known Familial Variant Analysis (Test Code: 8140679) for the specific variant detected.

METHODOLOGY

The total genomic DNA was extracted from the biological sample using the CE-IVD-marked Bead-based EZ1 DSP DNA Blood Kit (Qiagen, Hilden, Germany). After assessment of DNA quality and quantity using the Denovix DS-11 Spectrophotometer/Fluorometer system, the DNA was randomly fragmented, and ligated sequencing libraries were prepared

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using Twist Library Preparation EF 2.0 kit (Twist Biosciences, CA, USA). Regions of interest (coding exons and flanking intronic regions) were targeted by the hybridization-based target capture method using the Twist Exome 2.0 kit (Twist Biosciences). Captured DNA was sequenced to an average targeted depth of ≥100X on the Illumina NextSeq 2000 using 2x150 bp paired-end reads (Illumina, San Diego, CA, USA). Primary data analysis converting images into base calls and associated quality scores and secondary analysis aligning the sequencing reads against the reference human genome (GRCh37-hg19) and variant calling was carried out by Illumina's proprietary software (Dragen 3.10.12). Copy number variation (CNV) calling was performed using ClinCNV (1.18.3), Dragen CNV (3.10.12), and VS-CNV (2.6.2). Structural variation (SV) calling was performed using Dragen SV (3.10.12) and Delly (1.2.6). Variant annotation and filtering were performed on VarSeq 2.6.2 (Golden Helix, MT, USA). Variant interpretation was performed based on the published ACMG/AMP guidelines (Richard et al, 2015, PMID: 25741868) along with ClinGen gene/disease specifications. Variants classified as benign/likely benign are not reported.

The gene MEFV is part of the Familial hemophagocytic lymphohistiocytosis (NGS) panel which includes 43 genes: ADA; AP3B1; AP3D1; BLOC1S6; BTK; CD27; CD70; CORO1A; CTPS1; CYBB; FADD; FAS; FASLG; GATA2; IL2RA; IL2RG; ITK; LAMP1; LIPA; LYST; MAGT1; **MEFV**; MVK; MYO5A; NLRC4; NLRP3; PNP; PRF1; RAB27A; RAG1; RAG2; RECQL4; RHOG; SH2D1A; SLC7A7; STX11; STXBP2; TBXAS1; TNFRSF1A; UNC13D; WAS; XIAP; ZNFX1

COVERAGE METRICS

Average (median) coverage of MEFV gene: 122X
Minimum coverage of MEFV gene: 40X

A minimum coverage of ≥10X for each target/gene was used as the cut-off as the passing criteria. Any target region in a gene not meeting this cut-off is noted under the LIMITATIONS section.

PERFORMANCE CHARACTERISTICS

Single base substitution: accuracy >99%; reproducibility 100% (intra- and inter-assay). Small insertion/deletion events (up to 50 bp): accuracy >99%; reproducibility 100% (intra- and inter-assay). Larger single gene insertion/deletions with size ≥50bp that primarily encompass single to multi-exons will also be reported. Homozygous multi-exon deletion in single gene of size up to 21 kilobase (kb) is detectable.

LIMITATIONS

Next-generation sequencing may not detect all types of genomic variants. This test does not detect complex inversions, gene conversions, balanced translocations, repeat expansion disorders, and noncoding variants deeper than ±20 base pairs from the exon-intron boundary unless otherwise indicated. Additionally, this test may not reliably detect the following: low-level mosaicism, variants in the stretch of mononucleotide repeats, indels larger than 50bp, and single exon deletions or duplications. False-negative results may also occur in the setting of bone marrow transplantation, recent blood transfusion, or suboptimal DNA quality. In addition, the chance of a false positive or false negative result due to rare laboratory errors incurred during any phase of testing cannot be completely excluded. The depth of coverage may be variable for some target regions, but assay performance below the minimum acceptable criteria will be noted. In addition, some exons and intronic regions within ±20 base pairs are not analyzed due to inherent low quality due to homology/pseudogene and repetitive regions. Given these limitations, negative results do not rule out the diagnosis of a genetic disorder.

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Exons not analyzed: None
Intronic ±20 bp not analyzed (but splice-site ±2 bp included): None

DISCLAIMER

If testing was performed because of a clinically significant family history, it is useful to first test an affected family member. Detection of a reportable variant(s) in an affected family member would allow for more informative testing of at-risk individuals.

This test was developed, and its performance characteristics were determined by the Molecular Diagnostics & Genomics Laboratory of NRL. This test is used for clinical purposes and should not be regarded as investigational or for research. The test is currently not accredited by the Emirates International Accreditation Centre (EIAC) but will be added to the scope of accreditation for the next assessment cycle.

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