

Cholestasis Flex Panel Plus

REFERRING HEALTHCARE PROFESSIONAL

NAME **HOSPITAL**

PATIENT

NAME **DOB** **AGE** **GENDER** **ORDER ID**
 0 Female
PRIMARY SAMPLE TYPE **SAMPLE COLLECTION DATE** **CUSTOMER SAMPLE ID**
 DNA

SUMMARY OF RESULTS

TEST RESULTS

Patient is heterozygous for *JAG1* c.1452_1453del, p.(Cys484*), which is likely pathogenic.

Del/Dup (CNV) analysis did not detect any known disease-causing copy number variation or novel or rare deletion/duplication that was considered deleterious.

VARIANT TABLE: GENETIC ALTERATIONS

GENE	POS	TRANSCRIPT	NOMENCLATURE	CONSEQUENCE	GENOTYPE	CLASSIFICATION
JAG1	20:10629312	NM_000214.2	c.1452_1453del, p.(Cys484*)	stop_gained, frameshift_variant	HET	Likely pathogenic
	ID	gnomAD AC/AN	POLYPHEN	SIFT	MUTTASTER	
	OMIM	PHENOTYPE		INHERITANCE	COMMENT	
		Alagille syndrome	N/A	AD	-	

SEQUENCING PERFORMANCE METRICS

PANEL	GENES	EXONS / REGIONS	BASES	BASES > 20X	MEDIAN COVERAGE	PERCENT > 20X
Cholestasis Panel	2	60	13485	13485	230	100

TARGET REGION AND GENE LIST

The Blueprint Genetics Cholestasis Flex Panel (version 1, Aug 31, 2018) Plus Analysis includes sequence analysis and copy number variation analysis of the following genes: *JAG1* and *NOTCH2**. This panel targets protein coding exons, exon-intron boundaries (\pm 20 bps) and selected non-coding, deep intronic variants (listed in Appendix 5). This panel should be used to detect single nucleotide variants and small insertions and deletions (INDELS) up to 220 bps and copy number variations defined as single exon or larger deletions and duplications. This panel should not be used for the detection of repeat expansion disorders or diseases caused by mitochondrial DNA (mtDNA) mutations. The test does not recognize balanced translocations or complex inversions, and it may not detect low-level mosaicism.

Genes removed by the clinician: *ABCB11*, *ABCB4*, *ABCC2*, *AKR1D1*, *ATP8B1*, *BAAT*, *CFTR*, *CREB3L3*, *CYP7B1*, *DCDC2*, *DGUOK*, *EPCAM*, *FAH*, *HSD3B7*, *LCT*, *LMF1*, *MKS1*, *MYO5B*, *NEUROG3*, *NPC1*, *NPC2*, *NPHP1*, *NPHP3*, *NPHP4*, *NR1H4*, *PEX1*, *PEX10*, *PEX12*, *PEX2*, *PEX26*, *PEX5*, *PEX6*, *SERPINA1*, *SLC25A13*, *SLC26A3*, *SMPD1*, *SPINT2*, *TJP2*, *TMEM216*, *TRMU*, *TTC37*, *UGT1A1*, *VIPAS39* and *VPS33B*

*Some, or all, of the gene is duplicated in the genome. Read more: <https://blueprintgenetics.com/pseudogene/>
 The sensitivity to detect variants may be limited in genes marked with an asterisk (*).

STATEMENT

CLINICAL HISTORY

Patient is a 1-month-old baby girl. She was born in gestational week 36+3 with a birth weight of 1935g. The patient is generally of good health but she has elevated liver tests. The patient's father is diagnosed with Alagille syndrome and he was hospitalized long periods during his childhood. It is unclear whether the father's diagnosis has been genetically confirmed and the only test that has been performed through our laboratory regarding this syndrome is a FISH analysis that was carried out in year 1997 with a normal result. The cholestasis FLEX panel analysis plus (JAG1 and NOTCH2 genes only) is requested on the clinical suspicion of Alagille syndrome.

CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Cholestasis Flex Panel identified a heterozygous nonsense variant c.1452_1453del, p.(Cys484*) in *JAG1*. This variant has not been observed in the large reference population cohorts of the Genome Aggregation Database, (gnomAD, n>120,000 exomes and >15,000 genomes). Database curators have made every effort to exclude individuals with severe pediatric diseases from these cohorts. The 2 bp deletion generates a premature stop codon and is thus predicted to lead to loss of normal protein function either through protein truncation (484 out of 1218 aa) or nonsense-mediated mRNA decay. To our knowledge, the variant has not been published in the relevant medical literature or reported in the disease-related variation databases such as ClinVar or HGMD. However, loss-of-function mutations in the *JAG1* gene is a well established and the most common mechanism causing Alagille syndrome.

JAG1

The *JAG1* (MIM #601920) gene encodes a ligand of the Notch receptor, and their binding triggers a cascade of proteolytic cleavage that eventually leads to the release of the intracellular part of the receptor from the membrane, allowing it to translocate to the nucleus and activate transcription factors that play key roles in cell differentiation and morphogenesis. NOTCH signalling is activated through cell-cell contact. Ligand binding induces cleavage of NOTCH and translocation of the intracellular domain to the nucleus where it regulates gene expression in association with transcriptional cofactors.

Alagille syndrome (ALGS) is a multisystem disorder defined clinically by hepatic bile duct paucity and cholestasis in association with cardiac, skeletal, and ophthalmologic manifestations. ALGS is transmitted in an autosomal dominant pattern of inheritance with incomplete penetrance. ALGS comprises of two subtypes: the more common ALGS type 1 is caused by mutations in the *JAG1* gene and ALGS type 2 is caused by mutations in *NOTCH2* (MIM #610205). Sequence analysis of *JAG1* detects pathogenic variants in more than 89% of individuals who meet clinical diagnostic criteria; deletion/duplication analysis detects exon and whole-gene deletions, including microdeletion of 20p12, in approximately 7% of affected individuals. Pathogenic variants in *NOTCH2* are observed in 1%-2% of individuals with ALGS (ALGS - GeneReviews).

The prevalence of ALGS is approximately 1/70,000 (ORPHA52). Approximately 30%-50% of individuals with ALGS have an inherited pathogenic variant while about 50%-70% have a *de novo* pathogenic variant (ALGS - GeneReviews). ClinVar reports > 100 pathogenic or likely pathogenic mutations in the *JAG1* gene, including 18 missense, 40 frameshift, 26 nonsense, 7 splice site variants and 16 gross duplications/deletions. All truncations, consensus splice site variants and >100 bp deletions involving coding region are classified as pathogenic in ClinVar. There is only one individual with a radical (nonsense, frameshift, splicing variants, gross deletions) high-quality variant in *JAG1* in the ExAC reference population highlighting the rarity of such alterations in the population.

Mutation nomenclature is based on GenBank accession NM_000214.2 (*JAG1*) with nucleotide one being the first nucleotide of the translation initiation codon ATG.

CONCLUSION

JAG1 c.1452_1453del, p.(Cys484*) is classified as likely pathogenic considering the current evidence of the variant (established association between the gene and the patient's phenotype, rarity in control populations, variant type (nonsense)). However, additional information is still needed to confirm the pathogenicity of the variant. Genetic counseling and family member testing are recommended. Disease caused by *JAG1* variants is inherited in an autosomal dominant manner, and thus each child of an affected individual has a 50% chance of inheriting the variant. BpG offers targeted variant testing for the family if requested.

CONFIRMATION

JAG1 c.1452_1453del, p.(Cys484*) has been confirmed with bi-directional Sanger sequencing.

STEP	DATE
Order date	Aug 31, 2018
Sample received	Aug 31, 2018
Reported	Sep 19, 2018

On Sep 19, 2018 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



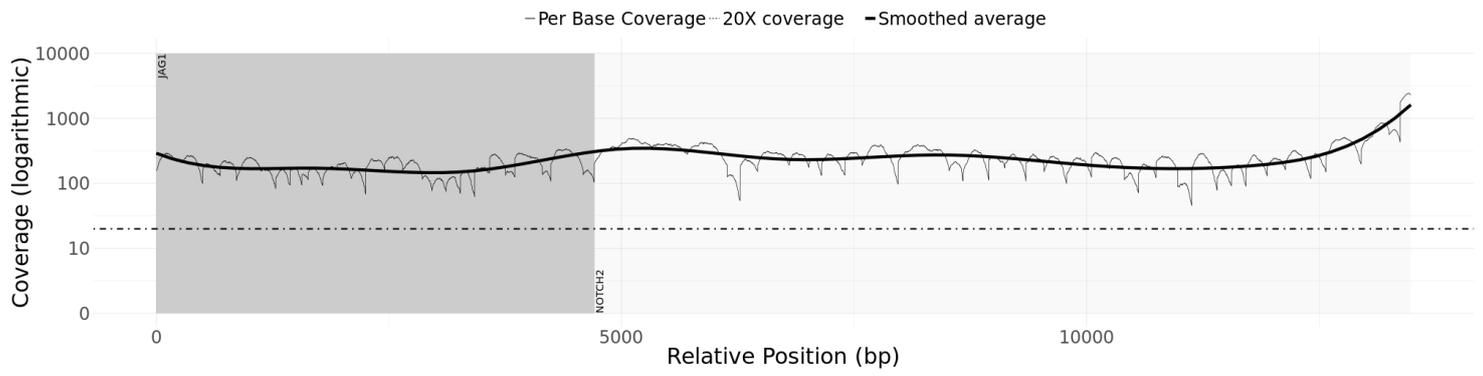
Milja Kaare, Ph.D.
Senior Geneticist



Juha Koskenvuo, MD, Ph.D.
Lab Director, Chief Medical Officer



Hannu Jalanko, MD, Ph.D.
Clinical Consultant



APPENDIX 5: SUMMARY OF THE TEST

PLUS ANALYSIS

Laboratory process: Total genomic DNA was extracted from the biological sample using a spin column method. DNA quality and quantity were assessed using electrophoretic methods. After assessment of DNA quality, qualified genomic DNA sample was randomly fragmented using non-contact, isothermal sonochemistry processing and purified with SPRI beads. DNA fragments were then end-repaired and sequencing adapters were ligated to both ends of the resulting fragments. Prepared DNA-Adapter libraries were size-selected with SPRI beads to ensure optimal template size and then amplified by ligation-mediated PCR (LM-PCR). The amplified sequencing library was purified using SPRI beads and a hybridization-capture method was applied for enrichment of whole exome and select non-coding regions (xGen Exome Research Panel with custom-designed capture probes, IDT). The enriched sequencing library was amplified by LM-PCR and purified using SPRI beads. The quality of the completed sequencing library was controlled by ensuring the correct template size and quantity and to eliminate the presence of leftover primer-dimers. Each captured library passing quality control was sequenced using the Illumina sequencing system with paired-end sequencing (150 by 150 bases). Sequencing-derived raw image files were processed using a base-calling software (Illumina) and the sequence data was transformed into FASTQ format.

Bioinformatics and quality control: The bioinformatics analysis began with quality control of raw sequence reads. Clean sequence reads of each sample were mapped to the human reference genome (GRCh37/hg19). Burrows-Wheeler Aligner (BWA-MEM) software was used for read alignment. Duplicate read marking, local realignment around indels, base quality score recalibration and variant calling were performed using GATK algorithms (Sentieon). The panel content was sliced from high-quality exome sequencing data acquired as presented above. The sequencing depth and coverage for the tested sample was calculated based on the alignments. The sequencing run included in-process reference sample(s) for quality control, which passed our thresholds for sensitivity and specificity. The patient's sample was subjected to thorough quality control measures as well, after which raw sequence reads were transformed into variants by a proprietary bioinformatics pipeline. Copy number variations (CNVs), defined as single exon or larger deletions or duplications (Del/Dups), were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads. The difference between observed and expected sequencing depth at the targeted genomic regions was calculated and regions were divided into segments with variable DNA copy number. The expected sequencing depth was obtained by using other samples processed in the same sequence analysis as a guiding reference. The sequence data was adjusted to account for the effects of varying guanine and cytosine content.

Interpretation: Our variant classification follows the [Blueprint Genetics Variant Classification Schemes](#) modified from the [ACMG guideline 2015](#). Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Likely benign and benign variants were not reported. The pathogenicity potential of the identified variants were assessed by considering the predicted consequence, the biochemical properties of the codon change, the degree of evolutionary conservation as well as the number of reference population databases and mutation databases such as, but not limited, to the [1000 Genomes Project](#), [gnomAD](#), [ClinVar](#) and [HGMD](#). For missense variants, *in silico* variant prediction tools such as [SIFT](#), [PolyPhen](#), [MutationTaster](#) were used to assist with variant classification. In addition, the clinical relevance of any identified CNVs was evaluated by reviewing the relevant literature and databases such as [1000 Genomes Project](#), [Database of Genomic Variants](#), [ExAC](#), [DECIPHER](#). The clinical evaluation team assessed the pathogenicity of the identified variants by evaluating the information in the patient referral, reviewing the relevant literature and manually inspecting the sequencing data if needed. Reporting was carried out using HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

Confirmation: Pathogenic and likely pathogenic variants that established a molecular diagnosis were confirmed with bi-directional Sanger sequencing unless all of the following criteria were fulfilled: 1) the variant quality score (QS) was above the internal threshold for a true positive call, 2) an unambiguous manual curation of the variant region using IGV was concordant with the variant call and 3) previous Sanger confirmation of the same variant has been performed at least three times in our laboratory. Reported variants of uncertain significance were confirmed with bi-directional Sanger sequencing only if the QS was below our internally defined score for a true positive call. CNVs (Dels/Dups) were confirmed using a quantitative-PCR assay if they covered less than 10 target exons or were not confirmed at least three times previously at our laboratory.

Analytic validation: This laboratory-developed test has been independently validated by Blueprint Genetics. The sensitivity of this panel is expected to be in the same range as the validated whole exome sequencing laboratory assay used to generate the panel data (sensitivity for SNVs 99.65%, indels 1-50 bps 99.07%, one-exon deletion 92.3% and two exons CNV 100%, and specificity >99.9% for most variant types). A normal result does not rule out the diagnosis of a genetic disorder since some DNA abnormalities may be undetectable by the applied technology. Test results should always be interpreted in the context of clinical findings, family history, and other relevant data. Inaccurate, or incomplete information may lead to misinterpretation of the results.

Regulation and accreditations: This test has not been cleared or approved by the FDA. This analysis has been performed in a CLIA-certified laboratory (#99D2092375), accredited by the College of American Pathologists (CAP #9257331) and by FINAS Finnish Accreditation Service, (laboratory no. T292), accreditation requirement SFS-EN ISO 15189:2013. All the tests are under the scope of the ISO 15189 accreditation.

NON-CODING VARIANTS COVERED BY THE PANEL:

NM_000214.2(JAG1):c.1349-12T>G

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant

AR = autosomal recessive

CNV = Copy Number Variation e.g. one exon or multiexon deletion or duplication

gnomAD = genome Aggregation Database (reference population database; >138,600 individuals)

gnomAD AC/AN = allele count/allele number in the genome Aggregation Database (gnomAD)

HEM = hemizygous

HET = heterozygous

HOM = homozygous

ID = rsID in dbSNP

MutationTaster = *in silico* prediction tools used to evaluate the significance of identified amino acid changes.

Nomenclature = HGVS nomenclature for a variant in the nucleotide and the predicted effect of a variant in the protein level

OMIM = Online Mendelian Inheritance in Man®

PolyPhen = *in silico* prediction tool used to evaluate the significance of amino acid changes.

POS = genomic position of the variant in the format of chromosome:position

SIFT = *in silico* prediction tool used to evaluate the significance of amino acid changes.
