

# Blueprint Genetics

## Ichthyosis Panel Plus

### REFERRING HEALTHCARE PROFESSIONAL

NAME	HOSPITAL
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### PATIENT

NAME	DOB	AGE	GENDER	ORDER ID
		0	Female	
PRIMARY SAMPLE TYPE	SAMPLE COLLECTION DATE		CUSTOMER SAMPLE ID	
DNA	2017-03-23			

### SUMMARY OF RESULTS

#### TEST RESULTS

The patient is heterozygous for two pathogenic variants in *TGM1*: c.424C>T, p.(Arg142Cys) and c.919C>G, p.(Arg307Gly).

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
<b>TGM1</b>	14:24730985	NM_000359.2	c.424C>T, p.(Arg142Cys)	missense	HET	<b>Pathogenic</b>
	<b>ID</b> rs121918716	<b>EXAC AC/AN</b> 1/121346	<b>POLYPHEN</b> probably damaging	<b>SIFT</b> deleterious	<b>MUTTASTER</b> disease causing	
	<b>OMIM</b>	<b>PHENOTYPE</b> Ichthyosis congenital		<b>INHERITANCE</b> AR	<b>COMMENT</b> -	
<b>TGM1</b>	14:24728975	NM_000359.2	c.919C>G, p.(Arg307Gly)	missense	HET	<b>Pathogenic</b>
	<b>ID</b> rs121918731	<b>EXAC AC/AN</b> 19/109474	<b>POLYPHEN</b> possibly damaging	<b>SIFT</b> deleterious	<b>MUTTASTER</b> disease causing	
	<b>OMIM</b>	<b>PHENOTYPE</b> Ichthyosis congenital		<b>INHERITANCE</b> AR	<b>COMMENT</b> -	

Please see [APPENDIX 2: Additional Findings](#)

#### SEQUENCING PERFORMANCE METRICS OS-SEQ

PANEL	GENES	EXONS	BASES	BASES > 15X	MEDIAN COVERAGE	PERCENT > 15X
Ichthyosis Panel	19	220	50334	50247	235	99.8

#### TARGET REGION AND GENE LIST

Blueprint Genetics Ichthyosis Panel (version 1, March 9, 2016) consists of sequence analysis of genes associated with congenital ichthyosis and lamellar ichthyosis: ABCA12, ALG8, ALOX12B, ALOXE3, CYP4F22, EBP, ERCC2, FLG\*, GJA1\*, GJB2, GJB3, KRT1, KRT2, KRT9, PEX7, PHYH, STS, SUMF1 and TGM1. The panel is targeting all protein coding exons and exon-intron boundaries of all target genes. It also covers a number of mutations located outside these coding regions. This test covers the majority of congenital ichthyosis and lamellar ichthyosis mutations known to date and it should be used to detect single nucleotide substitutions and small

insertions and deletions (INDELs). In addition, the Ichthyosis Panel includes OS-Seq Del/Dup (CNV) Analysis (version 1, updated November 15, 2016) for the same genes as listed above. It should be used to diagnose deletions and duplications (e.g. copy number variants) in protein-coding regions of the genes included in the panel. Detection limit of the test varies through the genome from one to six exons depending on exon size, sequencing coverage and sequence content.

\* Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions ([link to duplicated regions](#)): <http://blueprintgenetics.com/pseudogene/>. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out.

The test does not recognise balanced translocations or complex inversions, and it may not detect low-level mosaicism. The exact boundaries of the copy number aberration cannot be determined with this test. The test should not be used for analysis of sequence repeats or for diagnosis of disorders caused by mutations in the mitochondrial DNA.

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## STATEMENT

### CLINICAL HISTORY

The patient is a 2-month-old girl. She is a newborn collodion baby with negative family history.

### CLINICAL REPORT

Sequence analysis using the Blueprint Genetics (BpG) Ichthyosis Panel identified two heterozygous missense variants in the *TGM1* gene: c.424C>T, p.(Arg142Cys) and c.919C>G, p.(Arg307Gly). Both variants are rare in the Exome Aggregation Consortium (ExAC) data set, comprised in total of over 60,000 unrelated individuals; with 1 and 19 heterozygous individual, respectively. Both variants are predicted deleterious by used *in silico* tools.

The c.424C>T, p.(Arg142Cys) variant has previously been reported as homozygous or compound heterozygous in several patients. Initially the p.(Arg142Cys) variant was reported in a compound heterozygous state in a patient with autosomal recessive congenital ichthyosis (ARCI) by Huber et al. (PMID: 7824952). Subsequently, Laiho et al. analyzed the *TGM1* gene in Finnish patients with lamellar ichthyosis and identified homozygosity for p.(Arg142Cys) in affected members of 2 families. Compound heterozygosity for p.(Arg142Cys) and another mutation in *TGM1* was found in another 9 patients from 6 families, some presenting with lamellar ichthyosis and others exhibiting nonbullous congenital ichthyosiform erythroderma. (PMID: 9326318) The variant was also identified as compound heterozygous in a Korean collodion baby with ARCI (PMID: 24314425). In addition, several other missense variants in the same and adjacent codons has been listed in the HGMD professional database; p.(Arg142His), p.(Arg142Pro), p.(Arg143Cys) p.(Arg143His), p.(Gly144Arg) and p.(Gly144Glu).

The c.919C>G, p.(Arg307Gly) variant has previously been reported as compound heterozygous together with another pathogenic *TGM1* variant in at least five patients. Oji et al. described the variant in three patients who fulfilled the clinical criteria of bathing suit ichthyosis (BSI). The tested patients were born with collodion membrane and during the first to second month of life they developed a lamellar scaling on the trunk, whereas the four limbs and the face were almost completely spared. Patients with the p.(Arg307Gly) variant showed mild, brownish scaling that was most pronounced in the axillae and on the neck. (PMID: 16968736) Hellström et al. described the variant as compound heterozygous in one patient with self-improving collodion ichthyosis and in one patient with lamellar/pleiomorphic ichthyosis (PMID: 27025581). The variant has been reported in at least one patient in clinical testing (ClinVar ID 372534). In addition, another missense variant in the same codon: c.919C>T p.(Arg307Trp) is described as pathogenic in association to ichthyosis in the HGMD and LOVD databases.

The *TGM1* gene (MIM \*190195) encodes transglutaminase 1. This protein is a membrane-bound enzyme functioning in the formation of the epidermal cornified cell envelope. It catalyzes the cross-linking of proteins and conjugation of polyamines to proteins. Mutations in *TGM1* cause autosomal recessive congenital ichthyosis type 1 (ARCI, MIM #242300). ARCI is a heterogenous group of keratinization problems. The main symptom is abnormal skin scaling over the whole body. There are two main phenotypes, lamellar ichthyosis (LI) with large, adherent, often dark scales and nonbullous congenital ichthyosiform erythroderma (NCIE) with fine white, superficial and semiadherent scales. However, overlap of the phenotypes within the same patient and between the patients from the same family is rather common. There is also a minor subtype of ARCI associated with certain *TGM1* variants, in which collodion babies have nearly complete resolution of their ichthyosis already in infancy (PMID: 19890349). The prevalence of ARCI is estimated at 1:100000 to 1:200000. Certain populations, where consanguineous marriage is common, or that have had population bottle-necks, have higher frequencies. *TGM1* is the major gene behind ARCI and explains 35% to 55% of all the cases with ARCI and >90% of cases with severe LI (GeneReviews NBK1420, PMID: 19241467). Faraset et al. studied the genotype-phenotype correlations in >100 patients with ARCI (PMID: 18948357). Mutations in *TGM1* were observed in 55% (57/104) of cases. Patients with at least one truncating variant in *TGM1* were shown to have more severe hypohidrosis ( $p = 0.001$ ) and overheating ( $p = 0.0007$ ) at onset of symptoms than were those patients with only missense variants. There are 180 pathogenic/likely pathogenic variants in *TGM1* identified in patients with ARCI (HGMD database). Most of them are single-base changes including missense (approx. 58%), nonsense (16%) and splice-site (8%) variants. Small deletions or insertions are not very common (altogether 14%). Certain splice-site variants are common (c.877-2A>G, identified in approx. 40% of all patients) and > 40% of all pathogenic *TGM1* variants occur in arginine residues (especially at positions 142 and 143) that contain CpG dinucleotides (PMID: 18948357).

Mutation nomenclature is based on GenBank accession NM\_000359.2 (*TGM1*), with nucleotide one being the first nucleotide of the translation initiation codon ATG.

### CONCLUSION

Considering the current literature and the well-established role of both *TGM1* c.424C>T, p.(Arg142Cys) and *TGM1* c.919C>G, p.(Arg307Gly) as disease causing mutations, we classify them as pathogenic. Disease caused by *TGM1* mutations is inherited in an autosomal recessive manner. Testing of parental samples is needed to determine whether the variants occur in *cis* (the same copy of the gene) or in *trans* (different copies of the gene). Compound heterozygosity of the identified variants (variants in different alleles) would most likely explain the patient's disease. Thus, genetic counseling and family member testing is recommended. If the parents of the affected individual carry one pathogenic variant, then each sib of the affected individual has a 25% chance of being affected, a 50% chance of being an asymptomatic carrier of one of the variants, and a 25% chance of being an unaffected non-carrier. BpG offers mutation testing for the family if requested.

### CONFIRMATION

*TGM1* c.424C>T, p.(Arg142Cys) and *TGM1* c.919C>G, p.(Arg307Gly) were confirmed using bidirectional Sanger sequencing.

STEP	DATE
Order date	Mar 31, 2017
Sample received	Mar 31, 2017
Reported	Apr 21, 2017

On May 07, 2017 the statement has been prepared by our geneticists and physicians, who have together evaluated the sequencing results:



Milja Kaare, Ph.D.  
Geneticist



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



Katariina Hannula-Jouppi, MD, Ph.D.  
Clinical Consultant

## APPENDIX 2: ADDITIONAL FINDINGS

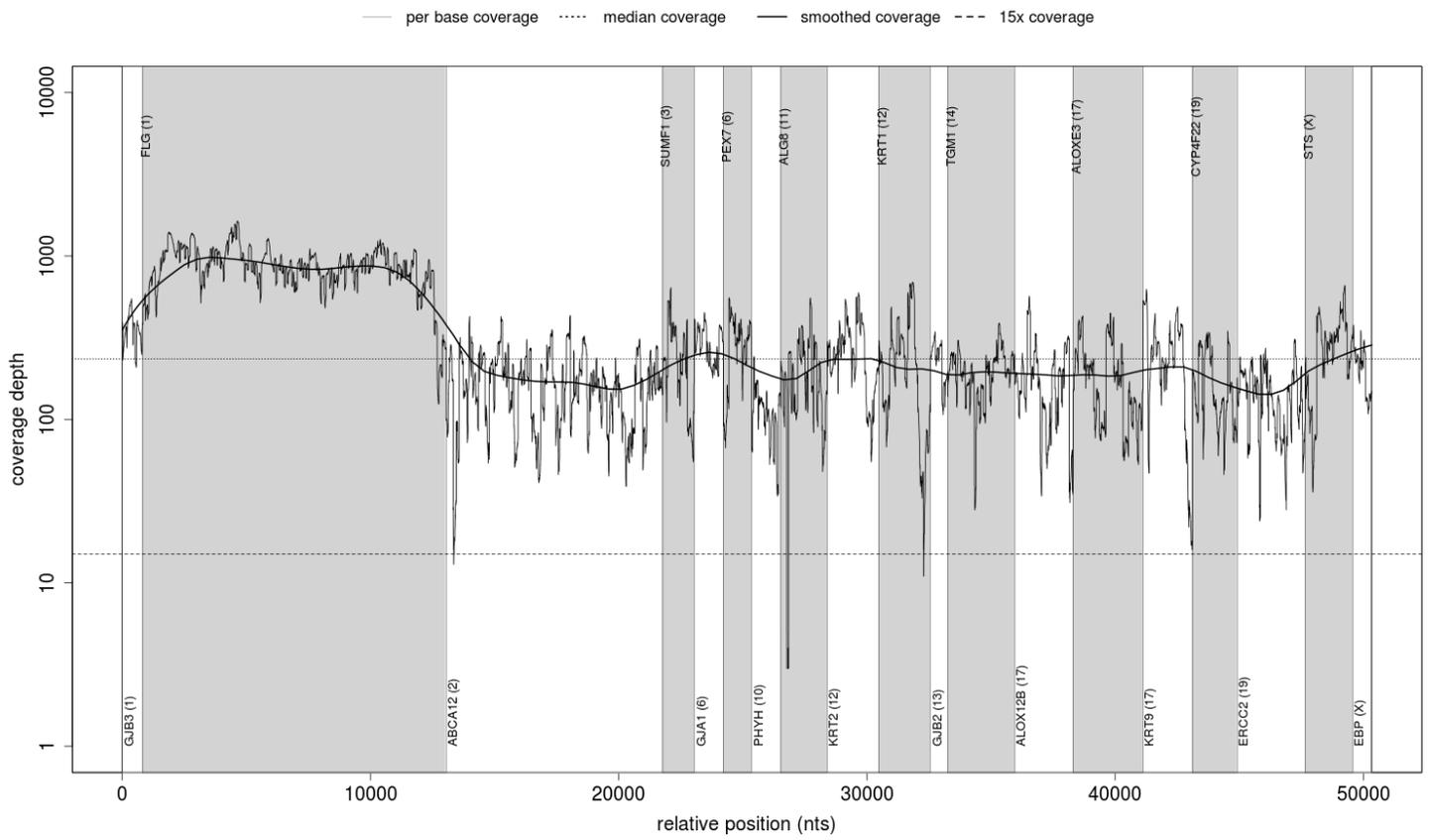
This table includes variants that either are not thought to be the likely cause for patient's phenotype (carrier status of variants of uncertain significance for recessive/X-linked disorders or heterozygous VUS variants for autosomal dominant disorders not likely related to the patient's phenotype), are secondary findings potentially relevant to patient's medical care (risk variants, heterozygous pathogenic or likely pathogenic variants for autosomal dominant disorders not related to patient's current phenotype) or carrier status for pathogenic or likely pathogenic variants for autosomal recessive or X-linked disorder not suspected in the patient.

## VARIANT TABLE: ADDITIONAL GENETIC ALTERATIONS

GENE <b>FLG</b>	POS 1:152280599	TRANSCRIPT NM_002016.1	NOMENCLATURE c.6763G>A, p.(Asp2255Asn)	CONSEQUENCE missense	GENOTYPE HET	CLASSIFICATION <b>Variant of uncertain significance</b>
	<b>ID</b> .	<b>EXAC AC/AN</b> 0/0	<b>POLYPHEN</b> possibly damaging	<b>SIFT</b> N/A	<b>MUTTASTER</b> polymorphism	
	<b>OMIM</b>	<b>PHENOTYPE</b> Ichthyosis vulgaris		<b>INHERITANCE</b> AD&AR	<b>COMMENT</b> -	

## NOTES REGARDING ADDITIONAL FINDINGS

As there is not enough data to support or rule out pathogenicity, we classify the identified *FLG* c.6763G>A, p.(Asp2255Asn) as a variant of uncertain significance (VUS). Additional information is needed to assess the clinical significance. Screening of the variant should not be used for risk evaluation within family members. Management of the patient and family should be based on clinical evaluation and judgment. According to our knowledge, the *FLG* c.6763G>A, p.(Asp2255Asn) variant has not previously been described in databases or in the literature. Heterozygous mutations in *FLG* have been associated with ichthyosis vulgaris and atopic dermatitis.



## APPENDIX 5: SUMMARY OF METHODS

### OS-SEQ (SEQUENCE ANALYSIS)

**Sequencing.** Total genomic DNA was extracted from the biological sample. DNA quality and quantity were assessed using a fluorometric electrophoresis method. Extracted total genomic DNA was mechanically fragmented and enzymatically end-repaired. DNA adapters were added using a ligation-based method and the sequencing library was amplified using PCR. Quality and quantity of the sequencing library DNA were assessed through electrophoresis and fluorometric analyses, respectively. A proprietary Oligonucleotide-Selective Sequencing (OS-Seq) method was used for capturing genomic targets and sequencing was performed using an Illumina sequencing system.

**Data analysis.** Raw sequence reads were filtered to exclude reads with ambiguous base calls and trimmed from the 3' ends based on base call quality and presence of adapter, poly-A or capture oligo sequences. The remaining high-quality reads were mapped to the human genome reference sequence (Hg19). Single nucleotide variants (SNVs) and short insertions and deletions (INDELs) were identified using a proprietary data analysis pipeline. The pathogenicity of the identified variants was predicted based on the biochemical properties of the codon change and the degree of evolutionary conservation using PolyPhen, SIFT and Mutation Taster. Identified variants were annotated using allelic frequencies from large population studies (e.g. 1000 Genomes Project and the Exome Aggregation Consortium), by matching with various genotype-phenotype association databases (e.g. ClinVar) as well as by searching from an in-house curated database of previously reported variants.

**Interpretation.** The clinical evaluation team assessed the pathogenicity of the identified SNV and INDEL variants by evaluating allele frequency, in silico predictions, the annotations from public variant databases and matches in the in-house mutation database and related medical literature. Information in the referral about the patient's phenotype was compared with experimental data in the relevant medical literature to link the identified variants to specific clinical phenotypes. Sequencing data was manually inspected to confirm the variant findings.

**Confirmation.** Novel SNV and INDEL variant(s) classified as pathogenic or likely pathogenic as well as variants of uncertain significance with quality score <500 were confirmed using direct Sanger sequencing of the PCR amplicons. Confirmation of recurrent pathogenic and likely pathogenic variants is initially performed for three consequent cases using Sanger sequencing and subsequently only, when variant quality so requires.

**Reporting.** Reporting was carried out using an HGNC-approved gene nomenclature and mutation nomenclature following the HGVS guidelines.

**Notes.** This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

**Accreditation.** 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.

### DEL/DUP (CNV) ANALYSIS

**Data analysis.** Deletions and duplications (Del/Dups) were detected from the sequence analysis data using a proprietary bioinformatics pipeline, which processes aligned sequence reads provided by the Blueprint Genetics OS-Seq data analysis pipeline. 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. 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. Identified variants were annotated using data from our in-house curated and maintained database and public databases (1000 Genome Project, Database of Genomic Variants, ExAC and DECIPHER).

**Interpretation.** The clinical evaluation team assessed the pathogenicity of the identified Del/Dups by reviewing the variant annotations. Clinical relevance of the identified variants was evaluated by relating the findings to the information in the patient referral and reviewing the relevant literature and databases.

**Confirmation.** Del/Dup variant(s) classified as pathogenic or likely pathogenic were confirmed using a quantitative-PCR assay if they cover less than 10 target exons or the sum of on-target exons and off-target bins (200kb) is < 10 (at least one on-target exon is required).

**Reporting.** Reporting was done using an HGNC-approved gene nomenclature.

**Notes.** This laboratory-developed test has been independently validated by Blueprint Genetics. This test has not been cleared or approved by the FDA. A normal result by this test does not rule out the diagnosis of a disorder since some DNA abnormalities may be undetectable by this 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.

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### GLOSSARY OF USED ABBREVIATIONS:

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

**ID** = rsID in dbSNP

**Transcript** = GenBank accession for reference sequence used for variant nomenclature

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

**ExAC AC/AN** = allele count/allele number in the Exome Aggregation Consortium Database

**AD** = autosomal dominant

**AR** = autosomal recessive

**OMIM** = Online Mendelian Inheritance in Man®

**ExAC** = Exome Aggregation Consortium Database (>60,000 unrelated individuals)

**het** = heterozygous

**hom** = homozygous

**Del/Dup** = Deletion and Duplication

**CNV** = copy number variation

PolyPhen, SIFT and MutationTaster are in silico prediction tools used to evaluate the significance of identified amino acid changes.

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