Report: Life Diagnostic
Patient: Albana Qemali
Date: 01.06.2023

G-Life
www.g-life.care
e-mail:contact@g-life.care

Analysis - Annotation with **Franklin** by **Genoox**, Palo Alto, CA, USA on raw FASTAQ files, created by Dante labs.

Result: Nine variants have been detected, one pathogenic, one likely pathogenic, and the rest are with uncertain significance.
Two of these variants are shared with the mother, two others are shared with the father.

Nr.	Gene / region	Variant	Variant type	Zygosity	Variant classification
1.	***CYP24A1***	CYP24A1:c.428_430delAAG (p.Glu143del) (rs777676129)	InDel, inframe deletion	Heterozygote	**Pathogenic**
2.	***CYP24A1***	CYP24A1:c.849A>T (p.Lys283Asn)	SNV, Missense	Heterozygote	**Uncertain significance**
3.	***SNC10A***	SCN10A:c.2485C>T (p.Arg829Cys) (rs755974168)	SNV, Missense	Heterozygote	**Likely Pathogenic**
4.	***SNC10A***	SCN10A:c.4089G>A (p.Val1363Val)	SNV, Splice region	Heterozygote	**Uncertain significance (Likely Patogenic)**
5.	***CLPB***	CLPB:c.1717C>T (p.Arg573Cys) (rs186989806)	SNV, Missense	Heterozygote	**Uncertain significance (leaning pathogenic)**
6.	***MT-ATP8***	MT-ATP8:c.116C>T chrM-8481 C>T (p.Pro39Leu) (rs1603221521)	SNV, Missense	Heterozygote	**Uncertain significance (leaning pathogenic)**
7.	***CACNA1G***	CACNA1G:c.4987G>C (p.Val1663Leu) (rs1450618466)	SNV, Missense	Heterozygote	**Uncertain significance (leaning pathogenic)**
8.	***ERF***	ERF:c.205G>A (p.Val69Ile) (rs745819984)	SNV, Missense	Heterozygote	**Uncertain significance (leaning pathogenic)**
9.	***KMT2C***	KMT2C:c.7443-7_7443-6delTT (rs746018833)	InDel, Intron variant	Heterozygote	**Uncertain significance (leaning benign)**
10.	***2q12.1 Deletion***	PANTR1|POU3F3	Deletion3.105 bp	Heterozygote	**Pathogenic**

Interpretation:

1. **Variant in the gene CYP24A1:c.428_430delAAG (p.Glu143del), Exon 2, autosomal recessive**

G-Life
www.g-life.care
e-mail:contact@g-life.care

This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This mitochondrial protein initiates the degradation of 1,25-dihydroxyvitamin D3, the physiologically active form of vitamin D3, by hydroxylation of the side chain. In regulating the level of vitamin D3, this enzyme plays a role in calcium homeostasis and the vitamin D endocrine system. Alternatively spliced transcript variants encoding different isoforms have been found for this gene.

- The mode of inheritance is autosomal recessive.
- The tested individual is a heterozygous carrier of this variant.
- Mutations in the gene are associated with infantile hypercalcemia.

  Hypercalcemia, infantile, 1 (MedGen UID: 934200) is characterized by severe hypercalcemia, failure to thrive, vomiting, dehydration, and nephrocalcinosis. An epidemic of idiopathic infantile hypercalcemia occurred in the United Kingdom in the 1950s after the implementation of an increased prophylactic dose of vitamin D supplementation; however, the fact that most infants receiving the prophylaxis remained unaffected suggested that an intrinsic hypersensitivity to vitamin D might be implicated in the pathogenesis.

- **The tested individual is a heterozygous carrier of the variant. This result is not sufficient to cause an autosomal recessive disease.** The variant is classified as **Pathogenic.**

- **This variant is shared with the father of the tested individual. The father is also a heterozygous carrier of this variant. This indicates that the variant is likely not the cause of disease.**

Variant details

- Located on chromosome 20, chr20-52789466 CCTT>C
- The variant type is an inframe deletion after Glutamic acid in codon 143
- The variant is present in the population databases (gnomAD 0.05%, rs777676129)
- The variant is reported in ClinVar, Variation ID: 29677
- There is evidence of pathogenic effect from the population data, effect on protein, and reputable source data
- Due to these reasons, the variant is classified as **Pathogenic**

2. Variant in the gene CYP24A1:c.849A>T (p.Lys283Asn), Exon 7, autosomal recessive

Another variant was detected in the gene CYP24A1.

- The mode of inheritance is autosomal recessive.
- The tested individual is a heterozygous carrier of this variant.
- Mutations in the gene are associated with infantile hypercalcemia (MedGen UID: 934200)

- The tested individual is a heterozygous carrier of the variant. This result is not sufficient to cause an autosomal recessive disease. At the current time being, there is limited information of the pathogenicity of this variant, and it is classified as a **Variant of Uncertain Significance.**

- **This variant is shared with the mother of the tested individual. The mother is also a heterozygous carrier of this variant. This indicates that the variant is likely not the cause of disease.**

Variant details

- Located on chromosome 20, chr20-52779397 T>A
- The variant type is a single nucleotide variant that causes a change in the amino acid sequence, where Lysine is replaced by Asparagine in codon 283
- The variant is not present in the population databases (gnomAD N/A)
- The variant is not reported in ClinVar
- There is evidence of pathogenic moderate classification from the population data
- Due to these reasons, the variant is classified as **Variant of Uncertain Significance**

3. Variant in the gene SCN10A:c.2485C>T (p.Arg829Cys), Exon 16, autosomal dominant

The protein encoded by this gene is a tetrodotoxin-resistant voltage-gated sodium channel alpha subunit. The properties of the channel formed by the encoded transmembrane protein can be altered by interaction with different beta subunits. This protein may be involved in the onset of pain associated with peripheral neuropathy. Alternative splicing results in multiple transcript variants.

- The mode of inheritance is autosomal dominant.
- The tested individual is a heterozygous carrier of this variant.
- Mutations in the gene are associated with episodic pain syndrome.

  Episodic pain syndrome, familial, 2 (MedGen UID: 816223) is an autosomal dominant neurologic disorder characterized by adult-onset of paroxysmal pain mainly affecting the distal lower extremities.Also known as feps2, it is related to neuropathy (hereditary sensory and autonomic), type v and causalgia, and has symptoms including hyperalgesia.

- The tested individual is a heterozygous carrier of the variant. This result is sufficient to cause an autosomal dominant disease. The variant is classified as **Likely pathogenic.**

- **This variant is shared with the mother of the tested individual. The mother is also a heterozygous carrier of this variant.This indicates that the variant is likely not the cause of disease.**

Variant details

- Located on chromosome 3, chr3-38770188 G>A
- The variant type is a single nucleotide variation where Arginine is replaced by Cysteine in codon 829
- The variant is present in the population databases (gnomAD <0.01%, rs755974168)
- The variant is reported in ClinVar, Variation ID: 1312974
- There is evidence of pathogenic effect from the population data and in-silico predictions
- Due to these reasons, the variant is classified as **Likely pathogenic**

4. Variant in the gene SCN10A:c.4089G>A (p.Val1363Val), Exon 23, autosomal dominant

Another variant was detected in the gene SCN10A.

- The mode of inheritance is autosomal dominant.
- The tested individual is a heterozygous carrier of this variant.
- Mutations in the gene are associated with Episodic pain syndrome, familial, 2 (MedGen UID: 816223)

- The tested individual is a heterozygous carrier of the variant. This result is sufficient to cause an autosomal dominant disease. At the current time being, there is limited information of the pathogenicity of this variant, and it is classified as a **Variant of Uncertain Significance (Leaning pathogenic).**

- **This variant is shared with the father of the tested individual. The father is also a heterozygous carrier of this variant. This indicates that the variant is likely not the cause of disease.**

Variant details

- Located on chromosome 3, chr3-38753652 C>T
- The variant type is a single nucleotide variant that causes a synonymous change in the amino acid sequence, where Valine is replaced by Valine in codon 1363
- The variant is not present in the population databases (gnomAD N/A)
- The variant is not reported in ClinVar

- There is evidence of pathogenic moderate classification from the population data, and pathogenic supporting evidence from the in-silico predictions
- Due to these reasons, the variant is classified as **Variant of Uncertain Significance (Leaning pathogenic)**

5. Variation of deletion in the region: 2q12.1, Del: PANTR1, POU3F3, 3105bp, autosomal dominant (AD)

The variation is 3.1kb long, located on chromosome 2, chr2: 105,470,669-105,473,774, exonic, and covers 1 gene or region (1 coding gene)

- The inheritance of diseases is autosomal dominant AD.
- The tested individual is a heterozygous carrier of the variant.

Relevant genes and regions:

POU3F3, (exonic 1), NM_006236

This gene encodes a POU-domain containing protein that functions as a transcription factor. The encoded protein recognizes an octamer sequence in the DNA of target genes. This protein may play a role in development of the nervous system

Calculated LOF score: 3
Decipher HI score: 23.83

HI disease: Snijders Blok-Fisher Syndrome (OMIM: #618604)
nijders Blok-Fisher syndrome (SNIBFIS) is a neurodevelopmental disorder characterized by global developmental delay, hypotonia, variable impaired intellectual development, and specifically impaired speech and language acquisition. Patients achieve independent ambulation and most have mildly to moderately impaired cognition with autistic features, although a few may develop seizures and have a more severe phenotype. Dysmorphic features include abnormal, cupped, or prominent ears and ocular anomalies. Mutations usually occur de novo, although 1 family with autosomal dominant inheritance has been reported (summary by Snijders Blok et al., 2019).

The gene POU3F3 is found to be Haploinsufficient by Franklin.

Details of the variant

- Located on chromosome 2, chr2: 105,470,669-105,473,774

- Contains protein-coding or other known functionally important elements. CNV overlaps with 1 protein-coding genes, 0 non coding genes, and 0 ClinGen dosage sensitivity regions.
- Overlap with established HI/LOF-sensitive genes or genomic regions.
  2A-2E (+1.00)
- Complete overlap of an established HI/LOF-sensitive gene/genomic region
  - Fully covered Haploinsufficient genes: POU3F3 : ClinGen Haploinsufficiency Score: No score, number of reported pathogenic null variants 20 , gnomAD o/e upper score 0.429, gnomAD PLI score 0.88278, DECIPHER HI score 23.83% .
  - Fully covered Haploinsufficient regions: No regions
  - Partially covered Haploinsufficient non coding genes: No genes
  - Partially covered Haploinsufficient regions: No regions
- Number of protein-coding RefSeq genes wholly or partially included in the CNV region is between 0-243A (0.00). Number of overlapping coding genes (not from the same family) is 1.

6. Variants with uncertain significance

A variant of uncertain significance is a change in the DNA sequence of a gene that has an unknown effect on a person's health. There is usually not enough information about a variant of uncertain significance to know whether it increases a person's risk of developing the disease, that is, there is not enough information in the literature about the particular variant to draw a conclusion related to the clinical manifestation.

The following table lists variants of uncertain significance, heritability and their potential association with certain clinical conditions.

Variant of uncertain significance detected in the proband

Variant	Zygosity	Mode of inheritance	Associated conditions
CLPB:c.1717C>T (p.Arg573Cys) (rs186989806)	Heterozygote	Autosomal recessive, Autosomal dominant	3-METHYLGLUTACONIC ACIDURIA, TYPE VIIA (AD) Severe Congenital Neutropenia (AD) CLPB-Related Neurodevelopmental Delay, Seizures, And Neutropenia (AR, AD) 3-Methylglutaconic Aciduria With Cataracts, Neurologic Involvement And Neutropenia (AR)

G-Life
www.g-life.care
e-mail:contact@g-life.care

MT-ATP8:c.116C>T chrM-8481 C>T (p.Pro39Leu) (rs1603221521)	Heterozygote	Autosomal recessive, Mitochondrial	Leigh Syndrome (M) NARP syndrome (M) Isolated ATP Synthase Deficiency (AR)
CACNA1G:c.4987G>C (p.Val1663Leu) (rs1450618466)	Heterozygote	Autosomal dominant	Spinocerebellar ataxia type 42 (AD) CACNA1G-Related Developmental Disorder (Monoallelic) (AD) Autism Intellectual disability
ERF:c.205G>A (p.Val69Ile) (rs745819984)	Heterozygote	Autosomal dominant	Chitayat syndrome Familial lambdoid synostosis
KMT2C:c.7443-7_7443-6delTT (rs746018833)	Heterozygote	Autosomal dominant	Kleefstra syndrome 2 Syndromic intellectual disability Autism

Conclusion: Deletion of POU3F3 gene as well as gene-gene interaction of the several variants that are still with uncertain significance although leaning to pathogenic outcome especially mitochondrial variant MT-ATP8:c116C>T can explain most of the symptoms of the affected proband.

Zvezdana Moneva Ackovska, MSc.
Biochemistry and Cell Biology BSc.
Human Biology MSc.
Genomic Medicine and Healthcare PgDip.

Dr. Robert Janevski
Biochemistry and Physiology BSc.
Molecular Biology, MSc.
Genetics, PhD