GENETIC REPORT

Professional: Dr. Jair Tenorio – Mendel@adntro.com

PATIENT INFORMATION

Name: XXXXXXXXXXX Gender: XXXXXX Date of birth: XXXXXXXXX

SAMPLE INFORMATION

ID: XXXXXX DNA extracted from saliva Reason for test: XXXXXXXXXXXXXX

REPORT CONTENT

This report is divided into three sections:

1. Rare variants with clinical significance / variants of clinical relevance to the patient's phenotype
2. ACMG Actionable gene list
3. Carrier screening

Report date: XXXXXXXXXX

1. RARE VARIANTS WITH CLINICAL SIGNIFICANCE / VARIANTS OF CLINICAL RELEVANCE TO THE PATIENT'S PHENOTYPE

Description of the section

*In this section you will find the genetic variants identified in yourself than can be associated with **an increased risk of a given disease.** Please, read carefully the description of each variant and the explanation. You may also be requested to set a genetic counseling consultation in order to know more about these results.*

RELEVANT GERMINAL GENETIC VARIANTS

Genomic coordinates (hg38)	Gene	HGVS cDNA	HGVS proteína	Classification	VAF*	DP*	GQ*	AD*
5:128259693	*FBN2*	NM_001999.4:c.8501A>T	NP_001990.2:p.Tyr2834Phe	VUS	0.5185	27	99	13, 14

According to the ACMG guidelines [Richards et al., 2015] this variant has been classified as a variant of **unknown significance** (VUS)

**VAF=Variant allele frequency DP=Filtered depth GQ=Genotype Quality AD= Allelic Depth*

Description of the variants

A heterozygous variant have been found in FBN2 that causes a missense change at exon 65 of the gene. This variant has not been found in pseudocontrol population databases (gnomAD exomes, gnomAD genomes, Kaviar, Beacon, 1000G phase III) although the majority of the in silico pathogenic prediction tools does not suggest a pathogenic effect for this variant. 191 out of 306 non-VUS missense variants in gene FBN2 are benign = 62.4% which is more than threshold of 33.1%.

*Heterozygous variants in FBN2 have been **associated with early-onset macular degeneration** [MIM# 616118] through an autosomal dominant pattern of inheritance. Only a few cases have been reported [PMID: 24899048]. Therefore, according to the ACMG guidelines [Richards et al., 2015] this variant has been **classified as a variant of unknown significance** (VUS). It is recommended to the patient to receive appropriate genetic counseling.*

2. ACMG Actionable gene list

Description of the section

*This section contains the analysis of a list of genes from which the American College of Medical Genetics and Genomics **recommend to analyze** in any exome and genome screening, **due to the potential clinical actionability** in case of find a candidate variant [Biesecker et al., Genet Med, 2013; Miller et al., Genet Med, 2023].*

The overall responsibility of the ACMG group is to provide recommendations for a minimum list of gene-phenotype pairs for opportunistic screening to facilitate the identification and/or management of risks for selected genetic disorders through established interventions aimed at preventing or significantly reducing morbidity and mortality.

No candidate variants were identified in the ACMG actionable gene list

3. Carrier testing

Description of the section

*In this section, you will find the genetic variants from which you are a healthy carrier, which means that **these variants will probably not affect you but can affect your offspring**. For more information about autosomal recessive disease please visit: https://adntro.com/en/blog/genetic-curiosities/the-laws-of-mendel/*

RELEVANT GERMINAL GENETIC VARIANTS

Genomic coordinates (hg38)	Gene	HGVS cDNA	HGVS proteína	Clasification	VAF	DP	GQ	AD
7:117540270	*CFTR*	NM_000492.4:c.1040G>C	NP_000483.3:p.Arg347Pro	P	0.5263	19	99	9, 10
16:8847797	*PMM2*	NM_000303.3:c.713G>A	NP_000294.1:p.Arg238His	P	0.6	30	99	12,18

According to ACMG guidelines [Richards et al., 2015], these variants have been classified as **pathogenic** (P).

Description of the variant - *CFTR*

*A heterozygous variant have been found in exon 8 of gene CFTR. This variant causes an amino acid change at position 347. The frequency of this variant in pseudocontrol population databases is zero or extremely low if present (gnomAD exomes: 0.0000239), representing an estimation of the percentage of healthy carriers. ClinVar classifies this variant as **Pathogenic**, 4 stars (practice guideline, reviewed May '23, 19 submissions), citing 11 articles (32429104, 31523618, 25583415, 24440181, 23974870 and 6 more). UniProt Variants classifies this variant as Pathogenic, citing 1937486, **associated with Bronchiectasis with or without elevated sweat chloride 1 and congenital bilateral aplasia of vas deferens** from CFTR mutation. LOVD classifies this variant as Pathogenic. Alternative variant chr7:117540270 G⇒T (Arg347Asn) is classified Pathogenic by UniProt Variants (confirmed using the germline classifier).*

Hot-spot of length 17 amino-acids has 24 missense/in-frame variants (6 pathogenic variants, 17 uncertain variants and 1 benign variant), which qualifies as moderate pathogenic. UniProt protein CFTR_HUMAN domain 'ABC transmembrane type-1 1' has 426 missense/in-frame variants (104 pathogenic variants, 318 uncertain variants and 4 benign variants), which qualifies as supporting pathogenic. The majority of the in silico pathogenic predictor tools suggested a pathogenic effect for this variant.

Therefore, according to the ACMG guidelines [Richards et al., 2015], ***this variant is classified as pathogenic, and the individual is considered a heterozygous healthy carrier****. It is recommended to seek genetic counseling to learn about these findings.*

Description of the variant – *PMM2*

A heterozygous variant have been found in PMM2 that causes a missense change at exon 8 of the gene. This variant causes the amino acid change from an Arginine to a Histidine at position 238 of the protein. The frequency of this variant in pseudocontrol population databases is zero or extremely low if present (gnomAD exomes: 0.000574), representing an estimation of the percentage of healthy carriers. ClinVar classifies this variant as Uncertain Significance, 1 star (criteria provided, reviewed Apr '23, 12 submissions), citing 3 articles (34652821, 34132027 and 27231023), with 13 submissions (1 LP, 10 VUS and 2 LB). Alternative variants chr16:8847797 G⇒C (Arg238Ser) and chr16:8847796 C⇒G (Arg238Ile) are classified as pathogenic by UniProt Variants (confirmed using the germline classifier). Also, this variant is located in a hot-spot region of length 17 amino-acids has 21 missense/in-frame variants (11 pathogenic variants, 10 uncertain variants and no benign), which qualifies as strong pathogenic. Therefore, according to the ACMG guidelines [Richards et al., 2015], this variant is classified as likely pathogenic.

Pathogenic and likely pathogenic variants in development of congenital disorder of glycosylation, type Ia *[MIM# 212065] by an autosomal recessive pattern of inheritance. Due to the fact the variant have been identified in heterozygous state****, the individual is considered as a healthy carrier****. It is recommended to seek genetic counseling to learn about these findings.*

TECHNICAL INFORMATION OF THE TEST PERFORMED

Genomic technology used	Agilent SureSelect Human All ExonV6 (58M) - Illumina NovaSeq6000
Average coverage	30x
Bioinformatics analysis	Data quality control (QC), Alignment with reference genome (GRCh38), sequencing depth and coverage statistics, germline SNP/InDel, annotation and statistics, somatic SNP/InDel, annotation and statistics (only applies to tumor-normal paired samples).
Files analyzed	Raw data (fastq), BAM files, vcf and annotations

How sequencing was carried out

*A **whole genome sequencing** (WGS) analysis was performed, and all the genes associated with different conditions in healthy individuals were selected.*

What has been analyzed

*All genetic analysis was divided into three different sections, with custom in-house developed genes for each section detailed **in annexes I and II**.*

Validation of variants

***No variants susceptible to validation** due to low quality have been detected.*

Limitations of the analysis

The analysis performed does not allow us to rule out:

- *The presence of mutations in regions of **low coverage**, or in other genes potentially associated with pathology currently unknown.*
- *The presence of large **deletions or duplications**, also known as large **CNVs or rearrangements**.*
- *Variants in other candidate genes not included in the analysis.*

Methodology used for variant classification

*All variants were classified following the **ACMG variant classification guidelines** [Richards et a., 2015].*

Interpretation

*All results should be interpreted by an expert in clinical and human genetics. In addition, **these results should be interpreted in the context of the patient's family and it will be the professional specialist who will advise the family in the most appropriate way** and explain the implications of these results, and if appropriate, the need to extend these studies. The interpretation of the results may change over time due to the increase in scientific knowledge.*

The analysis has been supervised and reviewed by the undersigned

Signed

Dr. Jair Tenorio Castaño
Scientific Director BitGenetic Lab
Certified by the Community of Madrid - Spain
European Board of Medical Genetics (EMBG)

ANNEX I – List of genes analyzed for ACMG Actionable list v3.2

Please note that in brackets it is included the ID of the disease associated with each analyzed gene [PMID: 37347242].

ACTA2 (MIM 102620), ACTC1 (MIM 102540), ACVRL1 (MIM 601284), APC (MIM 611731), APOB (MIM 107730), ATP7B (MIM 606882), BAG3 (MIM 603883), BAG3 (MIM 603883), BMPR1A (MIM 601299), BRCA1 (MIM 113705), BRCA2 (MIM 600185), BTD (MIM 609019), CACNA1S (MIM 114208), CALM1 (MIM 114180), CALM1 (MIM 614916), CALM2 (MIM 114182), CALM3 (MIM 114183), CASQ2 (MIM 114251), COL3A1 (MIM 120180), DES (MIM 125660), DES (MIM 125660), DSC2 (MIM 125645), DSG2 (MIM 125671), DSP (MIM 125647), DSP (MIM 125647), ENG (MIM 131195), FBN1 (MIM 134797), FLNC (MIM 102565), FLNC (MIM 102565), GAA (MIM 606800), GLA (MIM 300644), HFE (MIM 613609), HNF1A (MIM 142410), KCNH2 (MIM 152427), KCNQ1 (MIM 607542), LDLR (MIM 606945), LMNA (MIM 150330), MAX (MIM 154950), MEN1 (MIM 613733), MLH1 (MIM 120436), MSH2 (MIM 609309), MSH6 (MIM 600678), MUTYH (MIM 604933), MYBPC3 (MIM 600958), MYH11 (MIM 160745), MYH7 (MIM 160760), MYH7 (MIM 160760), MYL2 (MIM 160781), MYL3 (MIM 160790), NF2 (MIM 607379), OTC (MIM 300461), PALB2 (MIM 610355), PCSK9 (MIM 607786), PKP2 (MIM 602861), PMS2 (MIM 600259), PRKAG2 (MIM 602743), PTEN (MIM 601728), RB1 (MIM 614041), RBM20 (MIM 613171), RET (MIM 164761), RET (MIM 164761), RET (MIM 164761), RPE65 (MIM 180069), RYR1 (MIM 180901), RYR2 (MIM 180902), SCN5A (MIM 600163), SCN5A (MIM 600163), SCN5A (MIM 600163), SDHAF2 (MIM 613019), SDHB (MIM 185470), SDHC (MIM 602413), SDHD (MIM 602690), SMAD3 (MIM 603109), SMAD4 (MIM 600993), SMAD4 (MIM 600993), STK11 (MIM 602216), TGFBR1 (MIM 190181), TGFBR2 (MIM 190182), TMEM127 (MIM 613403), TMEM43 (MIM 612048), TNNC1 (MIM 191040), TNNI3 (MIM 191044), TNNT2 (MIM 191045), TNNT2 (MIM 191045), TP53 (MIM 191170), TPM1 (MIM 191010), TRDN (MIM 603283), TRDN (MIM 603283), TSC1 (MIM 605284), TSC2 (MIM 191092), TTN (MIM 188840), TTR (MIM 176300), VHL (MIM 608537), WT1 (MIM 607102)

ANNEX II – List of genes analyzed for carrier screening

This list has been in-house developed following the ACMG-ACOG guidelines. Adherence of 176 conditions to ACOG and ACMG panel design criteria and the expanded carrier screening: What conditions should we screen for? [PMID: 36624552, 34906503].

ABCC8, ABCD1, ACADM, ACADS, ACADVL, ADA, AGA, AGL, AGXT, AIRE, ALDH3A2, ALDOB, ALG6, ALMS1, ALPL, AMT, ANO10, ARG1, ARSA, ASL, ASPA, ASS1, ATM, ATP7A, ATP7B, BBS1, BBS10, BBS12, BBS2, BCKDHA, BCKDHB, BCS1L, BLM, BTD, CAPN3, CBS, CEP290, CFTR, CHRNE, CLN3, CLN5, CLN6, CLN8, CLRN1, COL4A3, COL4A4, COL4A5, COL7A1, CPS1, CPT1A, CPT2, CTNS, CTSK, CYP11B1, CYP21A2, CYP27A1, DBT, DHCR7, DLD, DMD, DYNC2H1, DYSF, ELP1 (IKBKAP), ERCC2, ERCC6, ERCC8, EVC, EVC2, F8, F9, FAH, FANCA, FANCC, FKRP, FKTN, FMR1, FXN, G6PC, GAA, GALC, GALK1, GALT, GBA, GBE1, GCDH, GJB2, GLA, GLB1, GLDC, GNE, GNPTAB, GNPTG, GRHPR, GRIP1, HADHA, HBA1, HBA2, HBB, HEXA, HEXB, HGSNAT, HLCS, HMGCL, HOGA1, HPS1, HPS3, HSD17B4, HYLS1, IDS, IDUA, IL2RG, IVD, KCNJ11, L1CAM, LAMA2, LAMA3, LAMB3, LAMC2, LIPA, LRPPRC, MAN2B1, MCOLN1, MEFV, MESP2, MKS1, MLC1, MMAA, MMAB, MMACHC, MPI, MTM1, MUT, MYO7A, NAGA, NAGLU, NBN, NEB, NPC1, NPC2, NPHS1, NPHS2, NR0B1, OCA2, OPA3, OTC, PAH, PC, PCCA, PCCB, PCDH15, PEX1, PEX10, PEX12, PEX2, PEX6, PEX7, PKHD1, PMM2, POMGNT1, PPT1, PROP1, PTS, RMRP, RS1, RTEL1, SACS, SGCA, SGCB, SGCD, SGCG, SGSH, SLC12A6, SLC17A5, SLC22A5, SLC26A2, SLC26A4, SLC37A4, SMN1, SMPD1, STAR, TAT, TCIRG1, TGM1, TH, TMEM216, TNXB, TPP1, TTPA, TYR, USH1C, USH2A, VPS13B, XPA, XPC, ZFYVE26

CONTACT INFORMATION

www.adntro.com
www.bitgenetic.com
mendel@adntro.com