



Cerebrum DX

Genekor Medical S.A. | 52, Spaton Ave., 15344, Gerakas, Athens, Greece ,G.E.MI. nr: 0007856001000
email: info@genekor.com, www.genekor.com | Tel. (+30) 210 6032138 Fax. (+30) 210 6032148
Scientific Director: George Nasioulas PhD

SAMPLE INFORMATION

Name :	-	Date Received :	-
Medical ID :	-	Date of Report :	-
Date of Birth :	-	Req. Physician :	-
Location :	-	Barcode :	-
Material :	WHOLE PERIPHERAL BLOOD (EDTA)	Reason of referral:	Ataxia

CerebrumDX analysis by Next Generation Sequencing

Results associated with the reason of referral

PATHOGENIC VARIANT IDENTIFIED

Gene	Variant	Clinical Significance	Zygosity
ATM	NM_000051.3:c.5932G>T, p.(Glu1978*)	Pathogenic variant	Homozygous



Electronically Signed by - Georgia Pepe, MSc Molecular Biologist, AMKA:09038403029

- George Nasioulas, PhD Molecular Biologist, Scientific Director, AMKA:26025301255

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Results associated with carrier status for autosomal recessive disorder

Gene	Variant	Clinical Significance *	Zygosity
CYP27A1	NM_000784.4:c.410G>A, p.(Arg137Gln)	Pathogenic variant	Heterozygous

* The absence of a second pathogenic/likely pathogenic variant in genes associated with autosomal recessive disorders in the analyzed sample suggests that these findings are unlikely to be related to the patient's phenotype.



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Variants Details

ATM, Exon 40, NM_000051.3:c.5932G>T, p.(Glu1978*)

ClinGen

HPO

ClinVar

This is a single base substitution, replacing Glutamic acid with a termination codon - p.(Glu1978*). This results in the production of a truncated, non-functional protein. Loss-of-function variants in ATM are known to be pathogenic ([PMID: 23807571, 25614872](#)). This alteration has been reported in the international literature, in patients with Ataxia-Telangiectasia (A-T) and breast cancer ([PMID: 15880721, 16266405, 17124347, 18807267, 19691550, 25614872](#)). The mutation database ClinVar contains entries for this variant ([Variation ID: 127414](#)). Based on the classification criteria set by the ACMG and AMP ([PMID: 25741868](#)) this variant has been classified as pathogenic.

The *ATM* gene (11q22.2) encodes a protein kinase that plays a central role in the DNA damage response, particularly in the recognition and repair of DNA double-strand breaks, as well as in cell-cycle regulation and the maintenance of genomic stability. The *ATM* gene is associated with autosomal recessive ataxia-telangiectasia (A-T), a rare inherited multisystem neurodegenerative disorder. The classic form of the disease typically manifests in early childhood and is primarily characterized by progressive cerebellar ataxia and oculocutaneous telangiectasias. The neurological spectrum may also include dysarthria, oculomotor apraxia, extrapyramidal manifestations, axonal peripheral neuropathy, and cognitive impairment. In addition, immunological and endocrine abnormalities, increased sensitivity to ionizing radiation, and an increased predisposition to malignancies may occur. A characteristic laboratory finding is an elevated serum alpha-fetoprotein (AFP) level. The clinical spectrum of A-T also includes milder forms (variant A-T), which may present with later onset, slower disease progression, and absence of some of the characteristic features of classic A-T. The disorder is inherited in an autosomal recessive manner and is caused by biallelic pathogenic/likely pathogenic variants in the *ATM* gene. Heterozygous carriers of pathogenic/likely pathogenic variants in *ATM* have an increased predisposition to certain malignancies, particularly breast cancer in women and pancreatic cancer. For heterozygous carriers, individualized cancer risk assessment and appropriate clinical surveillance in accordance with current clinical management guidelines (NCCN Guidelines) are recommended. ([PMID: 39622612, 35431147, 32958592, 35175286, 35084436, 31406321, 29719442](#)).

CYP27A1, Exon 2, NM_000784.4:c.410G>A, p.(Arg137Gln)

ClinGen

HPO

ClinVar

This sequence change replaces Arginine with Glutamine at codon 137 of the CYP27A1 protein. The arginine residue is located in a domain of the protein that is known to be functionally important, and there is a small physiochemical difference between arginine and glutamine (Grantham Score: 43). This variant is present in population databases at a very low frequency (rs587778818, ExAC <0.01%) and has been reported in individuals with cerebrotendinous xanthomatosis ([PMID: 20558929, 28623566, 29269672](#)). This variant disrupts the p.Arg137 amino acid residue in CYP27A1. Other variant(s) that disrupt this residue



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have been observed in individuals with CYP27A1-related conditions ([PMID: 8006521, 17697869](#)), which suggests that this may be a clinically significant amino acid residue. This variant is described in the mutation database ClinVar ([Variation ID: 65929](#)). Algorithms developed to predict the effect of missense changes on protein structure and function suggest that this variant is likely to be damaging to the protein. Based on the classification criteria set by the ACMG and AMP ([PMID: 25741868](#)) this variant has been classified as pathogenic.

The *CYP27A1* gene (2q35) encodes the mitochondrial sterol 27-hydroxylase, an enzyme belonging to the cytochrome P450 superfamily that plays an important role in bile acid synthesis and cholesterol metabolism. Biallelic pathogenic variants in *CYP27A1* cause autosomal recessive cerebrotendinous xanthomatosis (CTX), a rare but treatable disorder of lipid metabolism. The clinical spectrum of CTX is broad, and disease manifestations may begin from infancy to adulthood. Characteristic features include juvenile-onset cataracts, tendon xanthomas, and progressive neurological dysfunction, including cerebellar ataxia, pyramidal signs, peripheral neuropathy, cognitive and/or psychiatric manifestations, and, in some patients, seizures. Early diagnosis is particularly important, as specific treatment may prevent or limit the progression of neurological manifestations ([PMID: 36312475, 35858741](#)).



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* Upon consent of the individual tested, findings in the 84 clinically significant genes recommended by the ACMG guidelines may also be reported.

Additional findings (secondary findings) in clinically significant genes (based on the ACMG SF v3.3 recommendations)

The findings reported below concern the 84 genes defined as clinically significant according to international guidelines (ACMG) and for which identified findings are recommended to be reported, regardless of the reason for referral.

NO PATHOGENIC/LIKELY PATHOGENIC VARIANTS WERE IDENTIFIED.

Table 2: Genes analyzed for additional/secondary findings in clinically significant genes.

ABCD1	ACVRL1	ACTA2	ACTC1	APC	APOB	ATP7B*	BAG3	BMPR1A
BRCA1	BRCA2	BTD*	CACNA1S	CALM1	CALM2	CALM3	CASQ2	COL3A1
CYP27A1*	DES	DSC2	DSG2	DSP	ENG	FBN1	FLNC	GAA*
GLA	HFE**	HNF1A	KCNH2	KCNQ1	LDLR	LMNA	MAX	MEN1
MLH1	MSH2	MSH6	MUTYH*	MYBPC3	MYH11	MYH7	MYL2	MYL3
NF2	OTC	PALB2	PCSK9	PMS2	PKP2	PLN	PRKAG2	PTEN
RB1	RBM20	RET	RPE65*	RYR1	RYR2	SCN5A	SDHAF2	SDHB
SDHC	SDHD	SMAD3	SMAD4	STK11	TGFBR1	TGFBR2	TMEM127	TMEM43
TNNC1	TNNI3	TNNT2	TP53	TPM1	TRDN	TSC1	TSC2	TTN
TTR	VHL	WT1						

* Only in the presence of two pathogenic/likely pathogenic variants in homozygosity or compound heterozygosity.

** Only in the case of homozygosity for the HFE p.Cys282Tyr variant.



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Methodology

Genomic DNA was extracted from the sample under investigation. The DNA was analyzed using a target enrichment method (exome capture) covering all coding exons and flanking intronic regions of the human genome (21,285 genes), utilizing the Twist Human Core Exome EF Multiplex Complete kit (Twist Bioscience). Sequencing of the enriched targets was performed on the MGI DNBSEQ-T7 platform. Bioinformatic analysis and variant interpretation were carried out using the Breakthrough Genomics bioinformatics platform VG PLUS ver3.0.8, aligned to the reference genome GRCh37/hg19.

The mean coverage depth was >100X, with 99.5% of target regions sequenced at a depth $\geq 20\times$. Large genomic rearrangements (CNVs) were evaluated in silico using validated algorithms of the Breakthrough Genomics bioinformatics platform VG PLUS ver3.0.8.

Based on the available clinical data and the reported phenotype, a phenotype-driven analysis approach was followed.

-Genes were prioritized according to the OMIM and Human Phenotype Ontology (HPO) databases, selecting those associated with the patient's phenotype.

- Variant classification was performed according to the ACMG/AMP guidelines (PMID: 25741868).

-Variants predicted to have a deleterious impact (frameshift, nonsense, missense, or splice-site changes), as well as potential de novo variants, were evaluated. Only variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) relevant to the phenotype were reported.

- All clinically significant variants were confirmed by Sanger sequencing, when technically feasible.

RECOMMENDATIONS

1)Genetic counseling is recommended to discuss the implications of this test and to interpret the results in the context of the patient's overall clinical evaluation and family history.

2) Reinterpretation of genome sequencing data is recommended on an annual basis and may be requested by the referring clinician and one should be cautious about that variant classification and/or interpretation may change over time if more information becomes available and identification of new variants associated with disease phenotype during the re-assessment.

3) Targeted testing of the identified pathogenic variant in the VCP gene is recommended in the extended family members if deemed necessary, for identifying those at risk for the clinical condition or reproductive planning. Consult with the referring physician to discuss about risk assessment and disease management measures.



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*Note:

- Every molecular test has an internal 0,5-1% chance of failure. This is due to rare molecular events and factors related to the preparation and analysis of the samples.
- Unless otherwise stated, the target regions enriched in this assay include all coding exons and 15 base pairs of the flanking intronic sequences on either side. Therefore, this method does not detect variants located deep within introns, in regulatory regions (enhancers or promoters), or in non-coding RNAs.
- The applied methodology achieves >99% sensitivity and specificity for the detection of single-nucleotide variants (SNVs) and small insertions/deletions (INDELs) as well as >90% sensitivity for the detection of large genomic rearrangements (CNVs) using validated computational algorithms.
- CNV calls generated from sequencing coverage data should be interpreted with caution and confirmed by an independent method. In addition, due to limitations in technology, certain regions may either not be covered or may be poorly covered, where variants cannot be confidently detected.
- This methodology does not detect structural alterations such as translocations, balanced rearrangements, or nucleotide repeat expansions in genes associated with these disorders. In addition, it cannot detect low-level mosaicism (coverage <25%).
- Next generation sequencing technologies and our bioinformatics analysis significantly reduce the contribution of pseudogene sequences or other highly-homologous sequences, these may still occasionally interfere with the technical ability of the assay to identify pathogenic variant alleles in both sequencing and deletion/duplication analyses.

GLOSSARY OF USED ABBREVIATIONS:

AD = autosomal dominant

AR = autosomal recessive

HEM = hemizygous

HET = heterozygous

HOM = homozygous

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



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Details about non-pathogenic variants

Each individual carries a large number of genetic variants, most of which are not associated with an increased risk of disease. Variants that, according to bioinformatic analysis and the ACMG/AMP classification criteria, are considered benign or likely benign are not reported, as they are not known to confer an increased risk of disease and do not alter medical management beyond what is indicated by the patient's family and personal history. Only variants that are relevant to the reported clinical phenotype and that have been classified, according to the ACMG/AMP guidelines (2015) and ClinGen specifications (2021), as pathogenic or likely pathogenic are included in this report. Variants of uncertain clinical significance (VUS) are reported only when found in genes potentially related to the patient's phenotype and predicted by most computational algorithms (e.g., REVEL, MetaLR) to have a damaging effect on protein function. VUS identified in autosomal recessive genes are not reported unless another variant (pathogenic, likely pathogenic, or VUS) is detected in the same gene. Furthermore, variants that are not related to the indication for testing are not reported. Secondary findings are not included unless the patient has opted to receive such information, in accordance with ACMG SF v3.1 (2022) (PMID: 35802134).



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Literature

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- 7. et al. ([PMID: 29269672](#))
- 8. et al. ([PMID: 23807571](#))
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- 13. et al. ([PMID: 16266405](#))
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- 16. et al. ([PMID: 32958592](#))
- 17. et al. ([PMID: 8006521](#))
- 18. et al. ([PMID: 25741868](#))
- 19. et al. ([PMID: 35175286](#))
- 20. et al. ([PMID: 39622612](#))
- 21. et al. ([PMID: 36312475](#))
- 22. et al. ([PMID: 25614872](#))
- 23. et al. ([PMID: 17697869](#))
- 24. et al. ([PMID: 20558929](#))
- 25. et al. ([PMID: 17124347](#))
- 26. et al. ([PMID: 35084436](#))
- 27. et al. ([PMID: 15880721](#))
- 28. et al. ([PMID: 28623566](#))



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