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Whole Exome Sequencing (SOLO)

Full Name / Ref No:		Order ID/Sample ID:	
Gender:		Sample Type:	
Date of Birth / Age:		Date of Sample Collection:	
Referring Clinician:		Date of Report:	
Test Requested:	**ExomeMax**		

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

Family history of adrenoleukodystrophy. She is suspected to be carrier with X- linked adrenoleukodystrophy and has been evaluated for pathogenic variations.

RESULTS

Variant of unknown significance was detected as heterozygous in the proband. This mutation explains the family history of the proband from the maternal side. Patient is ready for pre-PGTM and PGTM for this variant.

SNV(s)/INDELS

Gene# (Transcript)	Location	Variant	Zygosity	Disease (OMIM)	Inheritance	Classifications
***ABCD1* (+)** NM_000033.4	Exon 1	c.661G>C **p.Asp221His**	Heterozygous	Adrenoleukodystrophy (OMIM#300100)	X-linked recessive	Uncertain Significance

COPY NUMBER VARIANTS CNV(s)

No significant CNVs for the given clinical indications that warrants to be reported was detected.

VARIANT INTERPRETATION AND CLINICAL CORRELATION

VARIANT (*ABCD1* gene):

Variant description: A heterozygous missense variant in exon 1 of the ***ABCD1*** gene (**chrX:g.153725927G>C; Depth: 85x**) that results in the amino acid substitution of Histidine for Aspartic acid at codon 221 (**p.Asp221His**; **ENST00000218104.6**) was detected (Table). The p.Asp221His variant has not been reported in the 1000 genomes, gnomAD (v3.1), gnomAD (v2.1), topmed and our internal databases. The *in silico* predictions# of the variant are probably damaging by PolyPhen-2 and damaging by SIFT and LRT. The reference codon/base/region is conserved across species.

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OMIM phenotype: Adrenoleukodystrophy (OMIM#300100) is caused by mutations in the *ABCD1* gene (OMIM*300371).

Adrenoleukodystrophy is an X-linked disorder which is secondary to a mutation in the ABCD1 gene and results in the apparent defect in peroxisomal beta oxidation and the accumulation of the saturated very long chain fatty acids (VLCFA) in all tissues of the body. The manifestations of the disorder occur primarily in the adrenal cortex, the myelin of the central nervous system, and the Leydig cells of the testes. ABCD1 is an ATPase binding cassette protein in the same category of transporter proteins such as the CFTR and MDR proteins. Identification of X-ALD as a lipid-storage disease, as a defect in the capacity to degrade VLCFAs, and its characterization as a peroxisomal disorder was reviewed by [PMID: 9278636]. [PMID: 16380594] provided a clinical review of ALD.[PMID: 18842627].

Based on the above evidence[$], ***this ABCD1 variation is classified as a variant of uncertain significance and has to be carefully correlated with the clinical symptoms.***

ADDITIONAL INFORMATION

- No other SNV(s)/INDELS or CNV(s) that warrants to be reported were detected. All the genes covered in this assay have been screened for the given clinical indications. To view the coverage of all genes Click here. NGS test methodology details of this assay are given in the appendix.
- [$]Genetic test results are reported based on the recommendations of American College of Medical Genetics and Genomics (ACMG) [PMID: 25741868, 31690835, 32906214].
- With regard to ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing (PMID: 35802134; ACMG SF v3.1), we report significant pathogenic and/ or likely pathogenic variants in the recommended genes for the recommended phenotypes, only if informed consent is given by the patient.
- Please write an email to genetic.counseling@viafet.com in case you need assistance for genetic counselling. For any further technical queries please write an email to techsupport@viafet.com

RECOMMENDATIONS

- **The *ABCD1* gene has a pseudogene in the human genome. Validation of the variant by an alternate technique is recommended to rule out false positives.**
- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Genetic counselling is advised for interpretation on the consequences of the variant(s).
- If results obtained do not match the clinical findings, additional testing should be considered as per referring clinician's recommendations.
- The sensitivity of NGS assay to detect copy number variants (CNV) is 70-75%. We recommend discussing alternative testing methodology options with Viafet Tech Support (techsupport@viafet.com) as required. In case clinician is suspecting CNV as an important genetic etiology, alternate tests like microarray/ MLPA or qPCR may be considered after discussing with the Viafet TechSupport team.

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APPENDIX

TEST METHODOLOGY

Targeted gene sequencing: Selective capture and sequencing of the protein coding regions and clinically relevant in the genome is performed. Variants identified in the exonic regions and splice-site are generally actionable compared to variants that occur in non-coding regions. Targeted sequencing represents a cost-effective approach to detect variants present in multiple/large genes in an individual.

DNA was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean depth of >80-100X on Illumina sequencing platform. We follow the GATK best practices framework for identification of germline variants in the sample using Sentieon [Sentieon]. The sequences obtained are aligned to human reference genome (GRCh38) using BWA aligner [Sentieon, PMID:20080505] and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels [Sentieon]. Sentieon haplotype caller is then used to identify variants in the sample. The germline variants identified in the sample is deeply annotated using VariMAT pipeline. Gene annotation of the variants is performed using VEP program [PMID: 20562413] against the Ensembl release 104 human gene model [PMID: 34791404]. In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the ExomeDepth method [PMID: 22942019]. This algorithm detects CNVs based on comparison of the read-depths in the sample of interest with the matched aggregate reference dataset.

Clinically relevant mutations in both coding and non-coding regions are annotated using published variants in literature and a set of diseases databases : ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar [PMID: 26582918, 18842627, 28349240, 21520333, 19344873, 20106818]. Common variants are filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38), 1000 Japanese Genome, TOPMed (Freeze_8), Genome Asia, and our internal Indian population database (MedVarDb v4.0) [PMID: 26432245, 32461613, 11125122, 26292667, 33568819, 31802016]. Non-synonymous variants effect is calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants are used for interpretation and reporting.

All samples are processed at Viafet labs in Bengaluru, India. Viafet Labs Ltd - Sy. Nos. 94/1C and 94/2, Tower 1, Ground Floor, Veerasandra Village, Attibele Hobli, Electronic City Phase-1, Electronics City, Bangalore, Bangalore South, Karnataka, India, 560100 Viafet Inc- 348 Hatch Dr,Foster City, CA 94404 United States

Average sequencing depth (x)	Average on-target sequencing depth (x)	Percentage target base pairs covered		
		0x	5x	20x
215	94.66	0.44	99.32	97.98

Total data generated (Gb)	13.41
Total reads aligned (%)	99.98
Reads that passed alignment (%)	86.20
Data Q30 (%)	98.00

[$]The classification of the variants is done based on American College of Medical Genetics as described below [PMID:25741868].

Variant	A change in a gene. This could be disease causing (pathogenic) or not disease causing (benign).

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Pathogenic	A disease-causing variant in a gene which can explain the patient's symptoms has been detected. This usually means that a suspected disorder for which testing had been requested has been confirmed.
Likely Pathogenic	A variant which is very likely to contribute to the development of disease however, the scientific evidence is currently insufficient to prove this conclusively. Additional evidence is expected to confirm this assertion of pathogenicity.
Variant of Uncertain Significance	A variant has been detected, but it is difficult to classify it as either pathogenic (disease causing) or benign (non-disease causing) based on current available scientific evidence. Further testing of the patient or family members as recommended by your clinician may be needed. It is probable that their significance can be assessed only with time, subject to availability of scientific evidence.

#The transcript used for clinical reporting generally represents the canonical transcript (MANE Select), which is usually the longest coding transcript with strong/multiple supporting evidence. However, clinically relevant variants annotated in alternate complete coding transcripts could also be reported.

Variants annotated on incomplete and nonsense mediated decay transcripts will not be reported.

#The *in-silico* predictions are based on Variant Effect Predictor (v104), [SIFT version - 5.2.2; PolyPhen - 2.2.2; LRT version (November 2009); CADD (v1.6); Splice AI; dbNSFPv4.2] and MutationTaster2 predictions are based on NCBI/Ensembl 66 build (GRCh38 genomic coordinates are converted to hg19 using UCSC LiftOver and mapped to MT2).

Diseases databases used for annotation includes ClinVar (updated on 17042023), OMIM (updated on 01092023), HGMD (v2023.1), LOVD (Nov-18), DECIPHER (population CNV) and SwissVar.

LIMITATIONS

- Genetic testing is an important part of the diagnostic process. However, genetic tests may not always give a definitive answer. In some cases, testing may not identify a genetic variant even though one exists. This may be due to limitations in current medical knowledge or testing technology. Accurate interpretation of test results may require knowing the true biological relationships in a family. Failing to accurately state the biological relationships in {my/my child's} family may result in incorrect interpretation of results, incorrect diagnoses, and/or inconclusive test results.
- Test results are interpreted in the context of clinical findings, family history and other laboratory data. Only variants in genes potentially related to the proband's medical condition are reported. Rare polymorphisms may lead to false negative or positive results. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.
- Specific events like copy number variants, translocations, repeat expansions and chromosomal rearrangements may not be reliably detected with targeted sequencing. Variants in untranslated region, promoters and intronic variants are not assessed using this method.
- Genetic testing is highly accurate. Rarely, inaccurate results may occur for various reasons. These reasons include, but are not limited to: mislabeled samples, inaccurate reporting of clinical/medical information, rare technical errors or unusual circumstances such as bone marrow transplantation, blood transfusion; or the presence of change(s) in such a small percentage of cells that may not be detectable by the test (mosaicism).
- The variant population allele frequencies and in silico predictions for GRCh38 version of the Human genome is obtained after lifting over the coordinates from hg19 genome build. The existing population allele frequencies (1000Genome, gnomAD-Exome) are currently available for hg19 genome version only. This might result in some discrepancies in variant annotation due to the complex changes in some regions of the genome.
- It is assumed that the clinician ordering a genetic test is fully aware of these limitations and Viafet shall not be responsible in case any inappropriate panel/test methodology is selected.