

Clinical Exome Sequencing Analysis

Patient name	: Master. XXX	PIN	: XX
Gender/ Age	: Male/6 Years	Sample number	: XX
Referring clinician	: XX	Sample collection date	: XX
Hospital/Clinic	: XX	Sample receipt date	: XX
Specimen	: Peripheral Blood	Report date	: XX

Clinical history

Proband, Master XXX is the fourth son born to a consanguineous couple. He is presented with features of ataxia, telangiectasia in the ocular region and recurrent sinopulmonary infections. Proband's elder sibling (female) had similar history of ataxia and recurrent sinopulmonary infection. She was diagnosed with Leukemia and succumbed to the illness. Master XXX is suspected to be affected with Ataxia-Telangiectasia and has been evaluated for pathogenic variations.

Results

Likely pathogenic variant causative of the reported phenotype was detected

List of significant variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
ATM (+)	Exon 58	c.8579C>A (p.Ser2860Tyr)	Homozygous	Ataxia-telangiectasia (OMIM#208900)	Likely Pathogenic	Autosomal recessive

Additional carrier variant identified:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
<i>HBB</i> (-)	Intron 1	c.92+5G>C 5' Splice site	Heterozygous	Beta thalassemia (OMIM#613985)	Likely pathogenic	Autosomal recessive

*Genetic test results are based on the recommendation of American college of Medical Genetics [1].
No other variant that warrants to be reported for the given clinical indication was identified.

Interpretation

ATM: c.8579C>A

Variant summary: A homozygous missense variation in exon 58 of the *ATM* gene (chr11:g.108345903C>A, NM_000051.4, Depth: 153x) that results in the amino acid substitution of Tyrosine for Serine at codon 2860 (p.Ser2860Tyr) was detected.

Population frequency: The variant has not been reported in gnomAD database and 1000 genomes database.

Literature evidence: This *ATM* gene has been previously reported in 6 cases for Classical Ataxia-Telangiectasia associated with Childhood-Onset Movement Disorders [3].

In-silico predictions: The *in-silico* predictions of the variant are damaging by PolyPhen-2 (HumDiv), SIFT, LRT and MutationTaster2. The reference codon is conserved across mammals in PhyloP tool and GERP++ tool.

OMIM phenotype: Ataxia-telangiectasia (OMIM#208900) is caused by homozygous or compound heterozygous mutation in the *ATM* gene (OMIM*607585). This disease follows autosomal recessive pattern of inheritance. This disorder is characterized by cerebellar ataxia, telangiectases, immune defects, and a predisposition to malignancy [2].

Variant classification: Based on the evidence, this variant has been classified as likely pathogenic.

HBB: c.92+5G>C

Variant summary: A heterozygous 5' splice site variation in intron 1 of the *HBB* gene (chr11:g.5226925 C>G, NM_000518.5, Depth:212x) that affects the invariant GT donor splice site of exon 1 (c.92+5G>C) was detected.

Population frequency: This variant has a minor allele frequency of 0.023% in gnomAD database and has minor allele frequency of 0.119% in 1000 genomes database.

Literature evidence: This variant has been classified as pathogenic in ClinVar database [4].

In-silico predictions: The reference codon is conserved across mammals in PhyloP and GERP++ tool.

OMIM phenotype: Beta thalassemia (OMIM#613985) is caused by homozygous or compound heterozygous mutation in the *HBB* gene (OMIM*141900). This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, the variant is classified as likely pathogenic. Master XXX is a carrier for this likely pathogenic variant.

Recommendations

- The *HBB* gene has pseudogene in the human genome. Validation of the variant(s) by Sanger sequencing is strongly 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 recommended.

Methodology

DNA extracted from the blood, was used to perform targeted gene capture using a custom capture kit. The targeted libraries were sequenced to a targeted depth of 80 to 100X using GenoLab M sequencing platform. This kit has deep exonic coverage of all the coding regions including the difficult to cover regions. The sequences obtained are aligned to human reference genome (GRCh38.p13) using Sentieon aligner and analyzed using Sentieon for removing duplicates, recalibration and re-alignment of indels. Sentieon haplotype caller has been used to call the variants. Detected variants were annotated and filtered using the VarSeq software with the workflow implementing the ACMG guidelines for variant classification. The variants were annotated using 1000 genomes (V2), gnomAD (v3.1.2,2.1.1), ClinVar, OMIM, dbSNP, NCBI RefSeq Genes. *In-silico* predictions of the variant was carried out using VS-SIFT, VS-PolyPhen2, PhyloP, GERP++, GeneSplicer, MaxEntScan, NNSplice, PWM Splice Predictor. Only non-synonymous and splice site variants found in the coding regions were used for clinical interpretation. Silent variations that do not result in any change in amino acid in the coding region are not reported.

Sequence data attributes

Total reads generated	8.28 Gb
Data ≥ Q30	93.37%

Genetic test results are reported based on the recommendations of American College of Medical Genetics [1], as described below:

Classification	Interpretation
Pathogenic	A disease-causing variation in a gene which can explain the patients' 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.

Disclaimer

- The classification of variants of unknown significance can change over time. Anderson Diagnostics and Labs cannot be held responsible for it.
- Intronic variants, UTR, Promoter region variants and CNV are not assessed using this assay.
- Certain genes may not be covered completely, and few mutations could be missed. Variants not detected by this assay may impact the phenotype.
- The variations have not been validated by Sanger sequencing.
- The above findings and result interpretation was done based on the clinical indication provided at the time of reporting.
- It is also possible that a pathogenic variant is present in a gene that was not selected for analysis and/or interpretation in cases where insufficient phenotypic information is available.
- Genes with pseudogenes, paralog genes and genes with low complexity may have decreased sensitivity and specificity of variant detection and interpretation due to inability of the data and analysis tools to unambiguously determine the origin of the sequence data in such regions.
- Incidental or secondary findings that meet the ACMG guidelines can be given upon request [5].

References

1. Richards, S, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine: official journal of the American College of Medical Genetics. 17.5 (2015): 405-424.
2. Amberger J, Bocchini CA, Scott AF, Hamosh A. McKusick's Online Mendelian Inheritance in Man (OMIM). Nucleic Acids Res. 2009 Jan;37(Database issue):D793-6. doi: 10.1093/nar/gkn665. Epub 2008 Oct 8.
3. Blanchard-Rohner G, et al. Childhood-Onset Movement Disorders Can Mask a Primary Immunodeficiency: 6 Cases of Classical Ataxia-Telangiectasia and Variant Forms. Front Immunol. 2022 Jan 28;13:791522. doi: 10.3389/fimmu.2022.791522. PMID: 35154108; PMCID: PMC8831727.
4. <https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000015447.88>
5. Kalia S.S. et al., Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med., 19(2):249-255, 2017.

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