Whole Exome Sequencing Analysis

Patient name	:	**Master. XXX**	**PIN**	:	**XX**
Gender/ Age	:	**Male/ 5 Years**	**Sample number**	:	**XX**
Referring clinician	:	**XX**	**Sample collection date**	:	**XX**
Specimen	:	**Dried blood spot card**	**Sample receipt date**	:	**XX**
			Report date	:	**XX**

Clinical history

Proband, Master XXX is the first-born male child to fourth degree consanguineous parents. He was born full-term by LSCS and presented with clinical features of skin lesions at birth, microcephaly, and corneal clouding. He had multiple bullae over both elbows, knees, oral cavity, and dorsum of feet, mild scarring with milia present over chest post-inflammatory hypopigmentation, normal fingernails, and total dystrophy of the right great toe. Skin immunopathology test revealed dystrophic epidermolysis bullosa. He has mitten hands and feet and an absence of speech at 6 years of age. His mother has a history of one spontaneous abortion at 12 weeks GA and his maternal grandparents are consanguineous. Proband, Master XXX has been evaluated for pathogenic variations.

Results

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
COL7A1 (-)	Exon 110	c.8053C>T **(p.Arg2685Ter)**	Homozygous	Epidermolysis bullosa dystrophica, localisata variant / Epidermolysis bullosa dystrophica inversa / Epidermolysis bullosa dystrophica, autosomal recessive **(OMIM#226600)**	**Pathogenic**	Autosomal Recessive

List of uncertain significant variant identified related to the phenotype:

Gene	Region	Variant*	Allele Status	Disease	Classification*	Inheritance pattern
CHST6 (-)	Exon 3	c.922C>T **(p.His308Tyr)**	Homozygous	Macular corneal dystrophy **(OMIM#217800)**	**Uncertain Significance**	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

***COL7A1*: c.8053C>T**

Variant summary: A homozygous stop gained variation in exon 110 of the *COL7A1* gene (chr3:g.48567184G>A, NM_000094.4, Depth: 108x) that results in the premature truncation of the protein at codon 2685 (p.Arg2685Ter) was detected. This variant is predicted to cause loss of normal protein function through protein truncation.

Population frequency: This variant has minor allele frequency of 0.0007% in gnomAD database and has not been reported in 1000 genomes database.

Clinical and Literature evidence: This variant has been previously classified as pathogenic in ClinVar database [3]. The observed variant has previously been reported in patients in affected with dystrophic epidermolysis bullosa in homozygous state [4].

***In-silico* prediction:** The *in-silico* predictions of the variant are deleterious by CADD. The reference codon is conserved across mammals in PhyloP tool.

OMIM phenotype: Epidermolysis bullosa dystrophica, localisata variant / Epidermolysis bullosa dystrophica inversa / Epidermolysis bullosa dystrophica, autosomal recessive (OMIM#226600) are caused by homozygous or compound heterozygous mutation in the *COL7A1* gene (OMIM*120120). It is a severe skin disorder beginning at birth and characterized by recurrent blistering at the level of the sublamina densa beneath the cutaneous basement membrane. This results in mutilating scarring and contractures of the hands, feet, and joints. Patients also developed strictures of the gastrointestinal tract from mucosal involvement, which can lead to poor nutrition. Affected individuals have an increased risk of developing aggressive squamous cell carcinoma. These diseases follow autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as a pathogenic variant. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the**

significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.

***CHST6*: c.922C>T**

Variant summary: A homozygous missense variation in exon 3 of the *CHST6* gene (chr16:g.75478907G>A, NM_021615.5, Depth: 129x) that results in the amino acid substitution of Tyrosine for Histidine at codon 308 (p.His308Tyr) was detected.

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

Literature evidence: This variant has previously been reported in patients in affected with macular corneal dystrophy in compound heterozygous state [5].

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

OMIM phenotype: Macular corneal dystrophy (OMIM#217800) is caused by homozygous or compound heterozygous mutations in the *CHST6* gene (OMIM*605294). This disorder is characterized by progressive punctate opacities in the cornea result in bilateral loss of vision, eventually necessitating corneal transplantation. MCD is classified into 2 subtypes, type I and type II, defined by the respective absence and presence of sulfated keratan sulfate in the patient serum, although both types have clinically indistinguishable phenotypes. This disease follows autosomal recessive pattern of inheritance [2].

Variant classification: Based on the evidence, this variant is classified as variant of uncertain significance. **In this view, clinical correlation and familial segregation analysis are strongly recommended to establish the significance of the finding. If the results do not correlate, additional testing may be considered based on the phenotype observed.**

Recommendations

- Sequencing the variant(s) in the parents and the other affected and unaffected members of the family is recommended to confirm the significance.
- Alternative test is strongly recommended to rule out the deletion/duplication.
- Genetic counselling is advised.

Methodology

DNA extracted from the dried blood spot card was used to perform whole exome using whole exome

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 DNAscope 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 (v3.1,2.1.1), 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	8.65 Gb
Data ≥ Q30	98.53 %

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 [6].

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. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000932076.9
4. van den Akker PC, et al. Long-term follow-up of patients with recessive dystrophic epidermolysis bullosa in the Netherlands: expansion of the mutation database and unusual phenotype-genotype correlations. J Dermatol Sci. 2009 Oct;56(1):9-18. doi: 10.1016/j.jdermsci.2009.06.015. Epub 2009 Aug 8. PMID: 19665875.
5. Park SH, et al. Molecular analysis of the *CHST6* gene in Korean patients with macular corneal dystrophy: Identification of three novel mutations. Mol Vis. 2015 Oct 26;21:1201-9. PMID: 26604660; PMCID: PMC4626780.
6. 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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