Identification of a novel start-loss mutation in the SLC29A3 gene by whole exome sequencing in an Iranian family with H syndrome

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Introduction

H syndrome, also known as histiocytosis-lymphadenopathy plus syndrome or PHID, is an autosomal recessive disorder caused by mutations in the SLC29A3 gene. SLC29A3 encodes the hENT3 protein, involved in nucleoside transport within lysosomes and mitochondria. Mutations in SLC29A3 lead to a range of clinical symptoms, including hyperpigmentation, hypertrichosis, hyperglycemia, hearing loss, hypogonadism, short stature, and organ involvement. This study aims to **elucidate the molecular basis of H syndrome in two affected individuals** from a single Iranian family by whole exome sequencing (WES) methodology[1,2,3].

Methods

Patients: Two siblings (16-year-old girl, 8-year-old boy) with symptoms were studied.
DNA Extraction: Blood samples collected from patients, parents, and sister. DNA extracted using Yekta Tajhiz kit, measured with NanoDrop.
WES: Agilent SureSelect probe set and Illumina NovaSeq platform used for sequencing (100X coverage).
Data Analysis: Alignment to hg19, duplicate reads identified (Picard), realignment (GATK), variant detection (GATK, Samtools), annotation (dbSNP, 1000 Genomes, ClinVar).
In-Silico Analysis: Tools (ACMG, CADD, Mutation Taster, Polyphen-2, SIFT, Panther, Fathmm, MUpro, I-Mutant) used for variant assessment.
Co-Segregation Study: PCR with specific primers, Sanger sequencing, Codon Code Aligner used for validation.
Protein Structure Prediction: I-TASSER generated 3D models, UCSF Chimera used for visualization.
Protein-Protein Interactions: STRING tool explored SLC29A3 interactions.
Conservation Analysis: Clustal Omega assessed SLC29A3 sequence conservation across species.

Results

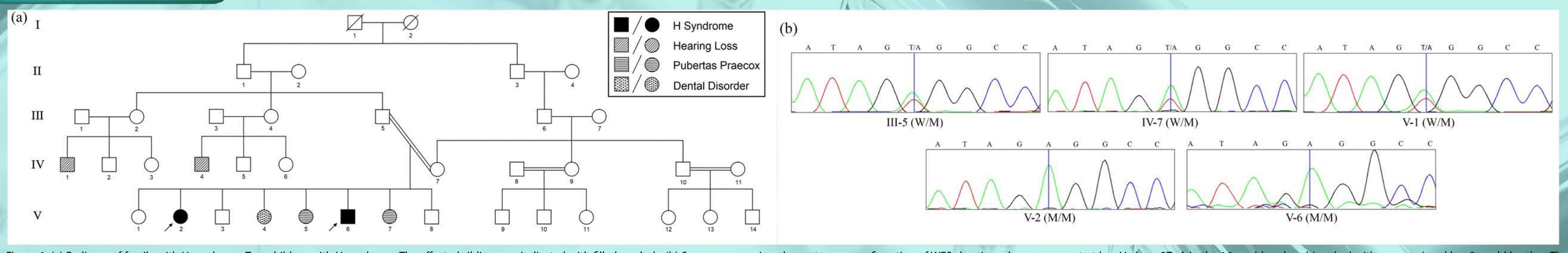

Figure 1. (a) Pedigree of family with H syndrome. Two children with H syndrome. The affected siblings are indicated with filled symbols. (b) Sanger sequencing chromatogram confirmation of WES showing a homozygous start loss Varian c.2T>A in the 16-yr old proband (marked with an arrow) and her 8-yr old brother. The mother, father and sister, who are healthy, are heterozygous for the mutation. (W/M: wild/mutant. M/M: mutant/mutant).

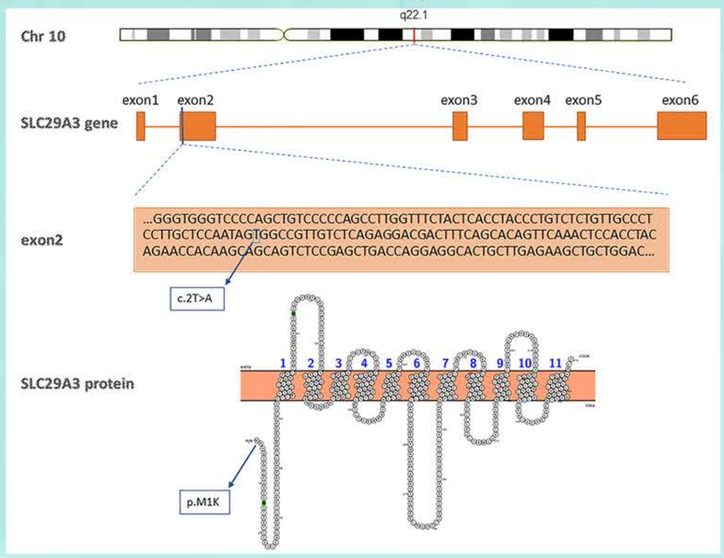

Figure 2. A graphical representation of the SLC29A3 gene structure and mutation position

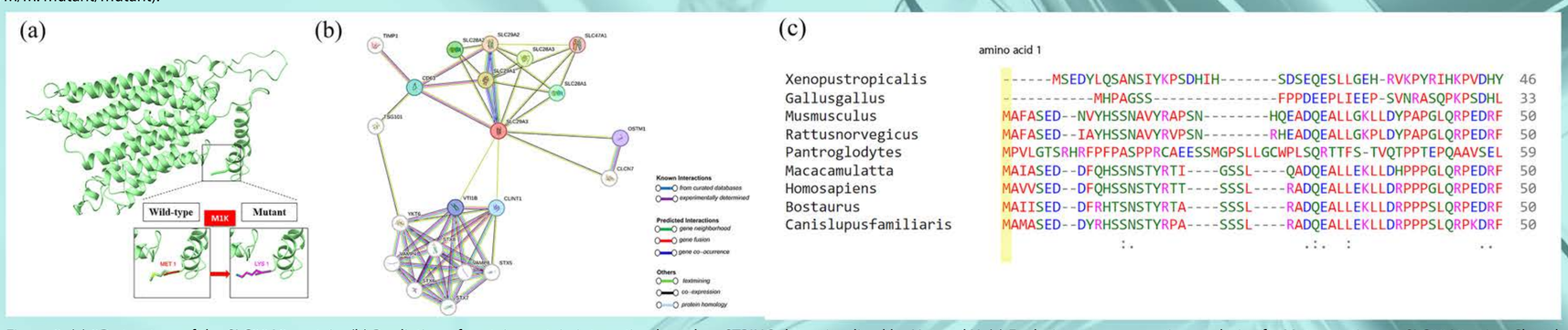

Figure 3. (a) 3D structure of the SLC29A3 protein. (b) Prediction of protein-protein interaction based on STRING data, visualized by NetworkX. (c) Evolutionary conservation analysis of p.Met mutation on SLC29A3 using Clustal Omega tool.

Table 1. Results of in silico analysis using computational predictors for the c.2T>A mutation in SLC29A3

Gene	Mutation	Protein	ACMG	CADD	Mutation Taster	Polyphon-2	panther	MUpro	I-Mutant 2	fathmm
SLC29A3	c.2T>A	p.M1K	Likely Pathogenic	23.2	Disease causing	BENIGN (score:0.261)	probably damaging (Pdel: 0.78)	Decrease (DDG:-2.23)	Decrease (-1.22)	Tolerated

Conclusion

In this study, we identified a novel homozygous missense mutation in exon 2, c.2T>A p.(Met1Lys), on SLC29A3 (NM_018344.6) that results in H syndrome. To validate our findings, we performed Sanger sequencing, which not only confirmed the presence of the identified mutation but also demonstrated its co-segregation with the mutation in the parents. Ultimately, the findings from the bioinformatics assessment were confirmed, establishing the mutation as pathogenic. these findings highlight the effectiveness of whole-exome sequencing as a valuable approach for identifying genes associated with genetic disorders in humansttt.

References

[1] Fraitag S, Emile JF. Cutaneous histiocytoses in children. Histopathology. 2022;80(1):196-215.
[2] Jacquot R, Jouret M, Valentin MG, Richard M, Jamilloux Y, Rousset F, et al. H syndrome treated with Tocilizumab: two case reports and literature review. Front Immunol. 2023;14.
[3] Hamsho S, Alaswad M, Sleiay M, Alhusseini A. Hyperglycemia with hypogonadism and growth hormone deficiency in a 17-year-old male with H syndrome: the first case report from Syria. BMC Endocr Disord. 2023;23(1):274.

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