Novel *EDA* mutations cause X-linked hypohidrotic ectodermal dysplasia: the first study from Venezuela

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Keywords: Ectodermal dysplasia, XLHED, *EDA*, Pathogenic variant,

Funding sources: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest:

The authors have no conflict of interest to declare.

Consent statement:

Written patient informed consent was obtained from the parents/guardians to publish photographs in scientific journals.

Data Availability:

The data related to this study are available from the corresponding authors upon reasonable request.

Ethics Statement:

None applicable.

Abstract

Hypohidrotic/anhidrotic ectodermal dysplasia (HED) is a rare genetic disorder affecting ectodermal tissues mainly including hair, teeth, sweat glands, skin and nails. It exhibits X-linked (XLHED) as well as autosomal dominant or recessive modes of inheritance. In the first study conducted from Venezuela, we analyzed two XLHED cases exhibiting classical clinical symptoms and identified a novel hemizygous *EDA* deletion (c.111delG) in one and a novel missense likely pathogenic variant (p.Gly192Glu) in the other. The current study adds to the growing repertoire of disease-causing *EDA* mutations with important implications for genetic screening in the affected families.

Hypohidrotic ectodermal dysplasia (HED) is characterized by developmental defects in ectodermal tissues and includes X-linked and autosomal recessive and dominant forms.[1] Of these, the X-linked HED (XLHED, OMIM 305100), caused due to mutations in *Ectodysplasin* (*EDA*, OMIM 300451), is the most common.[2] Here, we report the first clinical and molecular genetic analysis of HED from Venezuela; a study approved by Ethics Committee of the Department of Pediatrics, University of Los Andes (approval certificate number 19/0019, issued on August 10, 2019).

Informed consent was obtained from participants (from the parents/guardians in the case of minors). We used targeted exome sequencing including an ectodermal dysplasia-specific custom panel of genes[3]; sequencing libraries were generated using the Roche NimbleGen SeqCap Target Enrichment kit (Roche Diagnostic) and targeted sequencing was performed on the NextSeq550 (Illumina). Variants were prioritized using VariantStudio (Illumina);

after removing variants common with dbSNP (https://ncbi.nlm.nih.gov/SNP/) and gnomAD (https://gnomad.broadinstitute.org) databases as well as synonymous variants, only one potential disease-causing variant remained in both cases. The disease-causing potential of the variants were evaluated using VarSome (https://varsome.com), combined annotation dependent depletion (CADD; https://cadd.gs.washington.edu/) and MutationTaster (https://mutationtaster.org) databases. Sanger sequencing was performed to confirm the likely pathogenic variants.

Case 1: A 14-year-old boy presented with sparse eyebrows and eyelashes, scanty and thin hair, diffuse alopecia, dry skin, poor sweating, heat intolerance, periorbital hyperpigmentation, hypodontia and conical teeth (Figures 1a and b, IV:1). At the age of 4 years, he was hospitalized for cellulitis in the right buttock, bilateral epistaxis, and recurrent herpetic lesion. The proband's maternal grandmother (Figure 1b, II:4) showed scanty hair and hypodontia while the symptomatic maternal aunt (Figure 1b, III:6) exhibited heat intolerance. The maternal aunt's son (Figure 1b, IV:3) and maternal uncle-cousin exhibited similar phenotype (Figure 1b, III:10). We identified a novel *EDA* frameshift hemizygous deletion likely pathogenic variant c.111delG (NM_001399.5; p.Asn38ThrfsX19, NP_001390.1) in the proband and in heterozygous condition in the proband's symptomatic mother (Figures 1b, III:4 and 1c). The novel frameshift deletion, classified as 'likely pathogenic' (Table 1), is expected to result in a premature termination codon within exon 1, only 54 amino acids downstream of translation initiation codon (ATG) (Figure 1d). The mutated transcript is predicted to be degraded by nonsense-mediated decay (NMD)[4] possibly resulting in a loss of EDA protein expression.

Case 2: A 6-year-old male child presented with sparse scalp hair, eyebrows and eyelashes, poor sweating, heat intolerance and fever, periorbital hyperpigmentation and wrinkles, hypodontia, conical teeth, wide frontal region, excess skin folds around both eyes, bulbous nasal tip, and soft thin skin (Figures 2a and b, III:3), and a history of recurrent respiratory and skin infections, and bilateral epistaxis. Proband's elder male sibling (Figure 2b, III:2) presented with a similar phenotype. We identified a novel likely pathogenic *EDA* hemizygous missense variant c.575G>A (p.Gly192Glu, NP_001390.1) in the proband (Figures 2c and d) and affected male sibling (Figure 2d), and in heterozygous condition in the symptomatic mother (Figures 2b, II:4 and d) while the father was normal (Figures 2b, II:3 and d). Multiple sequence alignment (MSA) of EDA homologs, performed using T-coffee tools,[5] revealed significant conservation of the Gly192 residue (Figure 2e). The mechanism of pathogenicity of the p.Gly192Glu variant, classified as 'likely pathogenic' (Table 1), can be inferred by careful inspection of the MSA (Figure 2e) wherein the p.Gly192Glu can be seen to affect a conserved glycine in one of the Gly-X-Y triplets in the collagen-like domain of EDA (Figure 2e). These repeats, which characterize collagen proteins and are known to mediate their oligomerization, are necessary for the multimerization of EDA trimers. The p.Gly192Glu likely pathogenic variant is therefore predicted to alter the collagen-like domain, and consequently expected to adversely affect the multimerization of EDA trimers.

HED is clinically characterized by hypohidrosis and can lead to episodes of hyperthermia which may cause significant neurological damage and be potentially life-threatening[1]. The diminution or absence of several apocrine and eccrine glands[6] may result in chronic skin

issues, keratoconjunctivitis sicca, atrophic rhinitis, and recurrent airway infections, as described in case 2 as well as recurrent herpetic lesion in case 1. There is a greater risk of respiratory disease, due to the decrease in mucous glands in the respiratory tract, anatomic defects, atopy, and immune deficiencies[6].

Both the novel likely pathogenic variants were absent in the gnomAD and dbSNP databases. More than 364 disease causing pathogenic variants identified in *EDA* from HED-related cases (HGMD Professional 2021.4 version; https://www.hgmd.cf.ac.uk/), it is surprising that the first study from Venezuela has yielded novel likely pathogenic variants, underscoring the importance of screening in diverse populations. A molecular diagnosis is paramount as novel treatment in XLHED have been reported recently[7].

In conclusion, we present the first clinical and molecular genetic analysis of XLHED from the Venezuelan population. Our findings expand the genotype and clinical spectrum of this rare disorder besides highlighting the role of interdisciplinary molecular diagnosis and clinical evaluation for genetic counseling.

Acknowledgments

Authors gratefully acknowledge all patients and their family members who participated in this study. We are thankful to Dr. Ashwin B Dalal, Head, Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Hyderabad, India, for helping in generation of Table 1.

Funding information

This study did not receive any research grant from funding agencies or organizations.

Data availability statement:

The data related to this study are available from the corresponding authors upon reasonable request.

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Figure legends:

Figure 1. Clinical and molecular genetic analysis of XLHED (Case 1). Panel (a) shows proband with hypodontia and conical shaped teeth (top), sparse eyebrows and eyelashes with thin and scanty hair (bottom). Panel (b) shows the family pedigree; filled squares indicate affected males, pointed circles indicate female carriers, and arrow indicates the Proband. *, family members participated in the study for clinical and molecular testing; **, participated only for clinical evaluation. Panel (c) shows NGS based identification of a novel frameshift deletion likely pathogenic variant (left) and the corresponding electropherograms for confirmation through Sanger sequencing (right). Panel (d) shows depiction of deleterious effect of the novel *EDA* frameshift deletion c.111delG. The complete DNA and protein sequences corresponding to Exon 1 determined from normal (top) and mutant (bottom) samples are shown. The deleted nucleotide is underlined and shown in bold in the normal sequence. The premature termination codon generated due to the novel frameshift likely pathogenic variant is denoted in bold italic font in the mutant sequence.

Figure 2. Clinical and molecular genetic analysis of XLHED (Case 2). Panel (a) shows sparse scalp hair, eyebrows and eyelashes, and conical shaped teeth in the proband. Panel (b) shows the family pedigree; filled squares indicate affected males, pointed circles indicate female carriers (Proband's mother), and arrow indicates the proband. *, family members participated in the study for clinical and molecular testing. Panel (c) shows next generation sequencing based identification of the *EDA* novel missense likely pathogenic variant c.575G>A. Panel (d) shows sequence electropherograms for confirmation through Sanger sequencing. Panel (e) shows multiple sequence alignment (MSA) of human EDA protein with homologues from 15 species; sequence conservation of the region surrounding the mutated G192 residue (indicated by an arrow) is depicted and the amino acid position corresponding to the human protein sequence is also indicated. '*', identical; ':', conserved substitution; '.', Semi - conserved substitution.

Table 1. Evidences used for categorization of *EDA* pathogenic variants and their interpretation.

Case#	Pathogenic Variant	Variant classification as per ACMG guidelines	Evidences and their explanation as per ACMG guidelines		CADD Score	MutationTaster (Prediction/Score)	
			Evidence of pathogenicity	Explanation (Reproduced from VarSome)		Prediction	Score
Case 1	c.111delG (p.Asn38ThrfsTer19)	Likely pathogenic	PVS1 (Very strong)	Null variant (frame-shift) in gene EDA, predicted to cause NMD. Loss of function is a known mechanism of disease (gene	Not applicable	Disease causing (probably deleterious)	Not applicable

				has 155 reported pathogenic LOF variants). The exon contains 59 pathogenic variants. The truncated region contains 315 pathogenic variants.			
			PM2 (Supporting)	Variant not found in gnomAD genomes, good gnomAD genomes coverage = 24.6. Variant not found in gnomAD exomes, gnomAD exomes coverage is unavailable.			
Case 2	c.575G>A (p.Gly192Glu)	Likely pathogenic	PP3 (Strong)	MetaRNN = 0.989 is greater than 0.939 ⇒ strong pathogenic.	26.2	Disease causing (probably deleterious)	98 (Range from 0.0 to 215)
			PM1 (Moderate)	Hot-spot of length 17 amino-acids has 17 missense/in-frame variants (12 pathogenic variants, 5 uncertain variants and no benign), which qualifies as moderate pathogenic. UniProt protein EDA_HUMAN domain			

				'Collagen-like' has 54 missense/in-frame variants (42 pathogenic variants, 12 uncertain variants and no benign), which qualifies as moderate pathogenic. UniProt protein EDA_HUMAN region of interest 'Disordered' has 78 missense/in-frame variants (58 pathogenic variants, 17 uncertain variants and 3 benign variants), which qualifies as moderate pathogenic.			
			PM5 (Moderate)	Alternative variant chrX:70027904 G⇒A (Gly192Tyr) is classified Pathogenic by LOVD (confirmed using the germline classifier).			
			PM2 (Supporting)	Variant not found in gnomAD genomes, good gnomAD genomes coverage = 23.2. Variant not found in			

				gnomAD exomes, gnomAD exomes coverage is unavailable.			

Notes: Reference sequence *EDA* GenBank accession no. NM_001399.5. ACMG, American College of Medical Genetics and Genomics, PVS, pathogenic very strong; PM, pathogenic moderate; PP, pathogenic supporting; NMD, nonsense mediated decay; LOF, loss of function; gnomAD, Genome Aggregation Database; LOVD, Leiden Open Variation Database; CADD, Combined Annotation Dependent Depletion.

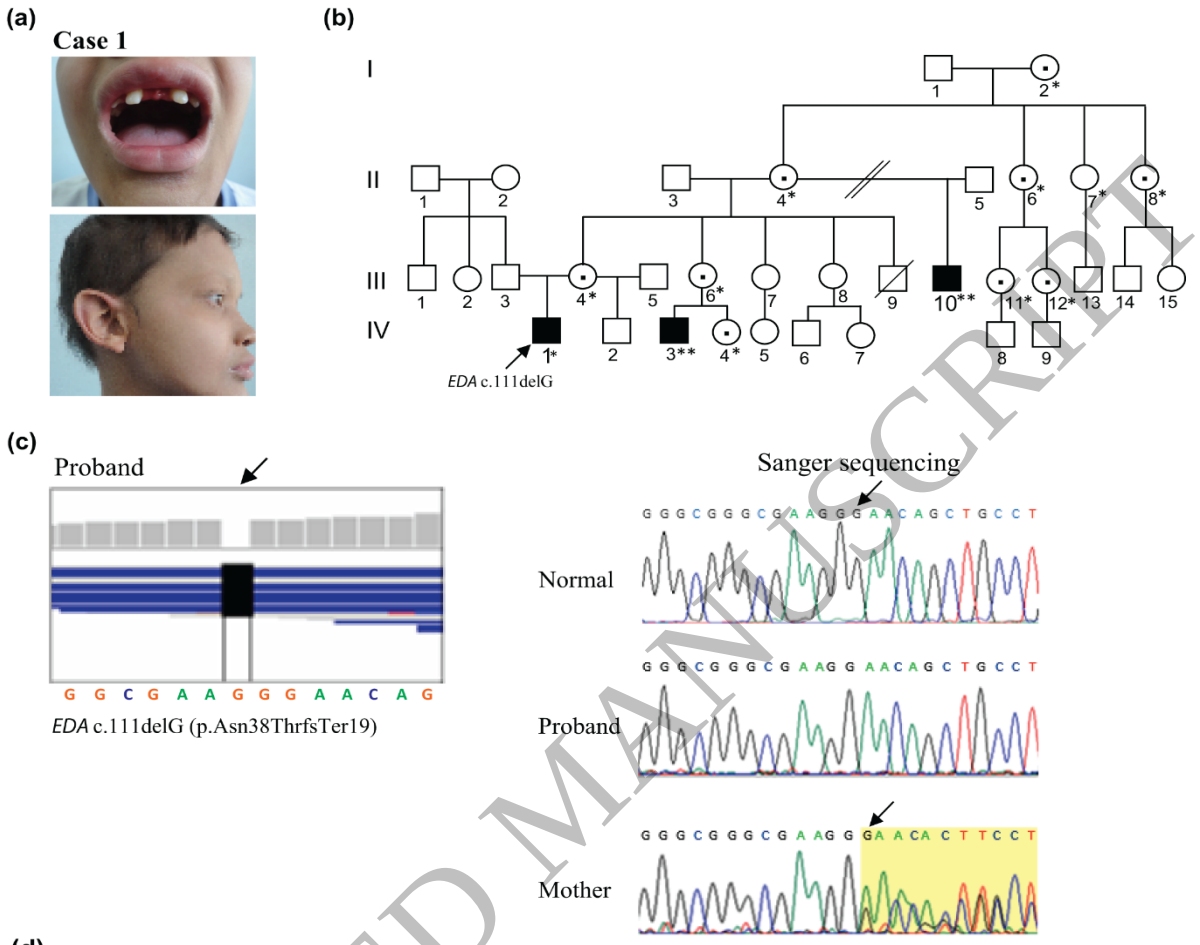

Figure 1
156x216 mm (x DPI)

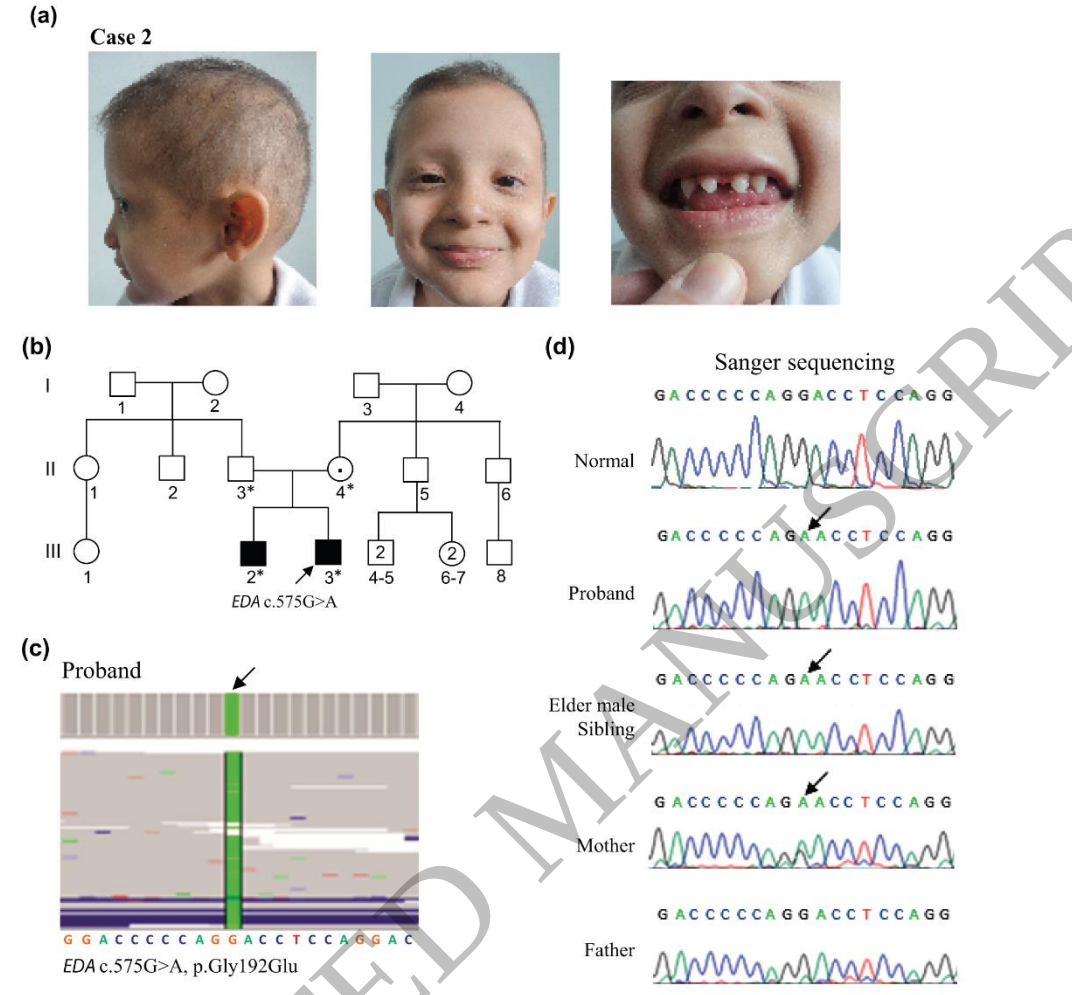

Figure 2
156x177 mm (x DPI)