Molecular Genetics and Metabolism Reports 29 (2021) 100796

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Case Report

A novel *EDA* variant causing X-linked hypohidrotic ectodermal dysplasia: Case report

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ARTICLE INFO

Keywords:
EDA
PGT-M
XLHED
Ectodermal dysplasia
Christ-Siemens-Touraine syndrome
X-linked recessive disorder

ABSTRACT

Hereditary ectodermal dysplasias are a complex group of inherited disorders characterised by abnormalities in two or more ectodermal derivatives (skin, nails, sweat glands, *etc.*). There are two main types of these disorders – hidrotic and hypohidrotic/anhidrotic ectodermal dysplasias. Hypohidrotic ectodermal dysplasia (HED) or Christ-Siemens-Touraine syndrome (OMIM: 305100) occurs in 1 out of 5000–10,000 births [19] and has an X-linked recessive inheritance pattern (X-linked hypohydrotic ectodermal dysplasia – XLHED) [2].

The main cause of XLHED is a broad range of pathogenic variants in the *EDA* gene (HGNC:3157, Xq12-13) which encodes the transmembrane protein ectodysplasin-A [4]. We report here the case of a patient with a novel inherited allelic variant in the *EDA* gene – NM_001399.5:c.337C>T (p.Gln113*) – in the heterozygous state. Targeted family member screening was conducted and other carriers of this *EDA* gene pathogenic variant were identified and phenotypically characterised. The patient subsequently underwent *in vitro* fertilisation with preimplantation genetic testing for monogenic diseases (PGT-M).

1. Case presentation

The patient, a 32-year-old Caucasian woman, was consulted by a geneticist at an infertility clinic based on suspicion of a possible ectodermal dysplasia. Clinical examination disclosed convex nails, brittle hair, partial one-sided ptosis and altered sweat gland distribution. She had only a few deciduous teeth in childhood that did not develop properly. Her adult teeth were pointed and many of them were missing due to decay. Despite the patient's inability to conceive, the underlying cause for her infertility had never been investigated.

Similar phenotypes were observed in the pedigree (Fig. 1). The patient's grandmother had pale fragile hair and sensitive skin. Her grandmother's sister was born with bright red skin and fine hair. The patient's mother had the same type of hair, spotted skin and scabrous nails. Other family members, including the patient's half-sister, demonstrated similar features to both the grandmother and the grandmother's sister. Distinct regions without hair were observed on the legs of the patient's niece. No male relatives displayed any of these characteristics.

According to the information available, ectodermal dysplasia was considered to be the underlying disease. All the observed clinical features closely resembled the pathology of the group of ectodermal dysplasia disorders. Specifically, nail abnormalities – convex, hypoplastic and thin fingernails and toenails – are one of the main clinical manifestations of ectodermal dysplasias. Consistent with the patient's brittle hair, another classic symptom of ectodermal dysplasias is thin, sparse, light-coloured and fragile scalp hair [11]. Eyelash ptosis, observed in our patient, is characteristic for 37% of all ectodermal dysplasia patients (Landau [5]). Furthermore, the patient's abnormal eccrine gland distribution indicated hypohidrotic ectodermal dysplasia (HED); HED is characterised by the partial or complete absence of sweat glands, causing anhidrosis or hypohidrosis. Additionally, the absence of

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https://doi.org/10.1016/j.ymgmr.2021.100796
Received 4 June 2021; Received in revised form 22 August 2021; Accepted 22 August 2021

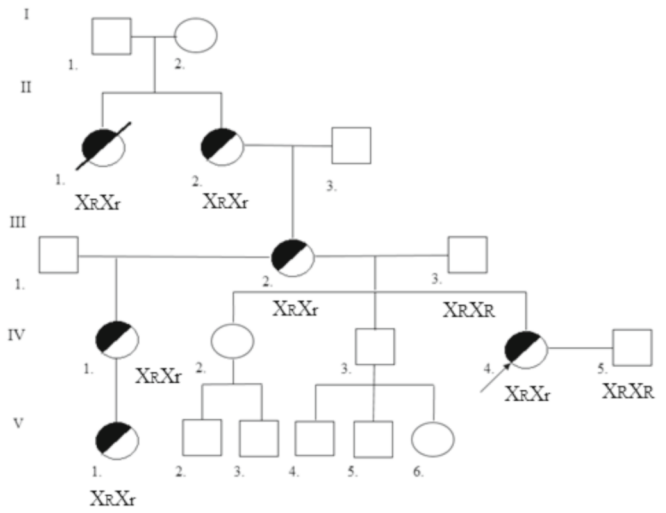

Fig. 1. Pedigree of XLHED family. The family members II-2, III-2, IV-1, IV-4 and V-1 displayed symptoms and their DNA was available for analysis. All five were confirmed to be carriers of the NM_001399.5:c.337C>T variant.
XRXR – individual with normal wildtype variants in both EDA alleles. XRXr – carrier of NM_001399.5:c.337C>T in one allele. An arrow in pedigree identifies described patient.

several teeth in the patient (anodontia or hypodontia) is also a common symptom of HED. Mosaic patchy distribution of body hair, caused by ectodermal dysfunction, was observed in a relative of the patient. Thin, pale and dry skin, noticed in several family members, is also a significant feature of HED [11]. Hence, the patient and her family were referred for targeted ectodermal dysplasia testing.

Based on the family history and clinical information, a single nucleotide variant in the *EDA* gene was suspected to be the cause of possible X-linked hypohidrotic ectodermal dysplasia (XLHED) in this family. Next-generation sequencing analysed all the exons and exon-intron junctions in the patient's *EDA* gene and compared them with the reference sequence NG_009809.2. An allelic variant NM_001399.5:c.337C>T (p.Gln113*) in exon 1 was found in the heterozygous state. It was located in the same region as that previously described for two other stop codons generated by a nucleotide substitution, namely NM_001399.5:c.329C>A (p.Ser110Ter) and NM_001399.5:c.347T>A (p.Leu116Ter). No known pathogenic changes were detected in the rest of the patient's *EDA* gene sequence. This novel allelic variant was interpreted as pathogenic according to the American College of Medical Genetics and Genomics guidelines [12]: 1) predicted as a null variant (nonsense) in the *EDA* gene for which loss-of-function is a known mechanism of disease (PVS1); 2) variant not found in gnomAD population database [Access 28.07.2021] (PM2); 3) *In silico* analysis (Mutation Taster, UMD-Predictor, SIFT, PolyPhen) indicated a possible adverse effect on the protein and the position was conserved (PP3); 4) Family sequencing demonstrated inheritance of NM_001399.5:c.337C>T in the heterozygous state in all five affected family members, including the patient's mother, patient's half-sister, patient's niece and patient's grandmother (PP1). The wildtype allele was confirmed in healthy family members (patient's father). Preimplantation genetic testing for monogenic diseases (PGT-M) was discussed with the patient and her partner and subsequently they consented to this procedure.

A broad spectrum of phenotypical variability was observed in affected members of the family, ranging from extreme changes in skin, pathological tooth count, hair defects and nail deformities to minor features such as sweat gland deficiency and hair follicle absence on certain skin regions being the only presenting clues. X chromosome inactivation status is the reason for this variability; however, their offspring bear the same risk. All family members with a characterised phenotype were female.

2. Materials and methods

2.1. Subjects and samples

Blood samples were obtained from the following family members: patient, patient's partner, patient's mother, patients's father, patient's half-sister, patient's niece and patient's grandmother. Genomic DNA was extracted using MagPurix Blood DNA Extraction Kit 200 (Zinexts, UK) according to the manufacturer's protocol.

2.2. Preimplantation genetic testing

An *in vitro* fertilisation cycle was performed according to standard controlled ovarian hyperstimulation protocols. Preimplantation genetic testing for aneuploidies (PGT-A) and monogenic diseases (PGT-M) was carried out as described elsewhere [9,18]. Selection of *EDA*-linked short tandem repeats (STR) flanking the *EDA* gene 2.5 Mb upstream and downstream was performed using the UCSC Genome Browser. Sequence-specific primers for each STR were designed and fluorescent PCR with capillary electrophoresis and fragment size analysis was conducted. Primer sequences and PCR conditions are available upon request. Family linkage analysis was performed for the determination of haplotypes.

2.3. Variant confirmation

To confirm the presence of *EDA* NM_001399.5:c.337C>T (p.Gln113*), Sanger sequencing was implemented using BigDye Terminator Kit v.3.1 (Thermo Fisher Scientific, USA) following the manufacturer's protocol. It was performed for the four affected family members, the patient's partner, the patient's father and all preimplantation embryos. PCR was performed with 10 nM primer mix (Table 1), nuclease-free water, 10× PCR buffer (20 mM $MgCl_2$), 10 mM dNTPs, betaine and 1 U/μl Taq polymerase. PCR conditions are available upon request. Further direct analysis was carried out using a standard Sanger sequencing protocol.

3. Results

Following the discovery of NM_001399.5(*EDA*):c.337C>T (p.Gln113*), confirmatory Sanger sequencing was carried out for all affected family members where a DNA sample was available, *i.e.* the patient's mother, patient's half-sister, patient's niece and patient's grandmother. The pathogenic variant was found in the heterozygous state in all four of these relatives. Sanger sequencing was also performed for the patient's partner and patients's father and in these two cases the wildtype variant was detected.

All preimplantation embryos underwent PGT-M to determine the inheritance of the mother's (carrier) and father's (non-carrier) haplotypes. Three embryos (A1, A2 and A3) were unaffected. One embryo (A5) was affected and carried the mother's allelic variant-related haplotype.

Sanger sequencing was performed for confirmation of the haplotyping results. The sequencing results corresponded with the STR haplotyping outcomes – embryos A1, A2 and A3 were declared as non-carriers, whereas A5 was acknowledged as affected.

PGT-A was also undertaken and unbalanced chromosomal

Table 1
Sequence-specific primers used for sequencing EDA NM_001399.5:c.337C>T (p.Gln113*).

Primer	Name	Fragment size, bases
GCTGACGTTGTGCTGCTACC	F	353
CCCTGGTCCTGCCCTCTAAAT	R	

F – forward, R – reverse.

aberrations were not found in embryos A1 and A3. These embryos were declared as suitable for transfer and A1 was selected. Pregnancy was achieved and the offspring was born after an uncomplicated pregnancy. A saliva sample was obtained and sequencing confirmed the wildtype variant in the newborn.

4. Discussion

The protein encoded by the *EDA* gene is involved in ectodysplasin signalling. It is important in the development of ectodermal structures such as nails, hair, teeth and sweat glands [11]. Currently, there are more than 100 pathogenic and likely pathogenic *EDA* allelic variants described in ClinVar. All variants are related to anhidrotic ectodermal dysplasias. We describe here the first case of the pathogenic *EDA* variant NM_001399.5:c.337C>T (p.Gln113*) and the successful preimplantation genetic testing of embryos in the affected family.

The *EDA* gene encodes ectodysplasin-A (EDA), a type II transmembrane protein with three functionally important regions: 1) cysteine-rich C-terminal tumor necrosis factor (TNF) homology domain; 2) collagen domain comprising 19 Gly-X_Y repeats and a gap of two amino acids between repeat 11 and 12; 3) large extracellular domain containing consensus furin protease recognition sequence [6].

EDA belongs to the TNF superfamily of ligands. These ligands participate in epithelial-mesenchymal interactions in the early embryonic stage and determine ectoderm-derived appendage formation. DNA sequence changes in the TNF homology domain are the most common. Such aberrations impede the binding of EDA to its receptor EDAR. Pathogenic variants in the collagen domain can impair multimerisation of the TNF homology domain. However, not all variants in *EDA* appear to be pathogenic – changes in the consensus furin recognition sequence prevent proteolytic degradation of EDA protein [13].

Different alternative splicing isoforms with 12 exons are known to exist. The two longest isoforms, EDA-A1 (NM_001399.5, 391 aa) and EDA-A2 (NM_001005609.2, 389 aa), contain the TNF homology domain. They vary by two amino acids. The TNF homology domain directly attaches to the extracellular part of EDAR – a death domain-containing member of the TNF receptor family. EDAR further binds to its adaptor, EDAR-associated death domain (EDARADD), and this interaction triggers the NF-κB pathway [20]. Allelic variants in both the *EDA* and *EDAR* genes cause tooth agenesis [1].

Pathogenic, novel and private pathogenic variants in *EDA* are common. Park et al. described 10 cases of XLHED with different phenotypes in the Republic of Korea. Nine of them were caused by private allelic variants in the *EDA* gene and three had been reported previously [8]. Phenotypes vary for female carriers with outcomes depending on inactivation of the normal X chromosome. For instance, of 54 genetically investigated female carriers in Denmark, 59% demonstrated no XLHED clinical features, 38% presented a reduced number of teeth (hypodontia) and, similar to the phenotype of our patient, 2% had a reduced number of sweat glands and hair follicles [7].

Distinct regions without hair were observed on the legs of the patient's niece, indicating somatic mosaicism with X chromosome inactivation. Female XLHED patients with identical pathogenic *EDA* variants can present phenotypic manifestations varying in expression which may result from preferential X chromosome inactivation. This is a random event in embryonic tissues. In such cases, any cell has a 50% probability of choosing to inactivate the mother's or father's X chromosome. Ultimately, every female is mosaic, with each cell expressing either the mother's or father's X chromosome genes [16].

The statistics on XLHED are inadequate due to exclusion of data on the size of cohorts in different regions [10,15]. In the literature, the prevalence of XLHED cases fluctuates from 1 to 287 per 100,000 [3,14,15]. Nguyen-Nielsen and colleagues estimated the prevalence of XLHED in Denmark to be around 1.6–22 cases per 100,000. The distribution of pathology may be even more ill-defined [7].

PGT-M with STR haplotyping was performed according to previously validated protocols [9,18]. The patient gave birth to a healthy boy who did not demonstrate any clinical symptoms linked to XLHED. A recent case report described the phenotype of a male patient with a novel *EDA* variant of unknown significance – NM_001399.5(*EDA*):c.1142G>C (p.Gly381Ala). He exhibited the following features: absent hair on the scalp and eyebrows; pale, thin, dry skin with eczematous dermatitis; linear wrinkled skin around the eyes and mouth [17].

Our case report is the first preimplantation diagnostics case for XLHED in Latvia. We anticipate that future PGT-M cases related to ectodermal dysplasias will benefit from our experience detailed here.

Ethics statement

Signed informed consent was obtained from the patient and her family.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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