RESEARCH ARTICLE

Genetic diagnosis for X-linked hypohidrotic ectodermal dysplasia family with a novel *Ectodysplasin A* gene mutation

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Aim: To make a gene diagnosis for a family with *Ectodysplasin A* (*EDA*) gene mutation as well as prenatal diagnosis, and report a novel *EDA* gene mutation.

Methods: All coding sequences and flanking sequences of *EDA* gene were analyzed by Sanger sequencing in the proband, and then, according to *EDA* gene mutation in the proband, the *EDA* gene sequencing was performed on the family members. Based on the results above, the pathogenic mutation in *EDA* gene was finally identified, which was used for making prenatal diagnosis.

Results: Sanger sequencing revealed c.302_303delCC [p.Pro101HisfsX11] mutation in *EDA* gene of the proband. This mutation induced *EDA* gene frame shift mutation which led to early termination of *EDA* gene translation because there was a termination codon TAA at the 11th codon behind the mutational site. Heterozygous deletion mutation (CC/--) at this locus was observed in the proband's mother and proband's grandmother, but the proband's aunt had no mutation at this locus. The analyses of amniotic fluid samples indicated negative sex-determining region on Y (*SRY*), and c.302_303delCC heterozygous deletion mutation.

Conclusion: We identified a pathogenetic mutation in *EDA* gene for the X-linked hypohidrotic ectodermal dysplasia family, made a prenatal diagnosis for the female carrier, and reported a novel *EDA* gene mutation.

KEYWORDS

Ectodysplasin A gene, gene diagnosis, hypohidrotic ectodermal dysplasia, novel mutation, Sanger sequencing

1 | INTRODUCTION

Congenital ectodermal dysplasia (CED) manifests as dysfunction of more systems/organs originating from the ectoderm such as skin and its associated structures, teeth as well as eyes, and occasionally affects the central nervous system. According to the condition of patients' sweat gland, CED includes hypohidrotic or anhidrotic ectodermal dysplasia (HED/AED, OMIM # 305100)¹ and hidrotic ectodermal dysplasia (HED, OMIM # 601375),² showing X-linked recessive inheritance, autosomal dominant inheritance and autosomal recessive inheritance. In clinical practice, X-linked hypohidrotic ectodermal dysplasia (XLHED) is relatively common, while autosomal dominant

HED and autosomal recessive HED are relatively rare. HED (hypohidrotic ectodermal dysplasia) incidence is about 1/100000, and infantile patients have obvious clinical symptoms including hypohidrosis or anhidrosis, high body temperature, and dry skin, especially for <1-year-old patients, high body temperature and pulmonary infection are life-threatening.³ HED severely affects human's health and brings heavy mental and economic burdens to both families and society. At present, there is no effective treatment for HED; thus, it is very important to avoid the birth of children with HED by effective prenatal diagnosis.

There are 3 subtypes of HED including X-linked (XLHED, OMIM 305100), autosomal dominant (ADHED, OMIM 129490, or

614940), and autosomal recessive HED (ARHED, OMIM 224900, or 614941).^{4,5} *Ectodysplasin A* (*EDA*; Gene ID: 1896) is the candidate gene for XLHED.^{4,6} The pathogenic genes of both ADHED and ARHED are *Ectodysplasin A receptor* (*EDAR*; Gene ID: 10913) and *EDAR-associated death domain* (*EDARADD*; Gene ID: 128178).⁴ The pathogenic gene of XLHED, *Ectodysplasin A* (*EDA*, OMIM 300451) gene located in Xq13.1 and containing 8 exons,⁷ encodes a tumor necrosis factor (TNF)-related ligand family type II transmembrane protein consisting of 391 amino acids. Due to its short coding region, Sanger sequencing was usually used in gene diagnosis of XLHED to obtain accurate and economical results, providing reliable experimental data for clinical diagnosis and genetic counseling. Therefore, in this study, we carried out gene diagnosis for the XLHED family and prenatal diagnosis for the proband's mother using Sanger sequencing.

2 | SUBJECTS AND METHODS

All study methods were approved by the Ethics Committee of Henan Provincial People's Hospital. All the subjects enrolled into the study gave written informed consent.

2.1 | Subjects

In July 2016, a family with suspected CED was transferred to the multidisciplinary consultation center of Henan Provincial people's Hospital from a basic hospital for definite diagnosis and genetic counseling. The proband was a 3-year-old boy. The boy had dry skin with little sweat and without eyelashes when he was born, and exhibited intermittent fever between 36.5°C and 38.3°C during his neonatal period. In summer, his body temperature was as high as 39°C, which could be reduced to about 37°C by physical cooling but did not respond to oral antipyretic. Physical examination indicated that the boy had no teeth, but his intelligence and physical development were normal. The proband's mother had less hair from the cradle, but she had no other abnormalities. No abnormality was found in the proband's grandmother and proband's aunt. The proband's uncle had the symptoms similar to the proband, and died 11 months after birth. Based on clinical symptoms and genetic pattern, the preliminary diagnosis of XLHED was made by the experts from stomatology, pediatrics, medical genetics institute, gynecology and obstetrics, and plastic surgery. The genealogical tree is shown in Figure 1. We subsequently carried out gene diagnosis for the *EDA* gene associated with XLHED, and prenatal diagnosis.

2.2 | Specimen collection and DNA extraction

Peripheral blood (3–5 mL) was collected and mixed with EDTA-K₂ for anticoagulation in family members. Amniotic membrane puncture was performed to obtain fetal exfoliated cells from the proband's mother under ultrasound guidance. Meanwhile, the peripheral blood samples (3–5 mL) was also collected and mixed with EDTA-K₂ for anticoagulation

FIGURE 1 Genealogical tree of the XLHED family. Notes: XLHED: X-linked hypohidrotic ectodermal dysplasia; □: Normal male; ⊙: Female XLHED carrier; ▤: Dead male XLHED patient; ○: Normal male; ■: Male XLHED patient and ◇: Fetus

during physical examination of new staffs of our hospital including 50 males and 50 females who had no XLHED family history and no blood relationship with this XLHED family. Total DNA of the peripheral blood and fetal exfoliated cells was extracted using Qiagen genomic DNA extraction kit (Qiagen, Germany). The DNA concentrations of normal control and samples were determined using NANODROP 2000 instrument (Thermo, USA) followed by storage at −20°C.

2.3 | Analysis of *EDA* gene mutation in the family by Sanger sequencing

Primers for 8 *EDA* exons were designed by Songon Biotech Co. Ltd (Shanghai, China) according to the *EDA* gene sequence, and detailed information about the sequences and primers could be found in the article.⁸ PCR amplification was performed on coding sequences and flanking sequences of *EDA* gene, and PCR amplification products were sequenced by Songon Biotech Co. Ltd (Shanghai, China). And then, according to the mutation locus of *EDA* gene in the proband, the sequences at the mutation locus in *EDA* genes from normal males of the family and 100 healthy individuals including 50 males and 50 females were analyzed by Sanger sequencing to exclude the possibility of gene polymorphism.

2.4 | Detection of maternal cell contamination in amniotic fluid

To make sure the accuracy of results, DNA (5 ng) from the proband's mother and amniotic fluid were amplified using PowerPlex® 21 HS genotyping system (Promega, USA) according to the manufacturer's instructions to analyze if there was the maternal cell contamination in amniotic fluid. Amplification was performed in a volume of 25 µL including 5 µL of PowerPlex® 21 5X Master Mix, 5 µL of PowerPlex® 21 5X Primer Pair Mix, 5 ng of Template DNA and H₂O. The cycling conditions were as follows: 96°C for 1 minute, 94°C for 10 seconds, 59°C for 1 minute, 72°C for 30 seconds, 30 cycles, and 60°C for 10 minutes.

2.5 | Fetal sex diagnosis

After establishment of normal male and female control groups, as well as blank control group, the amplification of fetal sex-determining region on

Y (*SRY*) gene was performed using 5'-GAATATTCCTCCGCTCTCCGGA-3' and 5'-GCTGGTGCTCCATTCTTG AG-3' primers in triplicate.⁹

2.6 | Analysis of *EDA* gene mutation in the fetus by Sanger sequencing

The sequences at the mutation locus in the fetal *EDA* genes were analyzed by Sanger sequencing, and then, PCR product was sequenced by Songon Biotech Co. Ltd (Shanghai, China) in triplicate.

3 | RESULTS

3.1 | Results of Sanger sequencing analysis

In the proband, c.302_303delCC [p.Pro101HisfsX11] mutation was detected in *EDA* gene. This mutation induced *EDA* gene frame shift mutation which led to early termination of *EDA* gene translation because there was a termination codon TAA at the 11th codon behind the mutational site. Heterozygous deletion mutation (CC/--) at this locus was observed in the proband's mother (II3) and proband's grandmother (II2); but in the proband's aunt (II4), grandfather and father, as well as 100 healthy individuals, no mutations (CC/CC) at this locus were found (Figure 2).

3.2 | Results detected by PowerPlex® 21 HS system

PowerPlex 21 HS system indicated that there was no maternal type in the fetal DNA typing, the fetus was female (sex chromosomes: XX), and the proband's mother as well as the fetus were in line with the maternal and daughter relationship. This suggested that there was no maternal cell contamination in the amniotic fluid specimens which might be used in future assays (Figure 3).

3.3 | Detection of *SRY* gene

Agarose electrophoresis did not show *SRY* gene bands amplified from the amniotic fluid samples, demonstrating that *SRY* gene was negative in the amniotic fluid samples, which was consistent with the results of fetal sex detected by PowerPlex 21 HS system.

3.4 | Detection of *EDA* gene in the fetal sample

There was c.302_303delCC heterozygous deletion mutation in the fetal *EDA* gene, the test was performed in triplicate, and these results were consistent in the 3 time tests (Figure 4). Based on above results, it was concluded that the fetus was a female XLHED carrier.

FIGURE 2 Results of Sanger sequencing. Notes: WT: Wild type; A, No mutation (CC/CC) in *EDA* gene of proband's aunt (II4), father (I1) and husband (II2), as well as 100 healthy individuals; B, c.302_303delCC heterozygous deletion mutation (CC/--) in *EDA* gene of the proband's mother (II3) and proband's grandmother (II2); and C, c.302_303delCC [p.Pro101HisfsX11] mutation in *EDA* gene of the patient (III1)

FIGURE 3 Detection of maternal components in the amniotic fluid using PowerPlex® 21 HS system. A, DNA typing of proband's mother (II3); B, Fetal DNA typing (III2)

FIGURE 4 c.302_303delCC heterozygous deletion mutation (CC/--) in the fetal *EDA* gene

4 | DISCUSSION

XLHED is the most common type of ectodermal dysplasia in clinical practice.¹ Its pathogenic gene is *EDA*. *EDA* mutation and its corresponding EDA-A1 protein abnormality lead to XLHED.¹⁰ The *EDA* gene was first cloned in 1996 and encodes a TNF-related ligand family type II trimer transmembrane protein which participates in intercellular signal communication during the process of ectodermal organ development.¹¹ To this day, about 300 species of *EDA* gene mutations have been reported, including *EDA* gene point mutation as well as splice site mutation, insertion or deletion, chromosomal changes, and so on.¹² In clinical practice, Sanger sequencing is usually used in gene test of XLHED because the length of *EDA* gene is short. With the development of gene detection tools and techniques, some scholars¹³ used the second generation high-throughput sequencing in *EDA* gene test of HED, and achieved good results. However, the

second-generation sequencing has higher requirements for hardware and software of laboratories, its subsequent analysis is more complex and difficult for developing countries, and its results still need to be verified by Sanger sequencing in order to ensure the accuracy of the results. Therefore, we recommend that Sanger sequencing is directly used in *EDA* gene test in order to make gene diagnosis and prenatal diagnosis more economical, effective, and accurate.

In this study, based on genetic pattern and the proband's clinical symptoms, the preliminary diagnosis of XLHED was made by multi-disciplinary consultation, so we used Sanger sequencing to carry out *EDA* gene test and prenatal diagnosis of XLHED. Sanger sequencing revealed c.302_303delCC [p.Pro101HisfsX11] mutation in the proband's (III1) *EDA* gene, c.302_303delCC heterozygous deletion mutation at this locus in the proband's mother (II3) and proband's grandmother (I2), and no mutation at this locus in the proband's

grandfather (I1) and father (II2), as well as 100 healthy individuals. These results of gene tests were in line with X-linkage recessive inheritance. This mutation found in this study has not been reported in the databases (<https://www.ncbi.nlm.nih.gov/clinvar>, <http://www.hgmd.cf.ac.uk/ac/index.php>).^{6,14-20} The Ectodysplasin-A protein encoded by *EDA* gene consists of 391 amino acids and contains 3 structural domains including N-terminal intracellular domain (1-41 amino acids), transmembrane domain (42-62 amino acids), and C-terminal extracellular domain (63-391 amino acids). In this family, the c.302_303delCC [p.Pro101HisfsX11] mutation in the proband's *EDA* gene induced *EDA* gene frame shift mutation which led to early termination of *EDA* gene translation because there was a termination codon TAA at the 11th codon behind the mutational site, so only 110 amino acids were translated and the 101-110 amino acids were also mismatched. Therefore, Ectodysplasin-A protein only contained intracellular domain, transmembrane domain, and partial extracellular domain, losing most of the C-terminal extracellular domain. This led to the loss of important cell signal transduction during the development of ectodermal organs, exhibiting ectodermal dysplasia. Combined with the similar mutation reported in previous literature,²¹ we believe that this mutation is the cause of this disease occurring in this family. In this study, tooth eruption was not seen in the proband on his admission to the clinic; and he had got high body temperature and eyelash had not been found since he was born, which may be related to the loss of excessive C-terminal extracellular domain of *EDA* gene. The proband's mother presented with mild clinical symptoms of XLHED, less hair from the cradle, which may be associated with random inactivation of X chromosome.²² However, the proband's grandmother, a XLHED carrier, had no significant clinical symptoms, suggesting that the XLHED carriers have clinical heterogeneity.

After identifying the pathogenic mutation of *EDA* gene in this family, we performed prenatal diagnosis for XLHED at the request of the proband's mother during mid-gestation. In prenatal diagnosis, we used the PowerPlex 21HS system to detect the maternal component in the amniotic fluid sample in order to avoid the influence of maternal contamination on the subsequent test results²³ and ensure the accuracy of the subsequent test results. Moreover, the PowerPlex 21HS system and SRY gene test could be validated mutually in the fetal sex, and the maternal and daughter relationship between the proband's mother and the fetal specimen could be confirmed by the PowerPlex 21HS system to ensure that the sample was exact. Prenatal diagnosis showed that the fetus was a female with c.302_303delCC heterozygous deletion mutation in the *EDA* gene, a XLHED carrier. Subsequently, we followed up the proband's mother. The proband's mother gave birth to a female baby with Apgar score of 10 by spontaneous delivery at 39W⁺⁶ gestational weeks. The baby had hair and eyelashes at birth without hyperthermia, which was consistent with our prenatal diagnosis. We will continue to follow up the baby until her adulthood.

In this study, we first made a gene diagnosis for the XLHED family and identified the pathogenic mutation in the proband. Based on this, we further made a prenatal diagnosis for the carrier of the family, which provided reliable genetic counseling for the XLHED family.

At the same time, we report a novel pathogenic *EDA* gene mutation, which provides useful information for *EDA* gene mutation database and the relationship between *EDA* gene mutation and XLHED phenotype.

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How to cite this article: Ma X, Lv X, Liu H-Y, et al. Genetic diagnosis for X-linked hypohidrotic ectodermal dysplasia family with a novel *Ectodysplasin A* gene mutation. *J Clin Lab Anal*. 2018;32:e22593. <https://doi.org/10.1002/jcla.22593>