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First report on an X-linked hypohidrotic ectodermal dysplasia family with X chromosome inversion: Breakpoint mapping reveals the pathogenic mechanism and preimplantation genetics diagnosis achieves an unaffected birth

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ABSTRACT

Background: To investigate the etiology of X-linked hypohidrotic ectodermal dysplasia (XLHED) in a family with an inversion of the X chromosome [inv(X)(p21q13)] and to achieve a healthy birth following preimplantation genetic diagnosis (PGD).

Methods: Next generation sequencing (NGS) and Sanger sequencing analysis were carried out to define the inversion breakpoint. Multiple displacement amplification, amplification of breakpoint junction fragments, Sanger sequencing of exon 1 of *ED1*, haplotyping of informative short tandem repeat markers and gender determination were performed for PGD.

Results: NGS data of the proband sample revealed that the size of the possible inverted fragment was over 42 Mb, spanning from position 26, 814, 206 to position 69, 231, 915 on the X chromosome. The breakpoints were confirmed by Sanger sequencing. A total of 5 blastocyst embryos underwent trophectoderm biopsy. Two embryos were diagnosed as carriers and three were unaffected. Two unaffected blastocysts were transferred and a singleton pregnancy was achieved. Following confirmation by prenatal diagnosis, a healthy baby was delivered.

Conclusions: This is the first report of an XLHED family with inv(X). *ED1* is disrupted by the X chromosome inversion in this XLHED family and embryos with the X chromosomal abnormality can be accurately identified by means of PGD.

1. Introduction

Ectodermal dysplasias (EDs) are a heterogeneous group of very rare genetic disorders characterized by certain shared structural and functional abnormalities in various tissues derived from the ectoderm, especially of eccrine sweat glands, teeth, and hair. X-linked hypohidrotic ectodermal dysplasia (XLHED, OMIM #305100), also known as anhidrotic ectodermal dysplasia (EDA) or Christ-Siemens-Touraine syndrome, is the most common form of EDs, with an estimated incidence of 1–287 per 100,000 births [1–3]. The XLHED affected patients, manifested as hypodontia, hypohidrosis, hypotrichosis and peculiar features, consist mainly of hemizygous male individuals, since most female heterozygous carriers are only mildly or moderately involved [4]. Hypohidrosis may lead to life-threatening or brain damaging hyperpyrexia, hypodontia may result in malnutrition, and the physical appearance may negatively impact the quality of life. Therefore, it is important to prevent pregnancies with affected male embryos.

The *ED1* gene, located at Xq12-q13 and encoding the ectodysplasin-A (EDA) protein, is responsible for XLHED through EDA-EDAR (EDA receptor)-EDARADD (EDAR-associated death domain) pathway, which is essential for the appropriate development and structure of ectoderm-derived organs [5]. Various mutations in *ED1* account for vast majority

Abbreviations: aCGH, array comparative genomic hybridisation; ADO, allele drop out; ALB, alkaline lysis buffer; ED, ectodermal dysplasia; EDA, anhidrotic ectodermal dysplasia; EDAR, EDA receptor; EDARADD, EDAR-associated death domain; FISH, fluorescence *in situ* hybridization; ICSI, intracytoplasmic sperm injection; ISCN, An International System for Human Cytogenetic Nomenclature; MDA, multiple displacement amplification; NGS, next generation sequencing; PBS, phosphate buffered saline; PGD, preimplantation genetic diagnosis; STR, short tandem repeat; TE, trophectoderm; 5′-UTR, the 5′-untranslated region; XLHED, X-linked hypohidrotic ectodermal dysplasia

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of XLHED cases [4]. In addition, chromosome structural abnormalities may also be involved. Sporadic cases have been reported about XLHED affected patients with balanced translocation between X chromosome and single or multiple autosomes [6–10], or with autosomal inversion containing an inserted fragment of the X chromosome [11]. All these cases show the same breakpoint on the X chromosome and deduce a disruption of the *ED1* gene.

Our research reported on an XLHED family accompanied by an inversion of the X chromosome [inv(X)(p21q13)]. Next generation sequencing (NGS) and preimplantation genetic diagnosis (PGD) were performed to identify the etiology and to achieve the delivery of a healthy child respectively.

2. Materials and methods

2.1. Subjects

A Chinese Han family with XLHED was investigated in this study. The Ethics Committee of Shenzhen Zhongshan Urology Hospital approved of this study. All the subjects participating in the study gave informed consent. The pedigree of the XLHED-affected family, comprising 13 members and spanning three generations, is shown in Fig. 1A. The proband (III:1, Fig. 1A) was a 19-year-old male who showed the typical triad of hypohidrosis, hypotrichosis and hypodontia (Fig. 1B), while the 4 female carriers (II:2, II:5, III:2, III:4, Fig. 1A) were all normal. Blood samples were obtained from 9 members of this family including 4 males II:1, II:4, III:1, III:3 and 5 females II:2, II:3, II:5, III:2, III:4 (Fig. 1A).

2.2. Cytogenetic studies

Karyotype analysis was carried out on at least 10 G-banded metaphases obtained from lymphocyte cultures of each specimen at the 320–340 band resolution level. All the chromosomal abnormalities were reported according to the International System for Human Cytogenetic Nomenclature (ISCN) 2009.

2.3. Molecular studies

Genomic DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini kit (Qiagen, Germany) according to the manufacturer's instruction.

NGS was performed on genomic DNA obtained from the proband. Whole genome sequencing library was prepared by the TruSeq Nano DNA kit (Illumina, USA) following the manufacturer's protocol, and was then sequenced on HiSeq X Ten System (Illumina, USA) with paired-end 150 base pair (bp) reads. Bioinformatics analysis of whole genomic sequencing data was based on Isaac Aligner and Fast QC, as well as was aligned to reference human genome hg19 from UCSC.

Sanger sequencing analysis of collected genomic DNA samples was done for both junctional fragments at the inversion breakpoint region delimited by NGS on derivative and normal X chromosome and for the 8 coding exons of *ED1* (NG_009809.1) including flanking intronic regions.

A panel of 15 short tandem repeats (STRs), located on the neighboring regions of *ED1* and on terminal regions of the X chromosome, were analyzed on genomic DNA from II:4, II:5, III:1 and III:4 in order to select informative STR markers for subsequent PGD. An STR marker was considered to be fully informative to determine segregation if II:5 was heterozygote for that marker and both alleles were different from II:4.

2.4. Preimplantation genetic diagnosis

For female carrier (II:5), the procedures of ovarian stimulation, oocyte retrieval, intracytoplasmic sperm injection (ICSI) and embryos culture were carried out step by step. As the day on which the ICSI was performed was defined as day0, biopsies were performed on the trophectoderm (TE) cells of blastocysts at day 5 or 6, depending on embryos development prior to cryopreservation.

After washed 3 times in 3 fresh phosphate buffered saline (PBS) droplets, the biopsied TE cells were lysed in 2.5 μl alkaline lysis buffer (ALB) by incubating at 65 °C for 10 min before the multiple displacement amplification (MDA) reaction, which was carried out using the REPLI-g Mini Kit (Qiagen, Germany).

Junctional fragments at the inversion breakpoint region, exon 1 of *ED1*, informative STR markers and *Amelogenin* for gender determination were amplified separately in singleplex PCR reaction with 2 μl of the MDA product. PCR products were analyzed by Sanger sequencing or fragment analysis as mentioned in the “molecular studies” section.

Unaffected blastocysts were kept frozen to be available for future transfer. With consent of the parents, all affected blastocysts were thawed and reanalyzed to confirm the TE cells diagnosis.

2.5. Prenatal diagnosis

Prenatal diagnosis including karyotype analysis and *ED1* genetic diagnosis was performed at 17 weeks of pregnancy following an amniocentesis.

3. Results

3.1. Cytogenetic studies

Karyotype analysis of peripheral blood samples showed that II:2, II:5, III:1, III:2 and III:4 had a balanced pericentric inversion of the X chromosome [inv(X)(p21q13)] (Fig. 2A, B).

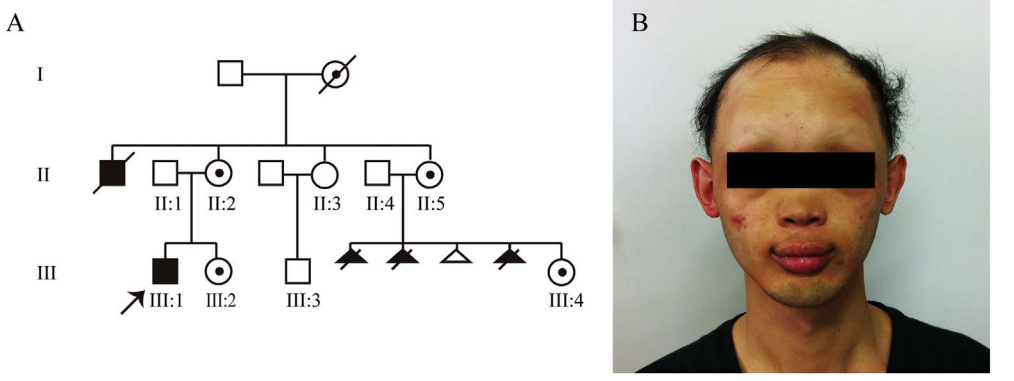

Fig. 1. (A) Pedigree of the family. Arrow indicates the proband and a slash indicates death. A white triangle indicates spontaneous abortion, while a black triangle with a slash indicates therapeutic abortion. (B) Typical XLHED facial appearance of the proband (individual III:1), including a saddle-shaped nose, sparse hair and missing eyebrows.