Case Report: Prenatal Diagnosis of a Fetus With Harlequin Ichthyosis Identifies Novel Compound Heterozygous Variants: A Case Report

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Background: Harlequin ichthyosis (HI) is the most severe form of the keratinizing disorders, and it is characterized by whole-body hard stratum corneum. ABCT12 has been identified as the major disease-causing gene of HI.

Methods: A case of HI was prenatally diagnosed by ultrasonography and genetic tests. The fetus had been found with dentofacial deformity and profound thickening of the palm and plantar soft tissues. Chromosomal microarray analysis (CMA) and whole exome sequencing (WES) were then performed on the amniotic fluid to identify germline pathogenic variants for the fetus. Candidate variants were verified by Sanger sequencing.

Results: Compound heterozygous frameshift variants (p.Q719QfsX21; p.F2286LfsX6) of ABCT12 were identified for the fetus, suggesting the former variants were maternally inherited and the latter paternally inherited. The fetus was terminated.

Conclusion: A prenatal molecular diagnosis is an important approach for the prevention of HI. In the study, we provided a successful case of genetic counseling for a family with an HI baby.

Keywords: harlequin ichthyosis, ABCT12 gene mutation, skin abnormalities, fetus, autosomal recessive

INTRODUCTION

Inherited ichthyoses are a group of genetic defects characterized by generalized dry skin, scaling, and hyperkeratosis. Harlequin ichthyosis (HI; OMIM 242500), the most severe form of ichthyoses, is a rare genodermatological disease. The clinical feature of HI is characterized by a markedly thickened stratum corneum covering the whole body, which cracks and forms erythematous fissures soon after birth. The skin abnormalities also affect the shape of eyelids, ears, nose, and lips (Thomas et al., 2006; Rajpopat et al., 2011).

HI is an autosomal recessive disease with an incidence of 1 in 3,00,000 births (Ahmed and O’Toole, 2014). Pathogenic variants of ABCT12 [OMIM*607800] have been demonstrated as the major causes of HI (Akiyama et al., 2005; Kelsell et al., 2005; Thomas et al., 2006).
The ABCA12 protein functions as a lipid transporter transferring lipids from the cytosol into lamellar granules in healthy skin. The lamellar granules fuse with the cell membrane and release their content into the intercellular lamellae. In the skin of HI patients, dysfunction of ABCA12 results in disordered lipid transfer. As a consequence, abnormal lipid-containing vacuoles form in the corneocyte cytoplasm. The skin turns into a defective formation of the lipid layer, and the stratum corneum grows remarkably thickened (Hovnanian, 2005).

Here, we report novel compound heterozygous variants in a Chinese HI case through prenatal molecular diagnosis.

CASE REPORT

Genetic counseling was provided to a 29-year-old Chinese woman (gravida 5, para 1) during her gestational 23 weeks since she had abnormal fetal ultrasonography results. The woman delivered a healthy female baby at term during her first pregnancy (Figure 1A). Ultrasonography at the 12th week of this pregnancy indicated that the fetus had multiple malformations, such as a short face, abnormal nasal bone, ear and mandible, and a cleft palate. At the 16th week of pregnancy, the fetus showed profound thickening of the palm and plantar soft tissues according to ultrasonography. The clinical diagnosis was not determined based on ultrasonography. Subsequent genetic tests were performed to make a definite diagnosis for the fetus. Combined with the results of whole-exome sequencing (WES) and ultrasonography, we provided sufficient information on genetic counseling for the family.

The results suggested that the sister, the mother, as well as the paternal grandmother carried the heterozygous c.2157delA (p.Q719QfsX21) and c.6858delT (p.F2286LfsX6) was identified. Both variants were frameshift deletions. The variants were verified using Sanger sequencing with a set of primers (ABCA12-17F: 5'-ATTATCAGGTTCCTTTCTCTGTTG-3', ABCA12-17R: 5'-CIATTTTTATCCTGTTGGGAAAAATTT-3', ABCA12-46F: 5'-GAGAGATACAAAAAGCAATGTCCTCA, ABCA12-46R: 5'-CTCATTTAGTATGTTGTACTCGT-3'). The results suggested that the sister, the mother, as well as the maternal grandmother carried the heterozygous c.2157delA, and the father and the paternal grandmother carried the heterozygous c.6858delT (Figures 1A, 2). 

DISCUSSION

The overall incidence of HI is 1 in 3,00,000 births (Layton, 2005) and the rate of recurrence is about 25% in subsequent pregnancies. HI can therefore largely be explained by genetic variations. With the development of medical techniques, HI with a family history could be successfully diagnosed at the prenatal stage (Yanagi et al., 2008; Ahmed and O'Toole, 2014; Rathore et al., 2015; Xie et al., 2016; Jian et al., 2018; Loo et al., 2018; Sheth et al., 2018; Liang et al., 2019). Here, we report a case without a family history of HI. Combining the information of ultrasonography and molecular diagnosis, we provided sufficient information on genetic counseling for the family.

More than 70 variants of ABCA12, accounting for autosomal recessive congenital ichthyoses, have been reported (Ahmed and O'Toole, 2014; Gurkan et al., 2015; Washio et al., 2017; Sheth et al., 2018). Pathogenic variants of the ABCA12 gene cause ARCI 4A (OMIM:601227, including congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI)) and 4B (HI, OMIM:242500). A previous analysis concerning genotype-phenotype correlation of ABCA12 variants indicated that the CIE...
FIGURE 1 | Clinical presentation of the proband. (A) Pedigree of the family. Transmission of the compound heterozygous variants of the HI baby were labeled in the three-generation family. (B) Clinical features of the HI baby. The fetus was covered in a thick, plate-like collodion membrane over the entire body surface at birth; the hands and feet in particular were enveloped by a wax-like cast of extremely tight skin. The features of this fetus were consistent with harlequin ichthyosis, displaying multiple malformations such as a short face, abnormal nasal bone, ear and mandible, and a cleft palate.

or LI phenotypes are often due to missense variants ABCA12 (Loo et al., 2018). HI was suggested to be caused by truncation or deletion variant in a conserved region of ABCA12, which leads to a severe loss of ABCA12 protein function (Akiyama, 2006). In this fetus, the c.6858delT variant inherited from the father is predicted to create a frameshift starting at codon Phe2286, resulting in a truncated protein. It has been previously reported in a female infant with HI who carries a novel missense variant in the gene (Loo et al., 2018), and in a male patient with atypical ARCI who also carries a missense variant in the gene (Scott et al., 2013a). The c.2157delA variant inherited from the mother is predicted to create a frameshift starting at codon Gln719 (p.Q719QfsX21). This novel variant has not been reported previously and is not listed in any population database.

In this report, we summarized published HI variants (splicing variants were not showed) that altered the ABCA12 protein sequence (Figure 2B). It is notable that most of these HI variants caused halt gain or frameshift, which should be damaging for the ABCA12 function. Multiple genes, such as ABCA12, NIPAL4, PNPLA1, LIPN, ST14, TGM1, ALOX12B,
# Molecular Diagnosis of Harlequin Ichthyosis

## TABLE 1 | Timeline of case report.

| Gestational age | Summaries | Diagnostic testing | Interventions |
|-----------------|-----------|--------------------|---------------|
| 12 weeks        | Multiple malformations such as short face, abnormal nasal bone, ear and mandible, cleft palate. | Ultrasound examination | |
| 12 weeks        | No abnormality. | CNV array tests | Amniocentesis |
| 16 weeks        | No abnormality. | Karyotype Analysis | Amniocentesis |
| 16 weeks        | Profound thickening of the palm and plantar soft tissues. | Ultrasound examination | |
| 16 weeks        | Two heterozygous frameshift variants (p.Q719QfsX21; p.F2286LfsX6) of ABCA12 were found in the fetus. | Whole exome sequencing | Amniocentesis |
| 23 weeks        | Diagnosed as HI. | Autopsy of the fetus | Rivanol-induced abortion |

## FIGURE 2 | Verification of variants in the HI baby. (A) Sanger sequencing verification of the HI compound heterozygous variants identified by WES in the family. (B) Summarization of published HI variants. The variants identified in this study have been highlighted in red.

ALOXE, and CYP4F22, have been known to be involved in congenital ichthyosis (Sheth et al., 2018). Among these genes, ABCA12 has been characterized as the major disease-causing genes of HI (Thomas et al., 2006). ABCA12 is a member of the adenosine triphosphate binding cassette (ABC) superfamily of active transporters (Uitto, 2005). Variants in ABC genes cause a variety of diseases such as cystic fibrosis, Tangier disease, pseudoxanthoma elasticum, Dubin-Johnson syndrome, and X-linked adrenoleukodystrophy (Hovanianian, 2005; Uitto, 2005). Dysfunction of the ABCA12 protein can directly affect the formation of the intercellular lipid layers, which is essential for epidermal barrier function (Hovanianian, 2005; Scott et al., 2013b). Although most HI cases are caused by ABCA12 variants, rare ABCA12 variants are difficult to identify using low throughput methods such as Sanger sequencing since this gene contains 53 exons spanning more than 200 kb genomic region. Therefore, WES should be the most cost-effective method for rare variant of HI at present.

The HI fetuses usually had a fatal outcome during the early neonatal period, usually within the first 2 weeks (Layton, 2005). In the past 20 years, the prognosis of HI infants has improved with advances in neonatal intensive care and retinoid therapy. However, the quality of life of HI patients is seriously affected in the long term. In the present study, the family chose to terminate the pregnancy after genetic counseling. The prenatal ultrasound diagnosis of HI was confirmed by molecular diagnosis and skin biopsy. Through WES, we identified two inherited heterozygous frameshift variants that would be helpful for the diagnosis of HI in the future.

## PATIENT’S PERSPECTIVE

The fetus was terminated at the 23rd gestational week according to the molecular diagnosis report. Although depressed, the mother was grateful to the genetic consultant for the genetic tests since she could avoid having a baby with HI in the future.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Lishui Maternity and Child Health Care Hospital. Written informed consent to participate in this study was provided by the participants’ legal guardian/next of kin. Written informed consent was obtained from the minor(s)’ legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.
AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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