CASE REPORT

Compound heterozygous mutations with novel missense *ABCA12* mutation in harlequin ichthyosisBenny Kai Guo Loo,¹ Melissa Jeanne Batilando,² Ene Choo Tan,³ Mark Jean Aan Koh⁴¹Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore, Singapore²Department of Neonatology, KK Women's and Children's Hospital, Singapore, Singapore³KK Research Centre, KK Women's and Children's Hospital, Singapore, Singapore⁴Department of Dermatology, KK Women's and Children's Hospital, Singapore, Singapore**Correspondence to**Dr Benny Kai Guo Loo,
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SUMMARY

Harlequin ichthyosis (HI) is the most severe form of autosomal recessive congenital ichthyosis, presenting at birth with distinctive facial features and thick, plate-like scales over the entire body. The abnormal skin barrier predisposes the patient to multiple complications, including dehydration and sepsis. Mortality rates of babies with HI have been greatly reduced since the introduction of systemic retinoid therapy. Mutations in *ABCA12* have been found to lead to HI. Most of these mutations are truncation or deletion mutations in the conserved region of the protein, leading to severe loss of *ABCA12* function. We report a case of HI caused by a compound heterozygous mutation (a known single nucleotide deletion and a novel single nucleotide substitution) in the *ABCA12* gene.

BACKGROUND

Harlequin ichthyosis (HI) is an extremely rare and severe form of autosomal recessive congenital ichthyosis (ARCI). It is characterised at birth, by large, thick, plate-like scales covering the entire body, separated by deep fissures. In addition, babies with HI show classical facial features, including ectropion, eclabium, flattened nose and ears.^{1–5} The abnormal skin barrier in HI predisposes to multiple problems after birth, including thermal dysregulation, dehydration, sepsis and feeding problems.^{2–5} Treatment with oral retinoids has greatly improved the survivability in recent years.^{3,4}

ABCA12 is associated with lipid transport in lamellar granules and *ABCA12* mutations can lead to defective lipid barrier in the stratum corneum which causes ARCI including HI phenotype.^{6,7} Most mutations associated with HI are due to truncation or deletion mutations in the conserved region leading to severe loss of function of *ABCA12*.^{7–9}

We describe a female infant with a novel *ABCA12* mutation and a known truncating mutation, and discuss the literature on HI.

CASE PRESENTATION

The patient, a female infant, was born to a 33-year-old gravida 4 para 3 Chinese mother at 40 weeks gestation via caesarean section for breech presentation. The baby weighed 3080 g and had APGAR scores of 5 at 1 min and 8 at 5 min. Antenatal ultrasonographic assessments did not detect any fetal abnormalities. Her parents were non-consanguineous, and the two older children were normal.

At birth, the baby's entire body surface was covered with a thick, plate-like collodion membrane. Her eyelids and lips were everted, and her hands and feet were small and constricted by extremely tight skin (figure 1).

INVESTIGATIONS

Genomic DNA was extracted from the patient using the Puregene DNA isolation kit (Qiagen, Hilden, Germany) and tested on the TruSight™ One sequencing panel (Illumina, San Diego, USA). Sequencing was performed in a MiSeq instrument at minimum target coverage of 20x. The sequences were mapped and analysed in comparison with the reference human sequence (hg19). Sequencing data were processed using the MiSeq Reporter software, and variants were annotated and filtered using wANNOVAR.¹⁰ The reported variants were confirmed by Sanger sequencing. Targeted sequencing was also performed on parental samples.

Analysis of patient's DNA detected a heterozygous single nucleotide deletion (c.6858delT (p.Phe2286fs)) in exon 46 and a heterozygous single nucleotide substitution (c.5884G>A (p.Gly1962Ser)) in exon 39 in the *ABCA12* gene (NM 173076.2). Parental DNA studies showed that the p.Phe2286fs variant was inherited from her father and the p.Gly1962Ser variant was inherited from her mother.

Treatment

She was started on total parenteral nutrition via an umbilical venous catheter. She was empirically started on intravenous cloxacillin and gentamicin, but both were discontinued when blood cultures returned negative. She did not require any respiratory support.

To prevent her skin from drying out, the baby was nursed in an incubator with humidity set at 80%, which was gradually reduced over the next few weeks. Frequent application of white soft paraffin to her entire skin surface was instituted within a few hours from birth, and Solcoseryl eye gel was applied to her eyes to prevent exposure keratitis. Acitretin was started at 0.5 mg/kg/day and gradually increased to 1.4 mg/kg/day over 3 weeks. Her skin progressively improved, with the scales and plaques gradually softening and falling off. However, her hands were auto-amputated at the wrists after becoming gangrenous, leaving behind stumps. With time, she was gradually able to suck and progressed to full oral feeds following a period of tube feeding.

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Figure 1 Patient was covered with thick plate-like collodion membrane over her entire body surface at birth. Her eyelids and lips were everted, and her hands and feet were enveloped by a wax-like cast of extremely tight skin. These features were consistent with harlequin ichthyosis. There was an orogastric tube inserted into her mouth.

She remained normothermic and continued to have good weight gain on expressed breast milk feeds and eventually progressed to direct breast feeding. She was discharged by day 53 of life.

OUTCOME AND FOLLOW-UP

At the 5-month review, she continued to grow well. Most of the thick scales had dropped off, leaving behind mild residual skin erythema with soft, thin scaling. Ectropion had improved significantly (figure 2), although she was still unable to completely close

Figure 2 The same patient was seen after 5 months of acitretin treatment. Most of the thick scales had dropped off, leaving behind alopecia, residual generalised skin erythema and thin scales. Her ectropion had also improved significantly, although she was still unable to completely close her eyes.

her eyes. Treatment was maintained on 1.4 mg/kg/day of acitretin and frequent application of moisturisers. She failed both the automated auditory brainstem response and otoacoustic emissions tests. Otoloscopic examination showed scales filling both ear canals, probably contributing to conductive hearing loss.

DISCUSSION

HI is an extremely rare and severe form of ARCI with a reported incidence of 1 in 300 000 births.¹ As infants with HI are at risk of developing multiple complications, they require multidisciplinary management in a tertiary paediatric hospital, equipped with specialised neonatal resuscitation facilities. In addition, HI is associated with premature birth, rendering them with the additional morbidities of prematurity.⁵

At birth, patients require nursing in incubators with high humidity as they are at risk of dehydration and electrolyte imbalance from increased transepidermal water loss.^{1 2 5} Close monitoring of body temperature is essential as dysregulation of body temperature can occur due to impaired sweating from obstruction of sweat glands by the thickened stratum corneum.² Ventilatory support may be required if the rigid chest wall restricts breathing and pulmonary function.¹ Due to the impaired skin barrier, there is an increased risk of skin infection and sepsis, requiring a low threshold for starting empirical systemic antibiotic therapy.

Frequent eye lubrication is necessary to prevent complications of ectropion such as keratitis and even blindness.^{1 3 5} Systemic retinoids are often required to prevent and treat these eye complications. Regular cleansing of external auditory canal may be needed to prevent hearing difficulties.³ Orogastric tube feeding may be required as these patients may have poor oral sucking skills. Additional caloric and protein supplementation may be required due to the extremely high rate of skin shedding, leading to increased loss of proteins.^{1 3 5} Orthopaedic problems include development of pseudocontractures and distal limb ischaemia, leading to distal limb gangrene and eventual autoamputation. Venepuncture can be extremely challenging and central line placement may be required for infusion of medication, fluids and parenteral nutrition.^{1 5}

Dermatological management includes frequent application of emollients, soap-free baths and systemic retinoids.^{1 4} The main causes of mortality include respiratory failure, infection or dehydration.¹⁻³ Systemic retinoids have been essential in treating disorders of keratinisation, including HI. Early treatment of HI with systemic retinoids hasten the shedding of hyperkeratotic plates, whereas continued use will reduce scaling and improve ectropion and eclabium. A multicentre survey showed that early systemic retinoid treatment in neonates with HI had better survival rate of 83% compared with 14% in those without systemic retinoid therapy.³ A review of systemic retinoid therapy in patients with HI showed that acitretin, etretinate and isotretinoin were administered at 0.5–1.0 mg/kg/day, 1.0–1.3 mg/kg/day and 0.5 mg/kg/day, respectively.¹¹ Side effects of systemic retinoid therapy include cheilitis, peeling of skin on hands, pruritus, hair loss and dry nose or nose bleed. Acitretin may be preferred as it has a better side effect profile.¹²

Since 2005, *ABCA12* mutations had been found in patients with HI.⁵⁻⁷ Pathogenic variants of the *ABCA12* gene cause ARCI 4A (MIM:601227) and 4B (MIM:242500), which includes HI, congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI). The c.6858delT variant found in our patient is predicted to create a frameshift starting at codon Phe2286, resulting in a truncated protein. It has been reported previously in a male patient with atypical ARCI who also carries a missense mutation in the

Table 1 Genotype–phenotype correlation of *ABCA12* mutations in harlequin ichthyosis (HI), congenital ichthyosiform erythroderma (CIE) and lamellar ichthyosis (LI)

Genotype	Phenotype
Truncation mutation+truncation mutation	HI
Truncation mutation+exon/amino acid deletion	HI
Truncation mutation+missense mutation	HI, CIE
Exon/amino acid deletion+exon/amino acid deletion	HI
Exon/amino acid deletion+missense mutation	HI, CIE
Missense mutation+missense mutation	CIE, LI

gene.¹³ The c.5884G>A variant is predicted to cause the substitution of an alternative amino acid at position 1962 (p.Gly1962Ser). Analysis using Alamut Visual showed high probability that it might lead to aberrant splicing as the substitution is at the last nucleotide of the exon. This novel mutation has not been reported previously and is not listed in any population database.

ABCA12 is a member of the large superfamily of the ATP-binding cassette transporters, which bind and hydrolyse ATP to transport molecules across the cell membrane or into a vesicle. It is associated with lipid transport in lamellar granules to the surface of granular layer keratinocytes and disruption of its function will cause malformation of intercellular lipid layers at the granular cornified layer interface, and hence epidermal lipid barrier disruption.^{6–8 14}

Reported *ABCA12* mutations in HI include truncation mutations, missense mutations, exon deletions and single amino acid deletions. To cause the HI phenotype, at least one mutation on each allele must be a truncation or deletion mutation in a conserved region, which results in severe loss of *ABCA12* protein function.^{7–9} Conversely, *ABCA12* mutations causing the CIE or LI phenotypes are often due to missense mutations which cause milder functional impairment of *ABCA12*. In CIE, at least one *ABCA12* mutation on each allele is a missense mutation, whereas in LI, the *ABCA12* mutations are due to homozygous or compound heterozygous missense mutations. The genotype–phenotype correlations for HI, CIE and LI are summarised in table 1.⁹ In contrast to HI, which is solely due to *ABCA12* mutations, CIE and LI are also caused by mutations in *TGM1*, *ALOX12B*, *ALOXE3*, *ICHTHYIN* and *FLJ39501*.⁹

Our patient presented with severe HI phenotype as a result of compound heterozygous mutations with a truncation mutation and a missense mutation. This combination of genotype is rare in patients with HI. There is only one other case with a truncation and a missense mutations, but no other detail is available on the patient's presentation.¹³ It appears that missense mutations that knock out protein function in the presence of another truncation or deletion mutation of *ABCA12* gene can lead to HI phenotype, although the missense mutation in this patient could also be a splice site mutation due to its position in the exon. Another possibility is the presence of a large deletion or a mutation in the promoter or regulatory region which would not be detected by our sequencing.

There is increasing evidence for the use of ultrasound to detect HI prenatally, with the most consistently described features being ectropion, flat nose, large open mouth and abnormally small hands or feet. Other associated sonographic features are findings of intra-amniotic debris, floating membrane, intrauterine growth restriction and polyhydramnios. However, most of these features can only be detected during the third trimester.¹⁵ Prenatal diagnosis from chorionic villus or amniotic fluid sampling can be offered to parents of an affected child for future pregnancies if the *ABCA12* mutation has been identified.¹⁶

In conclusion, HI is a devastating disease which will benefit from earlier prenatal diagnosis. Discovery of novel mutations can improve our understanding of the genotype–phenotype correlations in *ABCA12* mutations and HI with the aim of developing a reliable prenatal genetic test.

Learning points

- ▶ *ABCA12* mutations cause autosomal recessive congenital ichthyosis diseases including harlequin ichthyosis (HI).
- ▶ Most mutations in HI are due to truncation or deletion mutations in the conserved region leading to severe loss of function of *ABCA12*.
- ▶ HI may be caused by the combination of a missense mutation (c.5884G>A) and a truncating mutation in the *ABCA12* gene.
- ▶ Functional study could help to elucidate the significance of this c.5884G>A mutation in the conserved region of *ABCA12* gene and how it could result in the HI phenotype in the presence of a truncating mutation in the same gene.

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