

SHORT COMMUNICATION

ABCA12-deficient Congenital Ichthyosiform Erythroderma in a Boy with an Intellectual Developmental Delay

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Congenital ichthyosiform erythroderma (CIE) is an entity classified in non-syndromic ichthyosis of autosomal recessive congenital ichthyosis (ARCI). Genes responsible for CIE include *ABCA12*, *ALOX12B*, *ALOXE3*, *CERS3*, *CYP4F22*, *NIPAL4*, *PNPLA1* and *TGMI* (1–3). The genetic diagnosis of CIE could be achieved by conventional Sanger sequencing of multiple PCR-amplified cDNAs and/or genomic DNA fragments, but it is laborious to identify possible mutation(s) among all those genes. Alternatively, target-captured DNA analysis (4) and whole exome analysis (5) of genes using next-generation sequencing (NGS) can efficiently identify mutations involved in ichthyoses. Here we report a case of CIE with an intellectual disability, in which NGS of a panel of 54 genes involved in keratinisation disorders was used to establish an exact diagnosis of ABCA12 deficiency.

CASE REPORT

A 3-year-old boy was referred to us regarding his erythroderma with severe scales. The initial information was that he was born as a collodion baby, but the record of the NICU was suggestive of harlequin ichthyosis (HI). His parents and older sister were healthy and no other members of his pedigree had similar skin manifestations. On examination, his entire body surface was dry and mildly erythrodermic and was covered diffusely with large whitish scales (Fig. 1a, b). His scalp was covered with erosions and patchy thick scales and/or crusts (Fig. 1a). Scalp hairs were sparse, but there were no hair abnormalities. His eyebrows and eyelashes were also sparse and irregularly grown and mild ectropion and eclabium were observed (Fig. 1a). The nails were thickened and his palms and soles were also as hyperkeratotic as the dorsal sides of his hands and feet. He had just undergone an operation for cryptorchism but no major malformations were found. No neurological symptoms, paraplegia, ataxia, peripheral neuropathies and deafness were evident. However, the overall developmental quotient (DQ) measured using the Kyoto Scale of Psychological Development (6) was 55 at 3 years and 11 months of age (normal DQ = 70 at that age), indicating a delay in intellectual development.

Haematoxylin and eosin staining of a biopsy from his abdominal skin revealed epidermal acanthosis hypergranulosis and a markedly thickened homogeneous stratum corneum (Fig. 1c). Mild inflammatory cell infiltrates were seen in the upper dermis. Ultrastructural analysis of the epidermis showed aggregated abnormal vesicular structures, vesicles containing granules and lipid droplets in the granular layer and stratum corneum (Fig. 1d), comparable to CIE cases reported previously (7).

For the differential diagnosis of ichthyoses, coding exons and their flanking splice sites of 54 genes (Table S1¹) were exami-

ned by NGS, after approval of the Institutional Review Boards. Informed consent was obtained from his parents. The 8 genes responsible for ARCI and *ALDH3A2* and *APISI* for syndromic ichthyoses with neurological complications were included in the panel of 54 genes. For target capture, HaloPlex[®] probes of those 54 genes were obtained from Agilent Technologies Inc. (Santa Clara, CA, USA). A total of 137.419 kbp in 674 regions of those target genes were examined using a MiSeq NGS sequencer (Illumina Inc., San Diego, CA, USA). DNA samples from 10 other cases with keratinisation disorders and one case with pustular psoriasis were also examined. Sequences were analysed using a SureCall (Agilent Technologies Inc.) and human hg19 was used as a reference genome sequence. NGS revealed compound heterozygous mutations chr2: g.215876794_215876795delAA and chr2: g.215823087delG in *ABCA12* (Fig. S1¹). No other SNV (single nucleotide variation) and indels (insertions and deletions) compatible with the phenotype of the present case were found in any of the other genes examined. PCR products from genomic DNAs and/or cDNAs of the patient and his parents were subjected to Sanger sequencing and the mutations detected by NGS were confirmed. The maternal mutation was c.2021_2022delAA in exon 16 leading to p.Lys674Argfs*48, while the paternal mutation was c.6031delG in exon 41 leading to p.Glu2011Asnfs*16.

Immunofluorescence analysis revealed that ABCA12 was stained in the granular layer (Fig. 1e, upper) and glucosylceramide (GlcCer) staining was weak in the upper spinous layer and intense in the granular layer in healthy control skin (Fig. 1f, upper). In contrast, in the skin of the patient, ABCA12 was only faintly stained (Fig. 1e, lower) and weak staining of GlcCer remained in the upper spinous and granular layers of the skin (Fig. 1f, lower).

He was treated with etretinate 1 mg/kg daily, and his ectropion and eclabium had improved by the age of 4.5 years, but whitish scales were still diffusely distributed and he was treated topically with tacalcitol. Heat retention often occurred with a body temperature over 38°C in the summer season. We recommended a cool environment and cooling to avoid heat stress.

DISCUSSION

The present case corresponds to a survivor case of HI. The survival rates of HI range from 50% to 80% (8, 9). The mutations identified in this case cause compound heterozygous truncation mutations. One mutation is p.Lys674Argfs*48 in the N-terminal domain of ABCA12, and another one is p.Glu2011Asnfs*16 in the second transmembrane domain of the protein (Fig. 1g). Each mutation has been identified in an HI case (10) and a CIE case (11), respectively, but the compound

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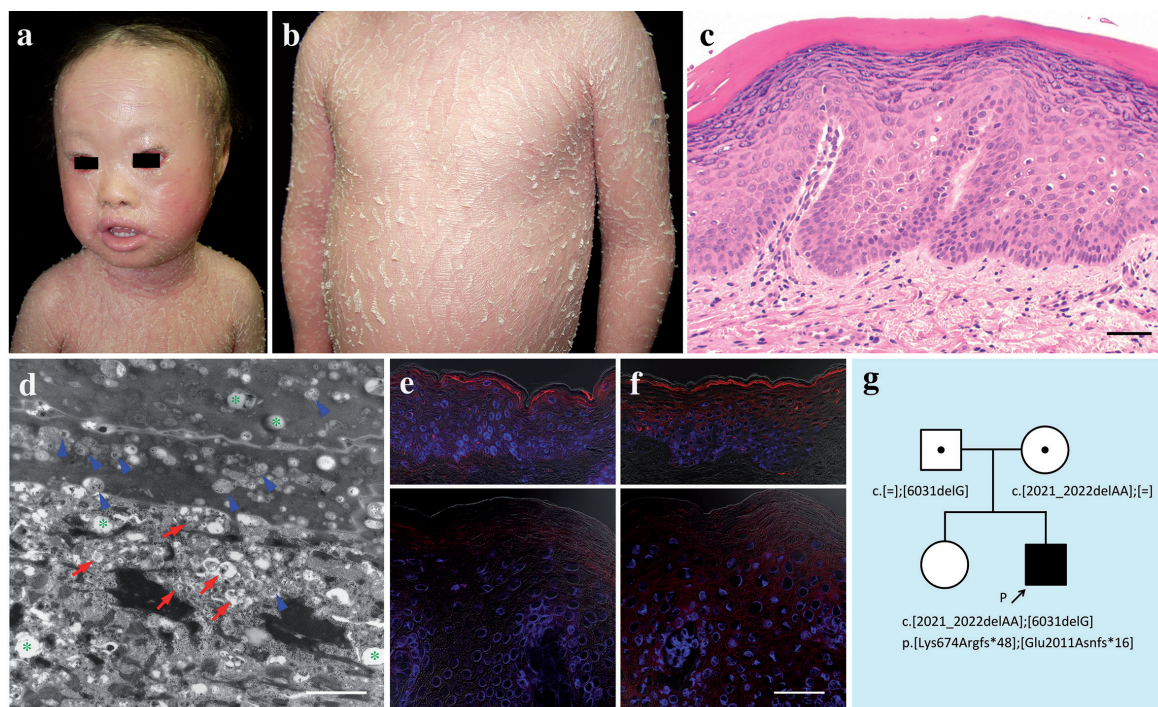


Fig. 1. Clinical findings, histology and ABCA12 mutations. (a) The facial skin of the patient was tight with thick scales and mild ectropion and eclabium were evident. The scalp was covered with some erosions with patchy thick scales and/or crusts and scalp hairs were grown but sparse. Eyebrows and eyelashes were sparse and irregularly grown. (b) The entire body surface was mildly erythrodermic and was covered diffusely with large whitish scales. (c) Haematoxylin and eosin staining. A biopsy of abdominal skin revealed acanthosis with hypergranulosis and homogeneously thickened stratum corneum. Mild inflammatory cell infiltrates were evident in the dermis. Bar = 50 μ m. (d) Ultrastructure of the skin. Abnormal vesicular structures (arrows), vesicles containing granules (arrowheads) and lipid droplets (asterisks) were evident in the granular layer and stratum corneum. Bar = 1 μ m. (e, f) Immunofluorescence of ABCA12 (e) and glucosylceramide (GlcCer) (f) in the skin. Cryosections were incubated with a rabbit polyclonal anti-human ABCA12 antibody (1:200 dilution) (Sigma, St. Louis, MO, USA), or with a rabbit polyclonal anti-GlcCer antibody (1:50 dilution) (Glycobiotech, Borstel, Germany) (for further experimental details contact KY). In healthy control skin (upper panels), ABCA12 was positive in the granular layer (e) and GlcCer was stained weakly in the upper spinous layer and intensely in the granular layer (f). In this patient (lower panels), ABCA12 was not evident (e) and GlcCer was weakly stained in the granular and spinous layers (f). Blue staining (DAPI) indicates nuclei. Bar = 50 μ m. (g) Pedigree and ABCA12 mutations.

heterozygosity of those truncation mutations have not been reported in survivors of HI with the phenotype of CIE. Using immunofluorescence, ABCA12 was only faintly detected by an antibody raised against residues 93–180 of the protein (Fig. 1e). This might be due to conformational changes of the mutant ABCA12 proteins. The defects in ABCA12 possibly impair the accumulation of GlcCer into lamellar granules in the granular layer (Fig. 1f), as suggested previously (12).

Reportedly, a developmental delay accompanies 32% of survivor cases with HI (8), but ABCA12 mutations associated with an intellectual disability have not been fully delineated. Eilers et al. (13) reported a German boy with severe psychomotor retardation. In that case, at 1 year of age, his mental development corresponded to that of a 3-month-old baby. He could not speak and his auditory skills were equivalent to those of a 6-month-old child. The Griffith-development score was 27, which is much lower than the normal range of 95–115. The mutation of that case was a homozygous mutation of c.7322del1C in exon 49 of ABCA12. That mutation causes p.Val12442Serfs*22, which is also a truncation mutation of the ABCA12 protein, but is more downstream

from the mutations found in our case. An intellectual delay is common in childhood and it is unknown whether those truncation mutations are associated with the complication or occurred by chance in those cases with HI. In follow-up of survivors of HI, it is necessary to carefully observe the intellectual development of infants to get help early in life by appropriate care in such cases.

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