




Recessive mosaicism in *ABCA12* causes blaschkoid congenital ichthyosiform erythroderma

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Summary

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We report the unique case of a 3-year-old girl who presented with linear erythematous lesions following the lines of Blaschko, suggestive of genetic mosaicism in the skin. Single-candidate gene analyses were performed on DNA from blood, excluding Conradi–Hünemann–Happle syndrome, erythrokeratoderma variabilis and a mosaic presentation of pityriasis rubra pilaris. With whole-exome sequencing (WES) on DNA from the patient's blood, a heterozygous missense mutation in exon 25 of the *ABCA12* gene was detected. By manually scrutinizing the WES data, another low-percentage pathogenic frameshift mutation was found in the adjacent exon 26 of the same gene. This frameshift mutation was confirmed with Sanger sequencing in DNA isolated from a lesional skin biopsy. A subsequent cloning experiment was performed to prove that the patient is compound heterozygous for both mutations in the affected skin, explaining the blaschkoid ichthyosiform erythrodermic phenotype. The patient's phenotype was elucidated by the combination of a germline mutation and an acquired postzygotic mutation in *ABCA12*, resulting in the diagnosis of a mosaic manifestation of autosomal recessive congenital ichthyosis. Postzygotic compound allelic loss in autosomal recessive disorders is extremely rare and will not appear as the typical phenotype of the known germline mutation-associated disease. This is the first report of a proven biallelic mosaic presentation of an autosomal recessive genodermatosis, and we propose the term 'recessive mosaicism' for this kind of manifestation.

What's already known about this topic?

- Specific mutations in the *ABCA12* lipid transporter are known to cause different phenotypes like harlequin ichthyosis, congenital ichthyosiform erythroderma and lamellar ichthyosis.
- In mosaicism, two or more cell populations that are genetically different arise postzygotically in the developing embryo.
- In the skin, mosaicism can present itself in different patterns of affected skin, often caused by a dominant genetic mutation.

What does this study add?

- We report a unique patient with blaschkoid congenital ichthyosiform erythroderma due to biallelic mutations, one inherited germline missense mutation and the other a postzygotic frameshift mutation in the *ABCA12* gene.
- This study describes the diagnostic approach and applied research that can be used if one encounters a similar diagnostic dilemma with manifestations suspected for genetic mosaicism.

- We propose the term 'recessive mosaicism' for this kind of mosaic presentation of an autosomal recessive genodermatosis.

ABCA12 belongs to a subfamily of ATP-binding cassette (ABC) transporters that are important in lipid transport.¹ ABCA12 transports ceramides such as glycosylceramides, which are the precursors of the corneocyte lipid envelope and intercellular lipid layer in cornified cells. Transport of these lipids is established through lamellar granules (LGs), which are present in spinous and granular keratinocytes. The LGs transport their contents to the apical regions of the granular keratinocyte, where they fuse with the cell membrane and effuse the lipids into the intercellular space.^{2,3} Defective ABCA12 leads to disturbed lipid transport in the LG, including ceramide transport. Homozygous or compound heterozygous truncating mutations in ABCA12 result in a severely impaired skin barrier, which causes the striking phenotype of harlequin ichthyosis (HI).^{4,5} This is in contrast to the clinically less severe congenital ichthyosiform erythroderma (CIE) or lamellar ichthyosis, where at least one of the ABCA12 allelic mutations is a missense mutation.⁶

Although combinations of truncating mutations with missense mutations have been described in HI, the exact location within the protein and the subsequent amino acid substitution of these mutations are most likely of importance for the resultant phenotype. Here we describe a patient with compound heterozygous ABCA12 mutations that did not represent the characteristic phenotype of any ichthyosis due to a postzygotic mutation resulting in an autosomal recessive ichthyosis in mosaic pattern. This phenotype, the causative mutations and this form of mosaicism have not been reported before in CIE or HI.

Case report

A 3-year-old girl was referred to our outpatient clinic with sharply demarcated linear erythematous plaques following the lines of Blaschko situated over the entire body and present from birth. Initially, the skin condition was confined to erythematous lesions only on the eyebrows, arms and legs, thereafter developing and stabilizing into lesions as described above and shown in Figure 1. There was no collodion membrane at birth. Furthermore, no growth or developmental delay was observed. Hair and teeth were not affected. She has no siblings, and with the exception of aunts with atopic eczema, her family history is unremarkable. Histology of the affected skin showed spongiotic dermatitis with parakeratosis. Topical treatment in recent months consisted of topical steroids, coal tar and emollients, resulting in a slight amelioration of scaling. Due to the typical clinical presentation a genodermatosis was suspected.

Single-candidate gene sequencing was performed in blood, excluding Conradi–Hünemann–Happle syndrome (EBP), erythrokeratoderma variabilis (GJB3 and GJB4) and mosaic presentation of pityriasis rubra pilaris (hotspots of CARD14). A mosaic presentation of an autosomal recessive congenital ichthyosis was suspected. As lesional skin at the time was not available we then performed whole-exome sequencing on DNA from blood and found that the patient was heterozygous for the missense mutation c.3679G>A (p.Glu1227Lys) in exon 25 of ABCA12. The glutamic acid at this position is highly conserved among species and across ABCA transporters. This mutation is not present in the Genome Aggregation Database (gnomAD) or the Human Genome Mutation Database (HGMD).

According to the prediction software tools PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2>), SIFT (<https://sift.bi.a-star.edu.sg>), MutationTaster (<http://www.mutationtaster.org>) and Align GVGD (<https://agvgd.iarc.fr>), this mutation is probably disease causing. However, the phenotype of the patient could only be explained if both alleles of ABCA12 were affected by recessive mutations. Therefore, the BAM file of ABCA12 was manually inspected for other low-percentage pathogenic variants. A second mutation was identified in 3.3% of the sequence reads (seven of 211 reads). This frameshift mutation c.3769dup in exon 26 of ABCA12, putatively present as low-grade mosaic, predicts a premature ABCA12 protein truncation (p.lle1257Asnfs*4). The mutation is not present in GnomAD and HGMD.

As the postzygotic mutation c.3769dup in exon 26 of ABCA12 could not be confirmed by Sanger sequencing in DNA isolated from blood, a biopsy of the lesional skin was taken with the hope of raising the mutation percentage for detection. Herein the mutation was confirmed (Fig. 2a). In order to assess whether the above-mentioned mutations were located on the different alleles (in trans, compound heterozygosity), a cloning experiment was performed. By means of amplification-refractory mutation system–polymerase chain reaction (primers: ABCA12_25F, GCATGGTTGTAAGG-GACTG, and ABCA12_26R_AS1, AAATGAAAGAGTCAGCTAG-GATTT; methods and data available on request), then cloning and sequencing (10 bacterial clones analysed), the c.3769dupA mutation was shown to be present on the same allele as the exon 25 wild-type reference sequence (NM_173076-2), which is consistent with the presence of the germline mutation c.3679G>A on the other allele (Fig. 2b). Thus, the patient is compound heterozygous for both mutations in the affected skin, explaining the blaschkoid ichthyosiform erythrodermic phenotype.



Fig 1. Clinical features of the lesions in the patient following the lines of Blaschko and resembling a clear presentation of genetic mosaicism. At the age of 9 months the lesions were less pronouncedly erythematous (a, b) and in time over 2 years intensified (c–h). We noted distal onycholysis on digits 3 and 4 of the right hand (e) and on digit 5 of the left hand (not shown).

Discussion

In mosaicism, two or more cell populations that are genetically different evolve from the initial zygote. In the skin, mosaicism can present in different patterns, where the affected skin often contains a dominant mutation that arose sometime at a postzygotic stage. These mutations can be either lethal or nonlethal. In case of lethal autosomal mutations, cells affected with such mutations can survive in mosaic state only in the presence of normal cells.^{7,8} In the present case, the recessive missense mutation in the *ABCA12* gene was paternally inherited. By sheer chance a different postzygotic frameshift mutation on the other allele occurred and resulted in genetic mosaicism. The clonal outgrowth of the compound heterozygous embryonic cells exhibits the phenotype of this autosomal recessive disease in the characteristic pattern following the lines of Blaschko. According to the archetypical classification of cutaneous mosaicism, this patient presented with narrow bands of Blaschko (type Ia).⁹ The unaffected skin should be considered only a 'carrier' of the germline recessive missense mutation and will not develop features resembling autosomal recessive ichthyosis.

Combinations of missense and truncating mutations have been described in both HI and CIE. However, the majority of cases of HI are caused by homozygous or compound heterozygous truncating mutations, while in CIE the

underlying biallelic mutations include at least one missense mutation.⁶ An exact classification of the ichthyosis in our patient is difficult, as the mutations are novel and not phenotypically linked to disease in the literature. However, based on the above-mentioned genotype–phenotype correlation and considering the severity of the presented ichthyosis, the patient is most likely affected with a mosaic manifestation of CIE.

First-degree relatives of our patient have a 50% likelihood of being a carrier of the germline recessive mutation c.3679G>A. Gonadal mosaicism in the patient cannot be excluded, so at this point there is no accurate prediction for the chance of passing on the postzygotic recessive mutation c.3769dupA. As the mutations are biallelic, the patient can only transmit one mutation to her offspring, giving only possible carriers. Preconception carrier screening for the partners of carriers can be offered to predict possible risk of affected offspring.

Postzygotic compound allelic loss in autosomal recessive disorders is extremely rare and will not manifest itself as the typical phenotype of the known associated disease due to mosaicism. Our patient presented with linear erythematous-squamous lesions following the lines of Blaschko, suggesting mosaicism in the skin. Mosaicism in recessive disorders has been described as a cause of postzygotic homozygosity.¹⁰ However, in this case the phenotype could eventually be

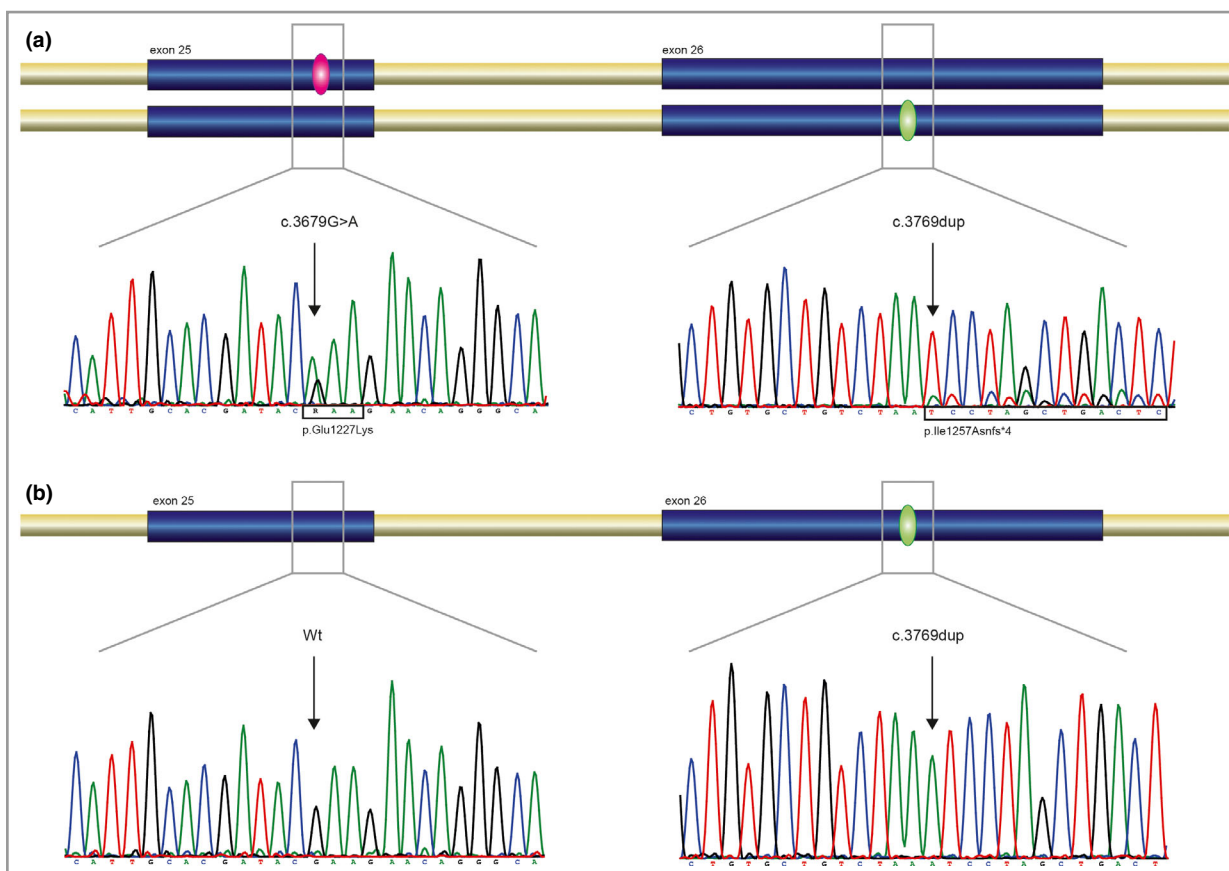


Fig 2. Genetic analyses of *ABCA12* in DNA isolated from lesional skin to demonstrate compound heterozygosity. (a) Polymerase chain reaction (PCR) amplification (fragment size 505 bp) and subsequent Sanger sequencing show the presence of both mutations, schematically on top and in biallelic traces below. The heterozygous missense mutation c.3679G>A in exon 25 is shown as a pink dot and the mosaic frameshift mutation c.3769dup in exon 26 as a green dot. (b) Through amplification-refractory mutation system-PCR, cloning of a single allele and subsequent sequencing of the cloned insert, the frameshift mutation c.3769dup in exon 26 (schematically on top, green dot) is present only in combination with a wild-type (Wt) exon 25 in the DNA fragment (traces below). The germline missense mutation should, by definition, be present on the other allele (not shown), therefore confirming compound heterozygosity in the affected skin.

explained by the combination of a germline mutation and an acquired postzygotic mutation in *ABCA12*, resulting in the diagnosis of a mosaic manifestation of autosomal recessive congenital ichthyosis. We propose the term ‘recessive mosaicism’ for this specific kind of manifestation.

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