Extensive Postzygotic Mosaicism for a Novel Keratin 10 Mutation in Epidermolytic Ichthyosis

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Accepted May 21, 2013; Epub ahead of print Oct 3, 2013

Epidermolytic ichthyosis (EI, MIM#113800) is a rare genetic disorder of keratinisation with the incidence estimated to be less than 1 in 100,000. The phenotype is characterised by congenital erythroderma, blistering and erosions of the skin, which cease in the first months of life and are followed by hyperkeratosis and verrucous scaling from early childhood (1). The disorder is dominantly inherited and caused by mutations in the genes encoding keratin 1 or 10 (KRT1, KRT10) (2), which are expressed in the suprabasal epidermal layers (3). The histological findings in EI, comprise thickened epidermis, epidermolytic within the suprabasal layer, and vacuolar degeneration and presence of keratinocyte granules in the granular layer (4). In addition to the generalised form of EI, epidermolytic naevi following the Blaschko’s lines, representing segmental mosaicism for KRT1 or KRT10 mutations have been described in a few patients. Mutations in KRT1 (5) and KRT10 (1, 6, 7) were found in the lesional but not in non-lesional skin, and in some (6), but not all cases (1, 5), the respective mutation was found at low allelic levels in DNA from lymphocytes. If the germ line is also affected, patients with epidermolytic naevi are at risk of transmitting EI to the offspring.

CASE REPORT

Here, we report on a 5-year-old boy with widespread hyperkeratotic, verrucous plaques being more pronounced in the folds, but affecting almost the entire body surface (Fig. 1). On this background, sharply limited normal-appearing areas were present next to severely verrucous skin in a band-like or patchy arrangement on the limbs and trunk (Fig. 1A–C). Palms and soles showed minimal focal keratoses. To address the molecular background of this extensive EI with patches of normal appearing skin, EDTA blood was obtained, as well as skin biopsies from an unaffected patch on the right flank (biopsy 1, Fig. 1A), a severely verrucous area next to it (biopsy 2, Fig. 1A), and a hyperkeratotic area on the chest (biopsy 3, Fig. 1B). Haematoxylin and eosin staining of the affected skin samples was consistent with epidermolytic hyperkeratosis, whereas the clinically healthy skin patch showed normal morphological features (Fig. 1D). Keratin 1 was strongly reduced in affected skin, but stained comparably to the control in unaffected skin (Fig. 1E). Keratin 10 staining revealed a perturbed filamentous cytoskeleton with aggregation of tonofilaments around the nucleus (Fig. 1F). Surprisingly, no KRT1 or KRT10 mutation was detected, suggesting that the disorder was due to a de novo postzygotic mutation mainly present in the affected skin. Therefore the analysis was extended to DNA from the skin of the patient. In both keratinocytes and fibroblasts from the affected skin the mutation c.1349G>C; p.Arg450Pro in exon 6 of KRT10 was disclosed in a heterozygous state. To the best of our knowledge, this allelic variant has not been reported before (Human Gene Database; HGMD Professional 2012.4). It is predicted to be disease-causing Polyphen 2 and it is not referenced as a SNP. This variant is located in the helix 2B domain of KRT10, in close vicinity to amino acids which are known to be mutated in patients with EI (Gln447Pro, Tyr449Asp, Tyr449Cys, Leu452Pro) and two residues N-terminally of the highly conserved LLEGEG sequence (amino acids 452–457) (1). When re-examined, the DNA sequence derived from lymphocyte DNA of the patient demonstrated the mutant allele at a low level, of about 10%, which had been considered as background in the initial analysis (Fig. S1). The same result was obtained with DNA extracted from keratinocytes and fibroblasts isolated from the normal-appearing skin (Sample 1, Fig. 1G). The mutation was absent in the DNA of both unaffected parents (Fig. S1). These findings confirmed that the patient had an epidermolytic naevus involving about 80% of the integument. Based on the presence of the mutation in keratinocytes, fibroblasts and lymphocytes, we speculate that it occurred very early during embryonic development. Since these tissues are derived from both ectoderm and mesoderm the time point was probably before the 4-cell stage (9). The extensive involvement of the skin suggested that mutant keratinocytes possess a selection advantage. To address this, we analysed epithelial proliferation, which clearly demonstrated significantly increased Ki-67-positive keratinocytes in areas with epidermolytic hyperkeratosis (Fig. 1H), probably as a response of the basal keratinocytes to the defective cytoskeleton caused by the KRT10 mutation (10), thus providing them with growth advantage.

Because of the proximity of the normal-appearing areas to markedly verrucous lesions (Fig. 1A) we investigated whether these result from a recombination event causing loss of heterozygosity (11), and leading to a “twin spot phenomenon” (12), as described in Darier’s disease and neurofibromatosis (13). Laser dissection microscopy was employed to isolate keratinocytes from a severely affected area, however no evidence for homozygosity of the mutation was found.

DISCUSSION

The hypothesis that normal skin patches in the affected skin represented revertant mosaicism, and together with neighbouring, severely affected areas result from a “twin spot phenomenon” (12, 13), could not be confirmed on a molecular level. Nevertheless, because of the extensive skin involvement, the term epidermolytic naevus is rather inappropriate in the present case, for which we propose the designation EI with postzygotic mosaicism.

1https://doi.org/10.2340/00015555-1695
In our patient, the mutation was initially missed because of the very low levels of the mutant allele in the lymphocyte DNA. The presence of the mutation in the germline determines the risk for transmission to the offspring, rendering the identification of the disease-causing mutation highly relevant for prenatal testing. These findings have also implications for mutation analysis in patients with genetic disorders, because postzygotic mosaicism may explain cases in which mutations are not disclosed by routine screening.

ACKNOWLEDGEMENTS

We thank the patient and his family. The excellent technical support by Kaethe Thoma, Margit Schubert, Ioannis Athanasiou and Annegret Bedorf is gratefully acknowledged. This work was supported by the Austrian National Bank (Grant No. 14819).
supported by the Network Epidermolysis bullosa grant from the Federal Ministry for Education and Research (BMBF) and the E-RARE grant “RevertantEB” to LBT., and the Excellence Initiative of the German Federal and State Governments and Freiburg Institute for Advanced Studies, School of Life Sciences to LBT, the “Theodor-Nasemann Scholarship” from Galderma Förderkreis and the DFG grant 1795/1-1 to D.K. and the DFG grant 5663/2-1 to C.H.

The authors declare no conflict of interest.

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