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Letter to the editor

A child with epidermolytic ichthyosis from a parent with epidermolytic nevus: risk evaluation of transmission from mosaic to germline

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***Short title*:** Epidermolytic ichthyosis from nevus

***Abbreviations*:**
EN, epidermolytic nevus; *KRT1*, keratin 1 gene; *KRT10*, keratin 10 gene; EI, epidermolytic ichthyosis; gDNA, genomic DNA

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(1) mosaicism, (2) epidermolytic nevus, (3) epidermolytic ichthyosis, (4) *KRT10*, (5) next-generation sequencing (NGS)

MAIN TEXT

Epidermolytic nevus (EN) is a rare subgroup of epidermal nevi, caused by somatic mutations in *KRT1* or *KRT10*. Epidermolytic ichthyosis (EI) is caused by autosomal dominant germline mutations in *KRT1* or *KRT10*. Rarely, EN can produce EI in the next generation (Chassaing et al., 2006, Nazzaro et al., 1990, Paller et al., 1994). In those cases, somatic mutations causative of EI in a parent involve germline cells and are transmitted to children. Here we report a female EI patient with a typical phenotype, and her father with EN caused by a mutation in *KRT10*. In her father, we investigated the level of mosaicism in some tissues, including semen, by next-generation sequencing (NGS). From the mutant rates in sperm, we evaluated the risk of disease transmission from a father with EN to a child at future pregnancies.

A 2-year-old Japanese girl, the proband, was born to unrelated parents as their first child. She showed diffuse erythema, multiple blisters and erosions on the whole body at birth. With growth, she showed scales and hyperkeratosis on the entire body surface (Figure 1a). Skin biopsy revealed hyperkeratosis, acanthosis, acantholysis and granular degeneration. She was clinically diagnosed with EI.

Her father presented hyperkeratotic lesions on the right upper limb and the right lumbar and groin regions (Figure 1b-d). The lesions on the upper limb seemed to follow the Blaschko's lines. The affected skin accounted for 0.5% of his whole body surface. The lesions had been present since birth. Histological features of the lesional skin were identical to those seen in his daughter (Figure 1e).

The local institutional review board approved this study, and all participants provided written informed consent. First, mutation analysis identified a heterozygous mutation, c.446C>T (p.Arg156Cys) in *KRT10* of genomic DNA (gDNA) from the proband (Figure 2a). The present mutation is a known causative mutation of EI (Rothnagel et al., 1993). Then, we investigated gDNA extracted from the skin sample of the EN lesion of her father and detected the identical mutation as a lower peak of the chromatograph than that of wild-type *KRT10*. The mutant allele frequency was estimated as approximately 14.9% from the ratio of the heights of each chromatograph peak (Figure 2a).

In considering future children, the parents wanted to know what percentage of the father's gametes had the mutation. Thus, we investigated the percentage of sperm with the pathogenic *KRT10* mutation in semen from the proband's father by NGS (amplicon sequencing) using the MiSeq system (Illumina, San Diego, CA) (details in the supplementary method). We evaluated that approximately 3.9% of his semen had the causative mutation (122,653 reads on average, mutant allele frequency: 3.9 ± 0.17%) and that the mutant allele frequency in his peripheral blood was 5.3 ± 0.11% (Figure 2b, c, S1 and Table S2), although we were unable to detect the mutation in either of the samples by Sanger sequencing (Figure 2 a). These results clearly indicate the limited ability of Sanger sequencing to find low-level mosaicism. Our results show that this system is able to accurately measure the mutant allele frequency from 0.27% to 12.8% (Figure 2b and Table S2). The mutant allele frequency in his lesional skin, including in the dermis, was 12.0%. In EN lesions, it was previously reported that the mutant rate of dermal fibroblasts is lower than that of epidermal keratinocytes even in the involved skin area (Paller et al., 1994). Thus, the mutant allele frequency in the lesional skin was much lower than 50% of that in EI patients.

To date, only five EI cases in which the parents have EN have been diagnosed by genetic analysis (Chassainget al., 2006, Paller et al., 1994). The causative mutation in a parent's gametes has not been proven directly in any case. In the present study, we investigated the precise prevalence of cells carrying the causative *KRT10* mutation in the skin lesions, the white blood cells in peripheral blood, and the gametes by NGS.

The prevalence of epidermal nevus is 1/1000 (Paller et al., 1994). EN is clinically indistinguishable from other types of epidermal nevi, but it can be histopathologically differentiated from them (Nazzaro et al., 1990). Thus, we recommend using a punch biopsy of the skin lesion to differentiate EN. In cases of EN, genetic diagnosis of the skin lesion and proper genetic counseling on the risk of disease transmission to the next generation would be beneficial to patients who wish to bear children. To determine the exact mutation transmission probability, we should consider not only the mutant allele frequency in sperm, but also the posibility that mutant sperm might have either a decreased or an increased chance of fertilising an egg. In addition, possible

contamination by wild or mutant leukocytes and genitourinary epithelial cells might have the potential to influence the results. However, contaminating leukocytes are thought account for less than 6.3% of all cells and the number of contaminating urogenital epithelial cells is considered to be small in semen samples (World Health Organization, Department of Reproductive Health and Research, 2010). Thus, we think the impact of any non-sperm cells contaminating the semen sample would likely be insignificant to the mutation allele frequency in the father's sperm. According to our trial calculation, even if the semen sample contained the maximum consensus number of leukocytes, the results of the mutant allele frequency in the father's sperm would deviate by only 0.2% (3.7%) from that of the present data.

The present study clearly demonstrates that the evaluation of rates of mutant gametes in semen samples by NGS is very effective and highly recommended even for a parent with only small, limited lesions, so that the parent can know the risk of transmitting the disease to the next generation in mosaic disorders, especially in mosaic diseases of the skin in which the mosaic conditions can be clinically recognized. However, this type of

examination is limited to affected fathers. Parental mosaicism has been found in several genetic non-dermatologic diseases (Biesecker and Spinner, 2013). However, in most cases, parental mosaicism was identified retrospectively only after affected children were born (Chen et al., 2014, Tan et al., 2015, Xu et al., 2015). In contrast, in mosaic skin disorders, parental mosaicism can be identified clinically before affected children are born. The present case suggests that we should take advantage of our ability to clinically recognize parent mosaicism in order to evaluate the risk of disease transmission and provide genetic counseling.

MEDICAL IMAGES

The patient/guardian gave consent to publish their photographs.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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FIGURE LEGENDS

Figure 1. Clinical and histopathological features of the skin lesions of epidermolytic ichthyosis (EI) in the proband and epidermolytic nevus (EN) in the father. (a) The proband has diffuse hyperkeratosis with scales and multiple erosions on the whole body. (b) Hyperkeratotic lesions appear on the right upper arm, the right hands and the right half of the lumbar and groin areas in the proband's father. The lesions on the trunk seem to follow Blaschko's lines. (c, d) Close-up photographs of the skin lesion on the right hand (c) and the groin areas (d) in the proband's father. (e) Histological examination of the scaly lesion on the paraumbilical abdomen of the proband's father reveals thick orthokeratotic hyperkeratosis and granular degeneration in the epidermis. Those features were identical to those seen in his daughter. Hematoxylin and eosin stain. Scale bars = 100 μm.

Figure 2. *KRT10* mutation detection in the proband and her parents by direct sequencing and mutant allele frequency measurements by next-generation sequencing. (a) The heterozygous mutation c.446C>T (p.Arg156Cys), which is in the

helix initiation region of the 1A domain and is critical for the assembly of keratin filaments, is detected in exon 1 of *KRT10* in the gDNA sample from the proband's peripheral blood. The identical *KRT10* mutation p.Arg156Cys is detected in the gDNA sample from the EN lesion of her father, as a lower peak of the chromatograph than that of wild-type *KRT10* (the mutant allele frequency, approximately 14.9% from the ratio of heights of each peak of chromatograph). A peak of the mutant allele is unrecognizable in gDNA samples from the father's blood and semen. No pathogenic *KRT1* mutation was found in the proband and her parents, and no pathogenic *KRT10* mutation was found in her mother. (b) The accuracy of mutant allele frequency measurements is confirmed by next-generation sequencing (NGS). Six points of the mutant allele frequencies of standard samples (12.8%, 5.16%, 2.35%, 1.12%, 0.55%, 0.27%) prepared by the mixture of gDNA from the proband and a healthy control were measured. Horizontal axis: theoretical values of the mutant allele frequency. Vertical axis: actual values of the mutant allele frequency (%) measured by NGS. The approximation curve is $y = 0.8724x – 0.0678$ and the determination coefficient is approximately 1. (c) The mutant allele frequency in gDNA samples from the saliva of

the proband and from the EN lesion, blood and sperm of the proband's father was determined by amplicon sequencing. Approximately 3.9% of the father's sperm has the causative mutation (mutant allele frequency, 3.9 ± 0.17%) and the mutant allele frequency in his peripheral blood cells is 5.3 ± 0.11%, as obtained by deep sequencing (122,653 reads for the semen and 100,053 reads for the blood cells). The mutant allele frequency in his EN lesion, including the dermis, as determined by deep sequencing (108,441 reads) is 12.0 ± 0.47%. The mutant allele frequency in the proband's saliva is 49.6 ± 0.47% as determined by deep sequencing (100,825 reads).