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Revertant Mosaicism in Ichthyosis with Confetti Caused by a Frameshift Mutation in *KRT1*

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TO THE EDITOR

Ichthyosis with confetti (IWC; MIM #609165) is a rare autosomal dominant disorder characterized by widespread ichthyosiform erythroderma and concomitant revertant skin patches resulting from the somatic loss of a disease-causing mutation (Choate et al., 2010). IWC is caused by frameshift mutations in *KRT10* (IWC-K10) or *KRT1* (IWC-K1) (Choate et al., 2010; Choate et al., 2015). Because the latter is extremely rare, with only one reported case in the literature, the clinicopathological features of IWC-K1 remain poorly understood. In this study, we analyzed a second family with IWC carrying a *KRT1* mutation. The local institutional review board approved this study, and all participants provided written informed consent.

In 2008, a 56-year-old Japanese woman was referred with widespread dry, flaky skin that had been present since birth (see Supplementary Figure S1a online). Her son was also affected (see Supplementary Figure S2a online). An initial physical examination showed palmoplantar keratoderma and mild generalized ichthyosis (Figure 1a, and see Supplementary Figure S1a). Moreover, her abdomen and extremities were diffusely erythematous. Skin biopsy results showed hyperkeratosis, parakeratosis, and acanthosis (see Supplementary Figure S1b). Cells with perinuclear vacuolization and two nuclei were also observed in the epidermis. Ultrastructurally, shell-like, electron-lucent areas around the nuclei of keratinocytes were observed (see Supplementary Figure S1c). Mutation analysis identified a heterozygous mutation, c.1758_1759insT (p.Tyr587-LeufsTer67) in *KRT1* (see Supplementary Figure S1d), previously unreported to our knowledge, in both the proband and her son. This mutation resulted in an erroneous C-terminal extension and an arginine-rich C-terminal domain: the mutant keratin 1 (KRT1) had 15 arginine residues after tyrosine 587, whereas wild-type KRT1 contained only five arginine residues (see Supplementary

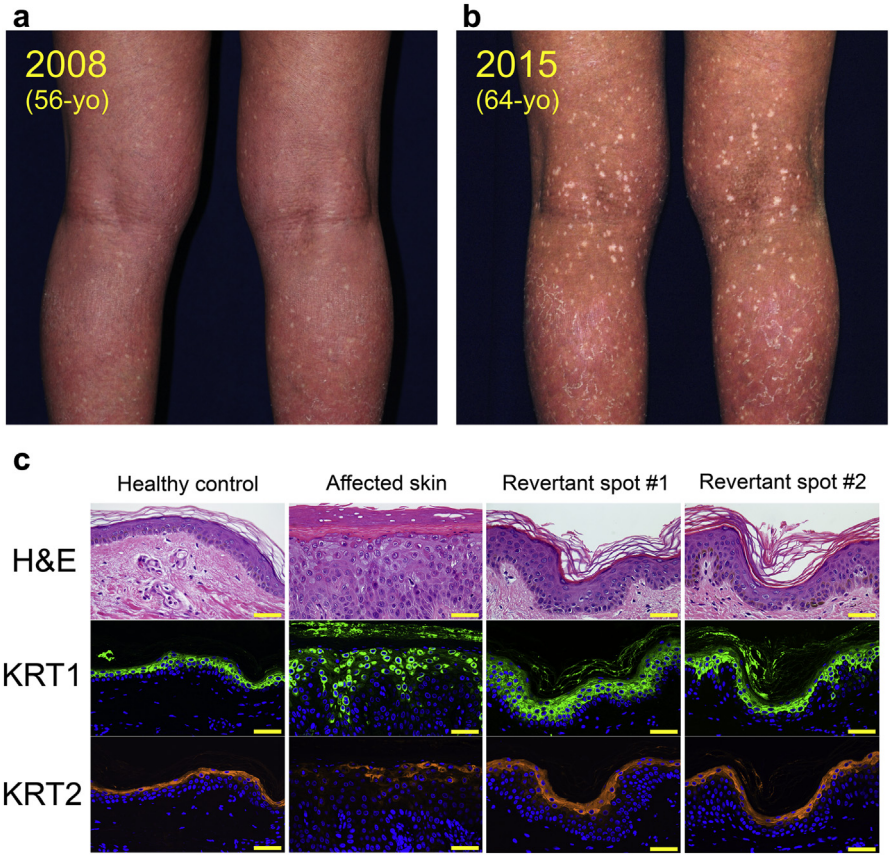

Figure 1. Clinical and histological features of revertant skin. (**a, b**) The proband showed marked increases in the number and size of normal-appearing spots between (**a**) 2008 and (**b**) 2015. The patient gave permission to publish her photographs. (**c**) Histological comparison between the affected epidermis and spots 1 and 2 on the epidermis. The affected epidermis showed hyperkeratosis, parakeratosis, and acanthosis with perinuclear mislocalization of keratin 1, with a marked reduction of keratin 2 expression. Spots 1 and 2 on the epidermis exhibited histological recovery. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Scale bar = 50 μm. H&E, hematoxylin and eosin; KRT1, keratin 1; KRT2, keratin 2; yo, years old.

Abbreviations: IWC, ichthyosis with confetti; IWC-K1, ichthyosis with confetti caused by frameshift mutations in KRT1*; IWC-K10, ichthyosis with confetti caused by frameshift mutations in* KRT10*; KRT1, keratin 1; LOH, loss of heterozygosity*

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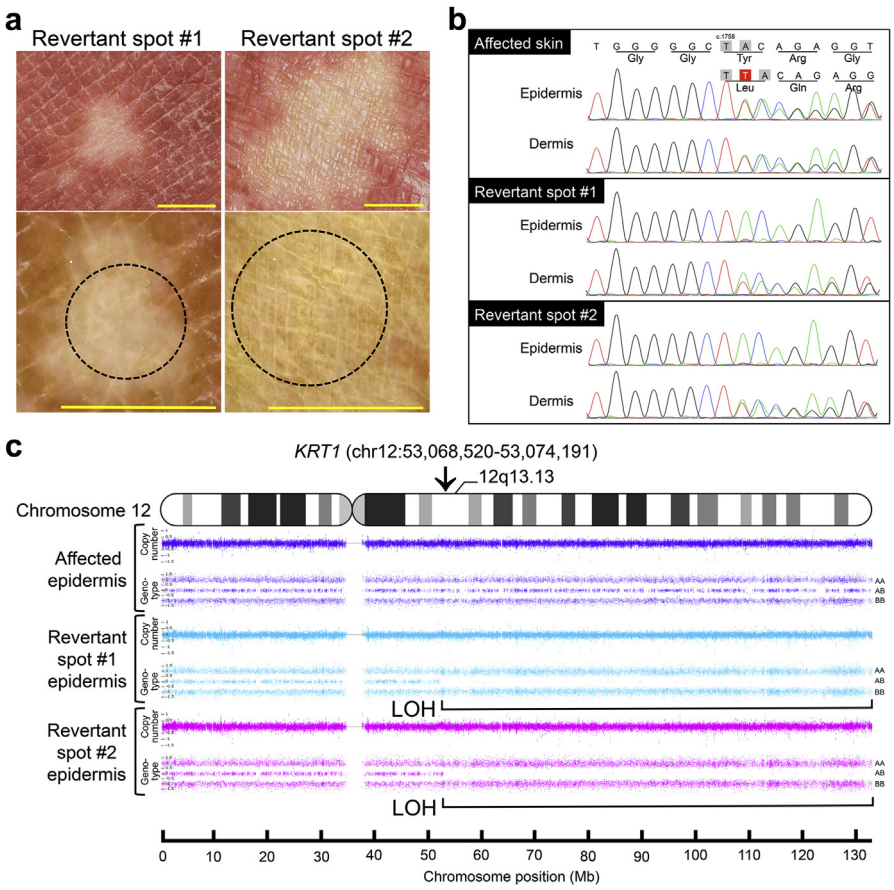

Figure 2. Somatic loss of the disease-causing mutation resulting from copy-neutral LOHs. (**a**) A dermoscopic view of revertant spots 1 and 2. Scale bar = 5 mm. The dotted circles illustrate the punch biopsy sites. (**b**) The disease-causing mutation c.1758_1759insT in *KRT1* (RefSeq: NM_006121.3) was present in both the epidermis and dermis in the affected skin. The mutation was absent in the epidermis of both spots of the unaffected skin, whereas it was present in the dermis. (**c**) Whole-genome SNP microarray identified copy-neutral LOHs on chromosome arm 12q in revertant spots 1 and 2, whereas no LOHs were detected in the affected epidermis. In both spots, the LOHs started from regions centromeric to *KRT1* and extended to the telomere of the chromosome. Arg, arginine; Gly, glycine; Leu, leucine; LOH, loss of heterozygosity; SNP, single nucleotide polymorphism; Tyr, tyrosine.

Figure S3 online). The overexpression of the N-terminal green fluorescent protein-tagged mutant KRT1 in the HaCaT cells resulted in an aberrant accumulation of KRT1 in or around the nucleus (see Supplementary Figure S4 online), suggesting the pathogenicity of the mutation.

In 2015, the patient revisited our hospital for the first time since 2008. She exhibited approximately 1,000 normal-appearing spots, up to 15 mm in size, on her extremities (Figure 1b). Looking back at the initial presentation in 2008, we noticed approximately one hundred 1- to 2-mm–sized white spots (Figure 1a). She recognized a few such spots for the first time at age 30 years. Her affected son (who was 34 years old in 2015) also showed 34 normal-appearing spots up to 2 mm in diameter; however, these spots were found only around his popliteal fossae (see Supplementary Figure S2b). Her 7-month-old granddaughter, who was also heterozygous for the mutation, did not present with any white spots, although hyperkeratotic erythema was evident on her extremities (see Supplementary Figure S2c). We performed skin sampling from two of the normal-appearing patches (Figure 2a) and from one of the affected patches of the proband. Notably, the stratum corneum of the normal-appearing patches showed a normal basket-weave pattern without hyperkeratosis, parakeratosis, or acanthosis (Figure 1c). Immunostaining of the affected skin showed aberrant perinuclear localization of KRT1 and reduced protein levels of keratin 2, whereas the normal-appearing skin exhibited normal patterns of these protein signals (Figure 1c). Thus, the white patches were histologically verified as cured. We next determined the *KRT1* genotypes of the two white spots using genomic DNA separately extracted from the epidermis and dermis. The mutation was absent in the epidermis of both spots but retained in the dermis (Figure 2b), suggesting that the somatic reversion of the mutation caused the phenotypic recovery. Collectively, we diagnosed the proband with IWC-K1.

To elucidate the mechanism that underlies the reversion of the mutation, we performed genome-wide single nucleotide polymorphism (SNP) genotyping, which identified copy-neutral losses of heterozygosity (LOHs) on chromosome arm 12q in only the revertant spots (Figure 2c). These LOHs started from regions centromeric to *KRT1* and extended to the telomere of the chromosome. In contrast, no large LOHs were detected elsewhere. These findings show that the reversion of the mutation resulted from mitotic recombination.

The affected individuals with IWC-K1 in this study displayed phenotypes distinct from those of IWC-K10. They showed lower intensities of erythema and hyperkeratosis, with a less extensive area of involvement and smaller revertant spots than those of IWC-K10. Moreover, ectropion, ear malformation, mammillae hypoplasia, and short stature, which are all common clinical signs of IWC-K10 (Guerra et al., 2015), were not observed in our patients. Our results, together with the first report of IWC-K1 that documented these differences (Choate et al., 2015), suggest that IWC-causing *KRT1* mutations might lead to phenotypes distinct from those of IWC-K10. Although the histology of the first IWC-K1 patient showed prominent coarse keratohyalin granules without parakeratosis (Choate et al., 2015), our proband exhibited widespread parakeratosis with fewer coarse keratohyalin granules. A mutation analysis of the gene encoding filaggrin (Nomura et al., 2008) identified a heterozygous nonsense mutation, c.8666_8667CC>GA, in our proband that likely explains the reduced keratohyalin granules.

In the present family, three affected individuals showed a marked difference in their number of revertant spots. Similarly, in the first IWC-K1 family, a 35-year-old proband presented with multiple revertant spots, whereas his three affected offspring aged 3 to 9 years showed no such spots (Choate et al., 2015). The probands in both the former and latter families noticed normal skin spots for the first time at ages 30 and 22 years, respectively, with the number and size of revertant spots increasing with age. These findings suggest that revertant spots are recognized by patients with IWC-K1 in their twenties or thirties, whereas such spots usually appear by age 3 years in patients with IWC-K10 (Choate et al., 2015). Remarkably, the number of revertant spots dramatically increased in our proband even after her late fifties. Thus, age might be a key factor for the development of a recognizable size of revertant spots in patients with IWC-K1.

Because the reversion of disease-causing mutations is not observed in the epidermolytic ichthyosis (MIM #113800) caused by missense or truncation mutations in *KRT1* or *KRT10*, this study suggests a possible link between C-terminal frameshift mutations in *KRT1* or *KRT10* and the high frequency of revertant mosaicism in patients with IWC. Elucidating the as yet uncovered molecular basis of revertant mosaicism might pave the way for the development of therapies for this intractable disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.05.109.

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Large Intragenic *KRT1* Deletion Underlying Atypical Autosomal Dominant Keratinopathic Ichthyosis

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TO THE EDITOR

Mutations in *KRT1* (keratin 1) or *KRT10* (keratin 10) underlie a spectrum of diseases known as keratinopathic ichthyoses (Hotz et al., 2016; Oji et al., 2010). Most commonly, heterozygous missense mutations within the head/tail domains result in autosomal dominant epidermolytic ichthyosis (EI), although a spectrum of mutations (some of which may be recessive) underlie a diverse collection of phenotypes that include generalized, annular, superficial (occasionally; mostly keratin 2), and nevoid forms of EI, as well as palmoplantar keratoderma (diffuse, focal, striate), severe variants such as Curth-Macklin ichthyosis, and most recently, ichthyosis with confetti (IWC) (for review on keratin diseases, see Knöbel et al., 2015). The latter usually results from downstream frameshift mutations that result in an arginine- or alanine-rich tail to the keratin leading to nuclear retention of mutant keratin and/or intermediate filament dysfunction (Choate et al., 2010, 2015). Almost all pathogenic variants reported, including in EI and IWC, have been point mutations (www.interfil.org/).

In this report, we describe an atypical heterozygous *KRT1* mutation, a large approximately 2.2 kb intragenic deletion mutation in an autosomal dominant pedigree containing six affected individuals over four generations with heterogeneous clinical features resembling IWC or EI. The proband is a 64-year-old woman. She was born as a collodion baby, but within a few weeks she became erythrodermic with just a few islands of sparing. Palm and sole skin became thicker although the rest of her skin was reported to be fragile. With increasing age, several pale macules developed on her trunk and limbs. She also reported a previous ulcerated basal

Abbreviations: EI, epidermolytic ichthyosis; IWC, ichthyosis with confetti

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