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# Accepted Manuscript

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Letter to the Editor

JID-2016-0405.R1

**Large intragenic *KRT1* deletion underlying atypical autosomal dominant keratinopathic ichthyosis**

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**Short title:** *KRT1* intragenic deletion causing ichthyosis

**Keywords:** ichthyosis with confetti; epidermolytic ichthyosis

**Abbreviations:** IWC, ichthyosis with confetti; EI, epidermolytic 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 (Oji et al., 2010; Hotz et al., 2016). 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/](http://www.interfil.org/)).

In this report, we describe an atypical heterozygous *KRT1* mutation, a large ~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 female. 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 cell carcinoma on the forehead (surgically excised). Examination of her skin as an adult revealed diffuse erythroderma with hundreds of confetti-like spots on the trunk and extremities in keeping with IWC (Fig. 1a), and palmoplantar keratoderma. Nails showed longitudinal ridging but no hair abnormalities were observed.

Following informed and written consent, skin biopsies were obtained from both the affected and unaffected areas of abdomen (Fig. 1b and c). Transmission electron microscopy from the affected area showed focal keratinocyte aggregation and early cytolysis through the spinous layer as well as perinuclear shells/vacuoles (Fig. 1d). In addition, there was an increased number of keratohyalin granules that were coarse and angulated noted from the upper-spinous layer to the granular layer (Fig. S1). Immunofluorescence microscopy on skin samples from affected and unaffected skin (Fig. 1e) and unrelated healthy control skin was performed using anti-keratin antibodies (Fig. 1f). There was a marked reduction in staining intensity for keratin 1 and keratin 10 in the affected epidermis compared to unaffected or control skin. Moreover,

no nuclear keratin 1/10 labeling was detected. Unaffected patient skin labeling resembled control skin apart from keratin 2 staining which was increased in the unaffected patient skin compared to both affected patient skin and control. Immunostaining for keratin 5 and 14 showed no major differences between any of the three skin samples. Within the rest of the family (Fig. 1g; proband is II-2), her late mother (I-2) and her daughter (III-2) had similarly abnormal skin, whereas the proband's three grandchildren (IV-1, IV-2 and IV-3, now aged 12 years, 9 years and 6 years, respectively) all had skin changes resembling EI with erythema, erosions and large islands of sparing on the face, neck, chest and abdomen (Fig. 1h), as well as moderate palmoplantar keratoderma (Fig. 1i). Indeed, keratoderma was present in all affected individuals but none of the youngest generation had clinical signs of IWC.

Next, after obtaining ethics approval and informed consent, we extracted genomic DNA from either peripheral blood or saliva samples from the patient, other family members and controls in compliance with the Helsinki Guidelines. Using four affected members' DNAs (I-2, II-2, III-2 and IV-2), whole-exome sequencing was performed on the Illumina HiSeq 2000 (San Diego, CA, USA), as described elsewhere (Campbell et al., 2014). Then, copy number variation analysis was performed by using four exome data sets. After excluding pathogenic mutations in genes implicated in

different forms of ichthyosis, the exome shared variant profile was filtered to look for novel heterozygous mutations. The only shared gene abnormality was a heterozygous deletion in *KRT1*. We then confirmed the accurate deletion points in intron 1 and intron 5 in *KRT1* of genomic DNA by PCR and Sanger sequencing (Fig. 2a and b; see Supplementary Methods for details). The deletion spans 2,209-bp of genomic DNA (chr12:53,071,005-53,073,213; GRCh37/hg19) and at a protein level it removes (in-frame) part of the 1A domain and all of the 1B and 2A domains (amino acids 197-375) (Fig. 2c). This deletion is not represented in the dbSNP database, the 1000 Genomes database, DECIPHER or in 2000 unrelated European in-house control exomes. By semi-quantitative PCR using genomic DNA we were also able to demonstrate reduced presence of the deletion mutation in the proband's pale skin spots compared to her erythematous skin or peripheral blood DNA (Fig. 2d; see also Supplementary Methods for details). Apart from generating a truncated keratin 1 peptide, it is also likely that the deletion mutation creates other transcripts that are degraded at RNA level since *KRT1* expression was reduced in red (lesional) skin in two subjects tested (Fig. S2). Microsatellite analysis (Fig. S3) provides support for mitotic recombination as the likely corrective mechanism for the IWC phenotype, as has been shown in detail elsewhere (Choate et al., 2010; 2015): typically there is copy neutral loss-of-heterozygosity from a

proximal breakpoint to the telomere with mitotic recombination allowing expression of the normal allele. In contrast to the reported cases of IWC, however, the causative pathology differs from that seen in our pedigree. The prototypic molecular pathology of IWC involves frameshift mutations towards the 3' end of the gene that lead to a polyarginine or polyalanine keratin tail, protein mislocalization to the nucleus, and intermediate filament collapse (Hotz et al., 2015; Lim et al., 2016). The much larger upstream in-frame deletion in our case appears to result in clinical features that overlap with IWC although the skin pathology, as well as the extended phenotype in the family, clearly incorporates some of the features of EI. Notably, transmission electron microscopy showed mixed findings with keratin filament aggregation and cytolysis in the spinous layer, as well as prominent keratohyalin granules, both consistent with EI, although some perinuclear shells/vacuoles supported IWC. Thus, although more than 50 mutations in *KRT1* have been reported, our pedigree appears to expand genotype-phenotype correlation and underscores the considerable heterogeneity underlying keratinopathic ichthyoses. Our findings also demonstrate that copy number analysis by next generation sequencing is a useful tool to identify pathogenic changes that elude conventional Sanger sequencing of keratin genes.

**The authors have no conflicts of interest to declare.**

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## Figure legends

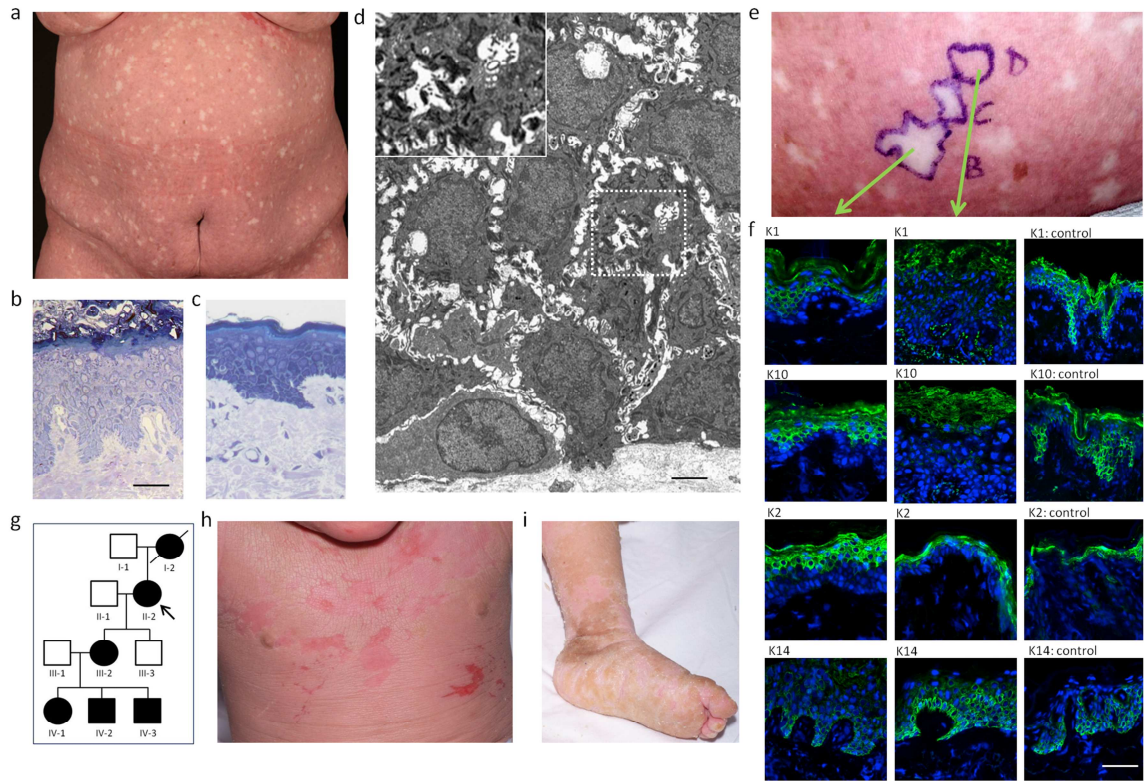
### Figure 1. Clinicopathologic features of this atypical *KRT1* keratinopathic ichthyosis.

(a) Diffuse truncal erythema with widespread pale macules of normal appearing skin in the proband resembling IWC; (b) light microscopy of affected (red) skin (trunk) showing acanthosis and hyperkeratosis (bar = 25  $\mu$ m); (c) light microscopy of reverted (pale) skin (trunk) demonstrating correction of the pathologic findings seen in (b) (bar = 25  $\mu$ m); (d) transmission electron microscopy of skin shown in (b) reveals spinous layer changes with both focal keratin filament aggregation and cytolysis (shown at higher magnification in boxed inset) as well as perinuclear vacuoles (bar = 2  $\mu$ m) (see also Fig. S1); (e) higher magnification of affected/reverted skin phenotype highlighting respective erythema and pallor; (f) immunofluorescence microscopy for keratin 1, 10, 2 and 14 in pale and red patient skin as well as normal control skin. Affected skin shows reduced expression of keratin 1 and 10 (bar = 25  $\mu$ m); (g) The pedigree shows autosomal dominant inheritance with 6 affected individuals over 4 generations (arrow indicates proband); (h) Clinical heterogeneity is noted in individual IV-2 whose skin (shown here aged 4 years) displays erythema and erosions in keeping with EI; (i) individual IV-2 also has acral hyperkeratosis involving the lower leg and sole. Individuals IV-1 and IV-3 also show features of EI and not IWC. Written consent and approval to

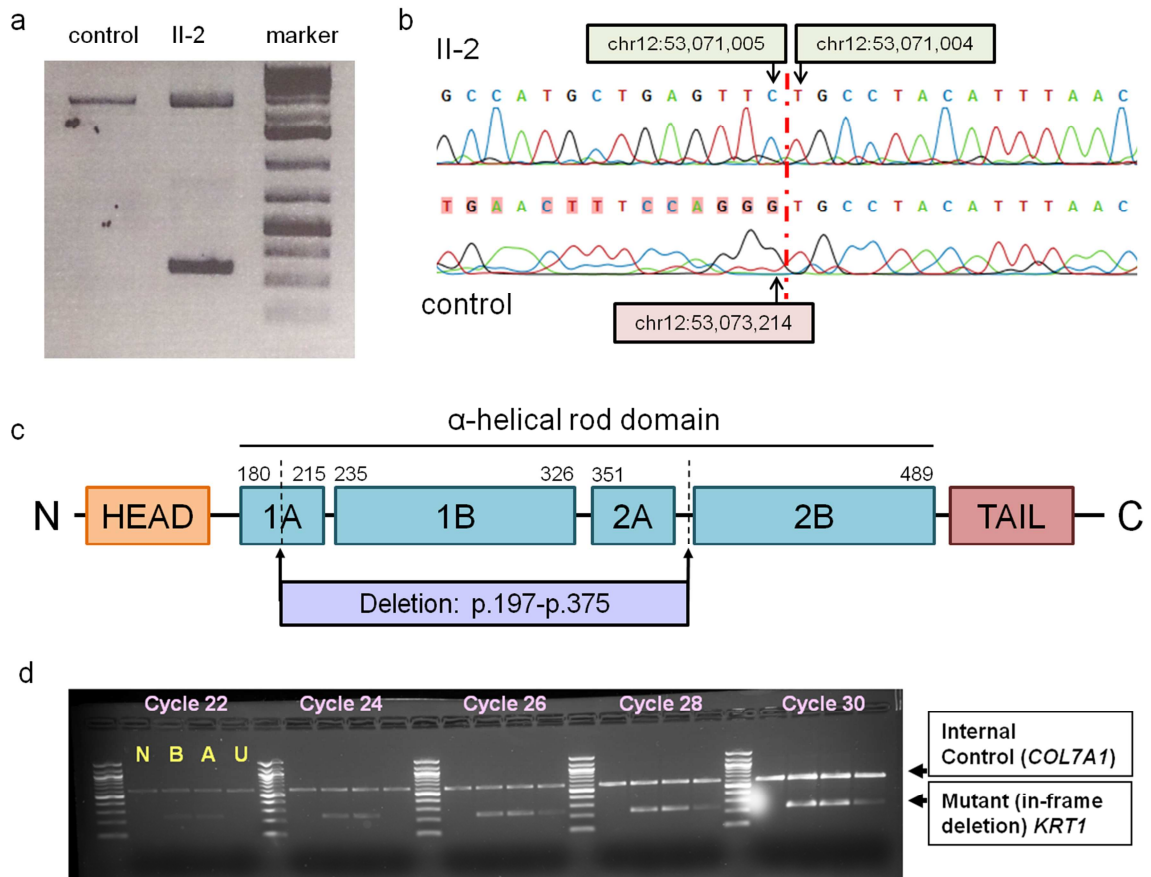
include these clinical images was obtained from the family members.

**Figure 2. Molecular pathology of the *KRT1* keratinopathic ichthyosis**

(a): Long-range PCR across the deletion indicated by the NGS data reveals a single band on agarose gel electrophoresis in control DNA but two bands in the Proband (II-2) consistent with a heterozygous internal deletion of ~2.2 kb; (b) Sanger sequencing shows the precise deletion point within *KRT1* on Chr 12 (see Supplementary Data for PCR Methods); (c) Schematic of keratin 1 and its functional domains illustrating that the deletion removes amino acids 197 to 375 inclusive; (c) Semi-quantitative PCR on genomic DNA using primers specific for the in-frame *KRT1* deletion (lower band) with an internal control (top band, *COL7A1*). Four templates were used (N = normal unrelated control; B = proband DNA from blood; A = proband DNA from affected skin; U = proband DNA from unaffected skin). The mutant transcript is evident for B and A from 22 cycles onwards but only starts to appear at cycle 26 in U (and not at all in N). Thus there is reduced presence of mutant DNA in the unaffected/reverted skin (see Supplementary Data for Methods).



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