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A novel microdeletion in *LOR* causing autosomal dominant loricrin keratoderma

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Dear Editor, The dramatic presentation of a collodion baby is most commonly associated with a diagnosis of autosomal recessive ichthyosis (ARCI). We investigated a family wherein the father and all four children were collodion at birth, implying an autosomal dominant inheritance pattern. Postnatally, the affected individuals presented with variable degrees of generalized ichthyosis and palmoplantar keratoderma (PPK) (Fig. 1). These clinical features were more pronounced in the father than in the children, and were reported to have become more prominent with age. The phenotype was of generalized nonerythematous xerosis and fine scaling, with adherent larger scale on the lower legs, and mild PPK that exhibited honeycomb patterning. The mother was unaffected and the parents were nonconsanguineous. One of the children also had moderate neurodevelopmental delay and microcephaly, and the father had asthma.

With research ethics committee approval, blood was taken from one child and both parents, and DNA was extracted using standard methods. Skin scrapings were obtained from the father and the child, with a 4-mm punch skin biopsy taken from the father. Next-generation sequencing of leucocyte DNA was performed using a Nextera Custom Enrichment Kit (Illumina, San Diego, CA, U.S.A.) targeting exonic regions of clinically relevant published dermatology genes, including known ichthyosis and PPK genes (Table S1; see Supporting Information). A 50-ng DNA sample was simultaneously fragmented using Illumina Enrichment Sample Prep and tagged with adaptors. Polymerase chain reaction (PCR) introduced sample-specific indexes to the library, and following quantification this was hybridized to the custom-capture probes. Streptavidin beads captured the probes containing the targeted regions of interest and a series of washes removed nonspecific binding from the beads. This enrichment and capture was repeated once to enrich the target regions further. The enriched library was amplified by limited rounds of PCR, quantified, diluted and sequenced on the Illumina MiSeq. Analysis was using an in-house pipeline, where FASTQ files were aligned using Burrows–Wheeler Aligner[1] and indexed with SAMtools (http://samtools.sourceforge.net/).[2] Variant calling was performed with VarScan (http://varscan.sourceforge.net/),[3,4] and VCF files were annotated with Variant Effect Predictor (http://www.ensembl.org). Annotated variants were then filtered in Microsoft Excel, BAM files reviewed manually and mutations confirmed using Sanger sequencing.

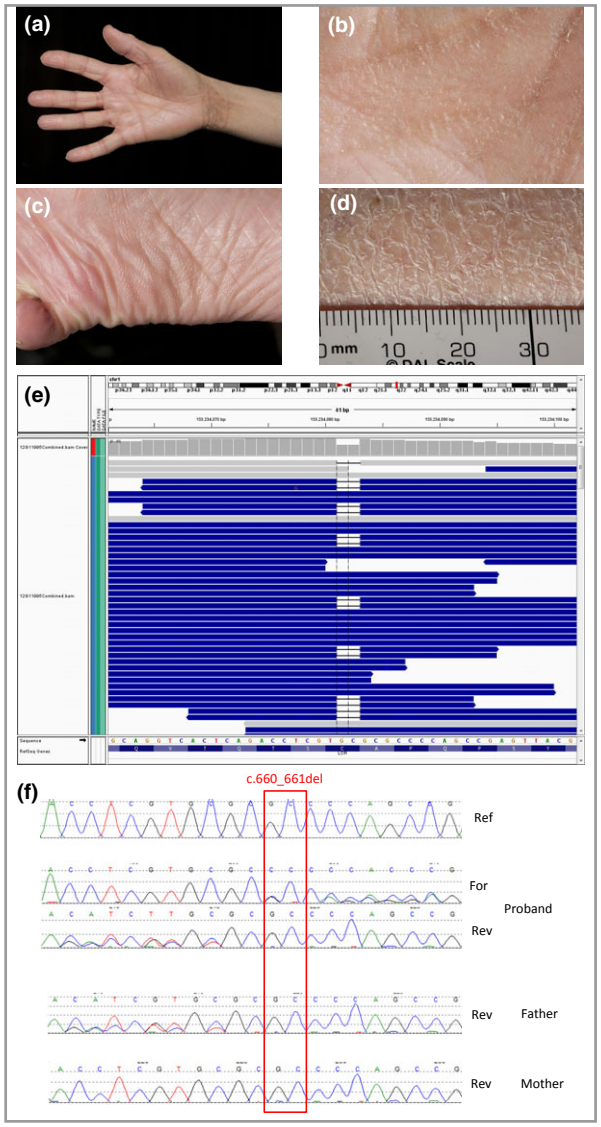

Fig 1. (a) Transgradient palmar keratoderma, (b) honeycomb patterning on the palm and (c) sole, (d) ichthyosis on the lower leg, (e) microdeletion in *LOR* shown by next-generation sequencing and (f) confirmed by Sanger sequencing.

A novel heterozygous deletion inducing a frameshift was found in the loricrin gene Chr1 (GRCh37): g.153234085_153234086del, *LOR* c.660_661delGC, p.Gln222Alafs*113,

predicted in *silico* as possibly damaging (Polyphen score 0.799; http://genetics.bwh.harvard.edu/pph2/) (Fig. 1). This established a diagnosis of loricrin keratoderma (previously known as Vohwinkel syndrome with ichthyosis, or Vohwinkel syndrome variant), distinct from classical Vohwinkel syndrome by the co-occurrence of generalized ichthyosis and the absence of hearing loss.[5] The two conditions are genotypically distinct, with Vohwinkel syndrome due to mutations in *GJB2* and Vohwinkel with ichthyosis due to mutations in *LOR*.[6] The occurrence of constrictive digital bands leading to autoamputation is variably reported to be a feature of the variant form, but was not present in this family.

Comparison of this novel mutation with previously described pathogenic mutations in this form of loricrin keratoderma revealed a common mechanism of frameshift indels affecting the C terminus of the protein,[7] with this being the first deletion reported in the literature. To characterize this mutation further we performed Western blotting for loricrin monomers and dimers on skin scale and whole-skin lysate, and immunofluorescence for loricrin, interleukin (IL)-1α, keratin 1 and mouse double minute 2 homologue (MDM2) on whole skin (Data S1; see Supporting Information). Monomeric and dimeric loricrin were clearly detectable in the patient's skin lysate compared with the normal skin lysate, where only the monomeric form was detected, There was weak expression of dimer in patient skin scale, compared with no free loricrin detectable in control scale (Fig. 2a). This defective incorporation of loricrin into the cornified envelope is analogous to that seen in ARCI.[8] The domain of loricrin expression was expanded in patient hyperkeratotic skin, with nucleolar expression of loricrin as previously reported with insertions (Fig. 2b).[7] Histological analysis of patient skin showed not only hyperkeratosis, but also focal areas of parakeratosis (Fig. 2c)

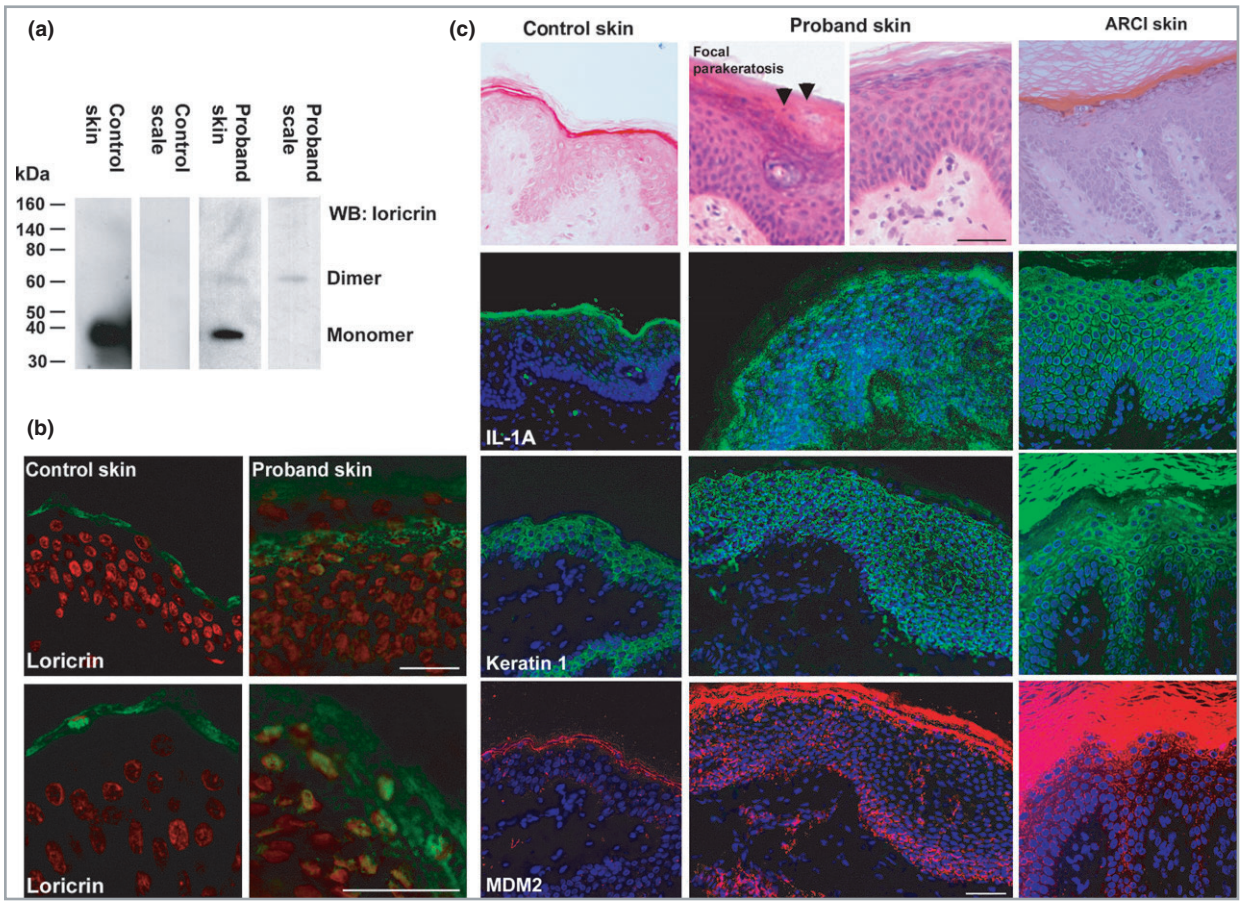

Fig 2. Western blots (WB) and immunofluorescence of the proband reveal features common to both loricrin keratoderma and autosomal recessive ichthyosis (ARCI). (a) Western blot of whole-cell lysates and scale from the proband and an unaffected control. Note the monomeric and dimeric loricrin in the proband skin lysate. No free loricrin is detectable in the control scale, while weak expression of loricrin dimer is detected in the proband scale. These data specifically indicate the change in expression of wild-type loricrin as a result of the presence of the mutant loricrin. (b) Immunofluorescence of loricrin in the proband and control skin. The domain of loricrin expression is expanded in proband skin. (c) Histology and immunofluorescence of interleukin (IL)-1A, keratin 1 and mouse double minute 2 homologue (MDM2) in control skin, proband skin and representative samples of ARCI skin.[9] Focal parakeratosis is indicated in the proband skin. Expression of both IL-1A and MDM2 is increased in the spinous and basal layers of both proband and ARCI skin, while keratin 1 increase is seen only in the ARCI skin. Bar 50 μm (b, both top and bottom panels, and c).

The upregulation of IL-1α and MDM2 occurs in all lamellar-type ARCIs irrespective of genotype,[9] and is consistent with the hyperkeratosis observed in MDM2-overexpressing mice.[10] The upregulation of these proteins in patient skin provides further support for a common mechanism for hyperkeratosis.[8,9] Interestingly keratin 1 expression was not increased in affected skin, further refining the hyperkeratosis signature common to ichthyoses due to different genetic defects.

These results describe a novel mutation and the first deletion leading to loricrin keratoderma unusually presenting with collodion membrane, described only once previously,[11] and provide further evidence of a common, MDM2-mediated gene expression pathway leading to ichthyosis and collodion membrane formation. This diagnosis should be considered as a rare cause of the clinical presentation of collodion baby.

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References

1 Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler Transform. *Bioinformatics* 2009; **25**:1754–60.
2 Li H, Handsaker B, Wysoker A *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 2009; **25**:2078–9.
3 Koboldt DC, Chen K, Wylie T *et al.* VarScan: variant detection in massively parallel sequencing of individual and pooled samples. *Bioinformatics* 2009; **25**:2283–5.
4 Koboldt D, Zhang Q, Larson D *et al.* VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res* 2012; **22**:568–76.
5 Camisa C, Rossana C. Variant of keratoderma hereditaria mutilans (Vohwinkel's syndrome). Treatment with orally administered isotretinoin. *Arch Dermatol* 1984; **120**:1323–8.
6 Maestrini E, Monaco AP, McGrath JA *et al.* A molecular defect in loricrin, the major component of the cornified cell envelope, underlies Vohwinkel's syndrome. *Nat Genet* 1996; **13**:70–7.
7 Ishida-Yamamoto A, Kato H, Kiyama H *et al.* Mutant loricrin is not crosslinked into the cornified cell envelope but is translocated into the nucleus in loricrin keratoderma. *J Invest Dermatol* 2000; **115**:1088–94.
8 O'Shaughnessy RF, Choudhary I, Harper JI. Interleukin-1 alpha blockade prevents hyperkeratosis in an *in vitro* model of lamellar ichthyosis. *Hum Mol Genet* 2010; **19**:2594–605.
9 Youssef G, Ono M, Brown SJ *et al.* Identifying a hyperkeratosis signature in autosomal recessive congenital ichthyosis: Mdm2 inhibition prevents hyperkeratosis in a rat ARCI model. *J Invest Dermatol* 2014; **134**:858–61.
10 Alkhalaf M, Ganguli G, Messaddeq N *et al.* MDM2 overexpression generates a skin phenotype in both wild type and p53 null mice. *Oncogene* 1999; **18**:1419–34.
11 Matsumoto K, Muto M, Seki S *et al.* Loricrin keratoderma: a cause of congenital ichthyosiform erythroderma and collodion baby. *Br J Dermatol* 2001; **145**:657–60.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Data S1. Supplementary methods.

Table S1. Ichthyosis and palmoplantar keratoderma genes covered by the custom-designed next-generation sequencing panel at the time of investigation.