Supplemental Data

Frequent somatic reversion of *KRT1* mutations in ichthyosis with confetti

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Supplementary Methods:

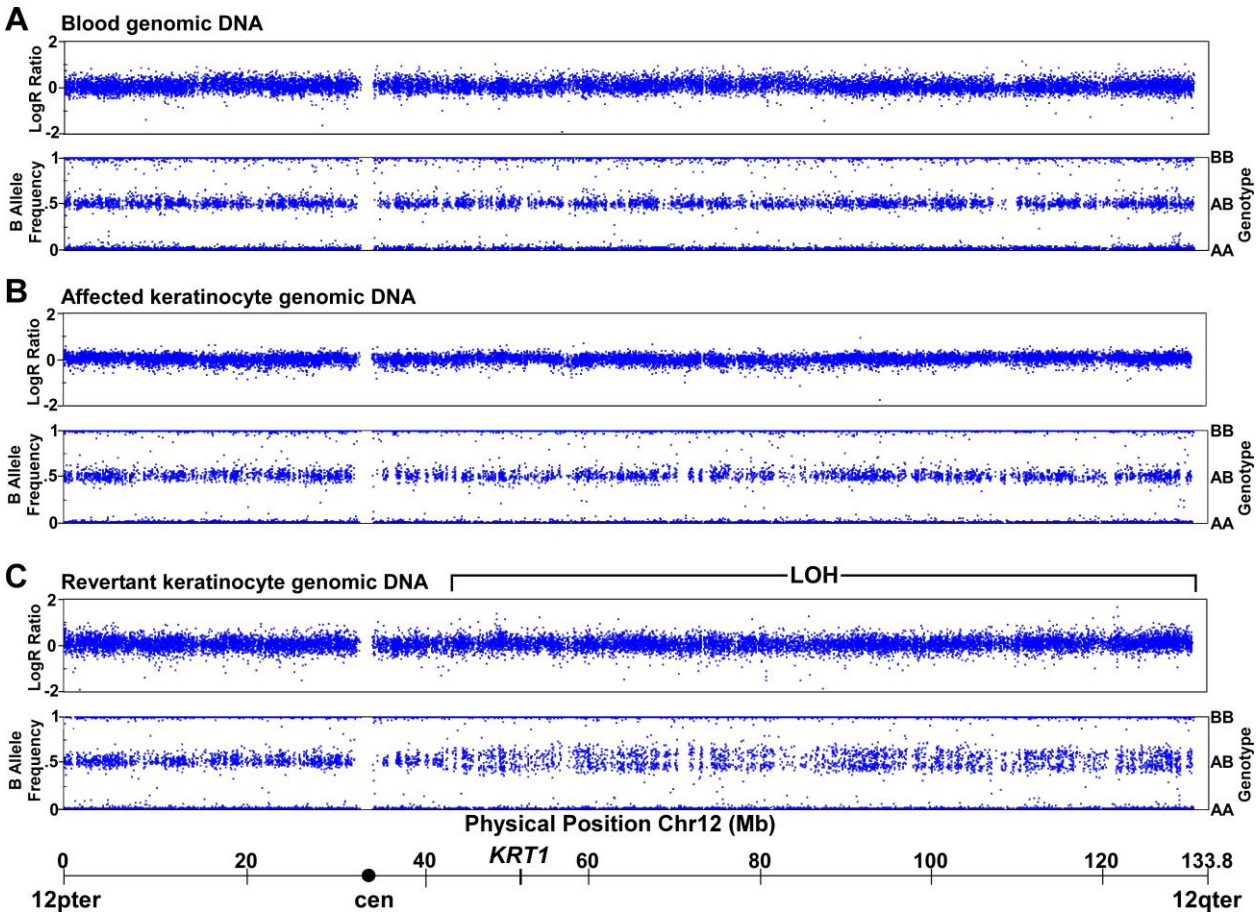
Genomic DNA preparation. Genomic DNA was prepared from blood and keratinocyte cultures via alkaline lysis and phenol/chloroform extraction.

KRT1 sequencing. The Primer3 web interface was used to generate primers for the *KRT1* coding region (1). DNA from affected subjects, family members, and control samples was used as a template in PCR performed using 2G Fast polymerase (Kapa Biosystems). Bi-directional Sanger sequencing was performed, and resulting traces were analyzed using Sequencher 9.0 (Genecodes).

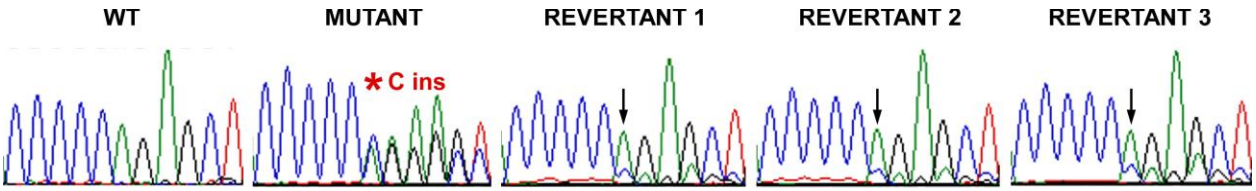
Expression construct generation. Total RNA was isolated from affected and revertant keratinocytes using the RNeasy Mini kit (Qiagen). Reverse transcription was performed using Superscript II (Invitrogen) using oligo-dT priming per standard protocols. *KRT1*-specific products were generated by PCR using KAPA HiFi polymerase (Kapa Biosystems) and specific primers in the 5' and 3' UTRs. Products were TA-cloned into pCR2.1TOPO vector (Invitrogen). Plasmid DNA was isolated from the resulting clones and subjected to Sanger sequencing. Wild type and mutant cDNAs were subcloned into pcDNA3.1 (Invitrogen) and sequence confirmed using gene and vector specific primers in Sanger sequencing. Quikchange site-directed mutagenesis of the wild-type construct was employed to generate a construct with a stop codon at the affected individuals' mutation position (Agilent).

Transfections. Primary liver hepatoma cells (PLC, CRL-8024, ATCC) were plated on 4-well chamber slides (BD Biosciences) and transfected using Lipofectamine 2000 (Invitrogen) per standard protocols and harvested at 48 hours post-transfection for analysis.

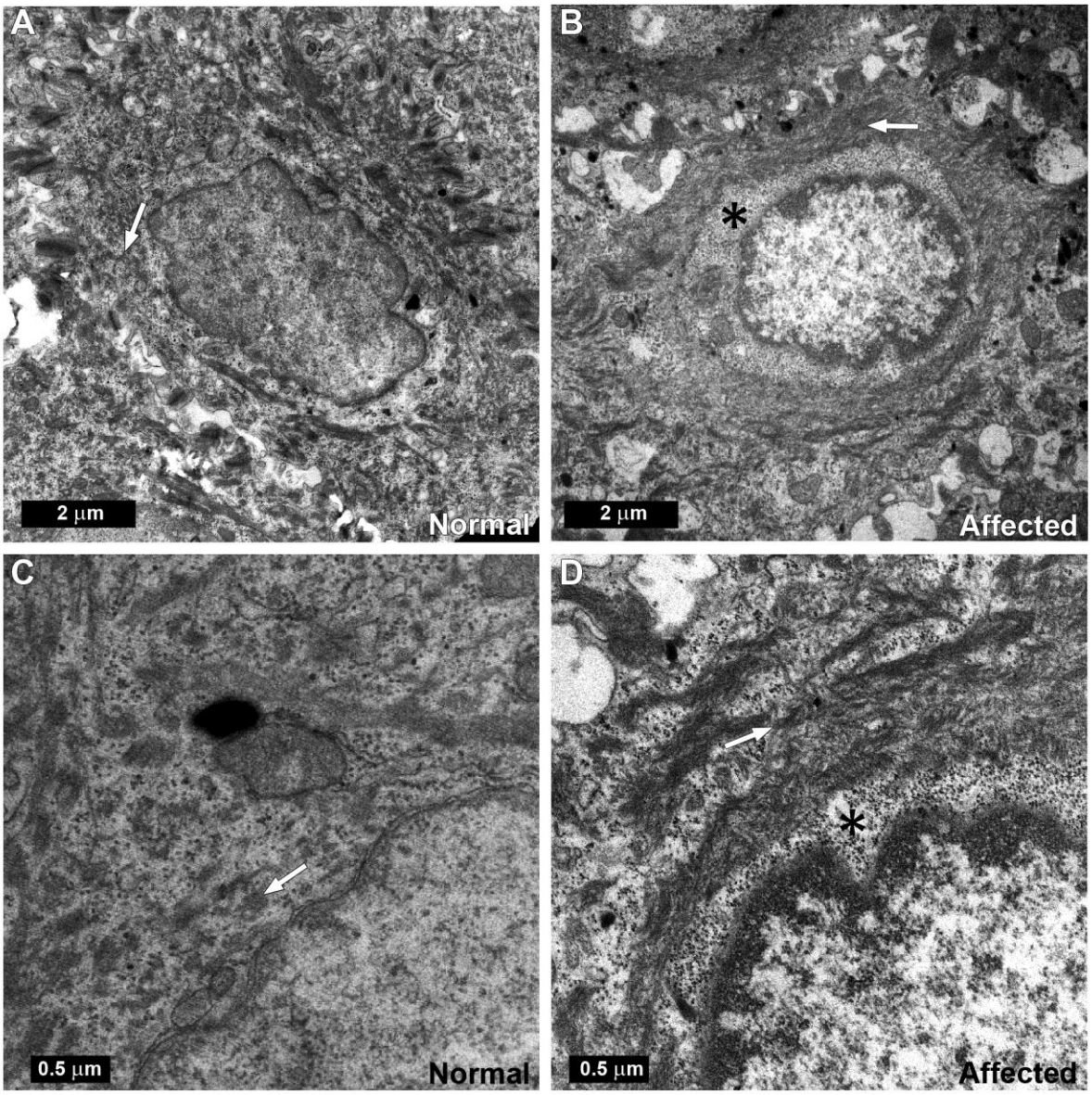
Immunofluorescence microscopy. 5 micron sections were cut from skin biopsy tissue embedded in OCT (Sakura). Tissue sections or cells grown on chamber slides were fixed with 2:1 methanol/acetone for seven minutes at -20˚C. Blocking with 10% donkey serum/1% BSA was performed and slides were incubated with primary antibody overnight. They were then washed with PBS, incubated with secondary antibody, and again washed with PBS before mounting with Mowiol (Polysciences) containing 1% n-propyl gallate (Sigma). Primary antibodies used include: 1:200 rabbit anti-human KRT1 (HPA017917, Sigma), mouse anti-human KRT1 (34ß34, Covance). Secondary antibodies used include CY3 donkey anti-rabbit IgG and CY2 donkey anti-mouse IgG (Jackson Immunoresearch). DAPI was used as a nuclear counterstain (Santa Cruz Biochemicals).



Supplementary Figure 1. IWC-K1 revertant keratinocytes show LOH on chromosome 12q. The logR ratio of the SNP intensity and the genotypes of SNPs along chromosome 12 from Illumina 370K arrays are shown in each panel for index case 101-1. (**A**) Results from peripheral blood DNA. (**B**) Results from affected keratinocyte DNA. (**C**) Results from revertant keratinocytes. Given small revertant spot size, it was difficult to isolate pure revertant cell clones, but two revertant keratinocyte cultures showed B-allele deviation beginning near the centromere and extending to the telomere without commensurate change in LogR ratio, suggesting LOH via mitotic recombination. The incomplete B-allele deviation observed suggests that revertant keratinocyte cultures are contaminated with affected cells.



Supplementary Figure 2. Revertant keratinocytes show loss of the mutant allele. Revertant keratinocytes were isolated from frozen tissue sections using laser capture microdissection in three biopsies of revertant skin. DNA was isolated and PCR with gene-specific primers was performed. Sequencing revealed that the mutant allele is present in a 1:1 ratio in affected cells (MUTANT) but is reduced to < 20% in laser microdissected tissue from each of these biopsies (REVERTANT 1, 2, 3).



Supplementary Figure 3. Electron microscopy of normal and IWC-K1 affected skin. (**A**) Normal spinous layer skin shows keratin intermediate filaments (white arrows) throughout cytoplasm extending to nucleus. (**B**) In affected IWC-II spinous layer skin, there is perinuclear filament detachment (*). (**C**) Cytoplasmic intermediate filaments in wild type tissue. (**D**) Perinuclear filament detachment in IWC skin. Scale bars are shown and black and are labeled in each panel with their size.

Supplementary References:

1. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Methods Mol Biol*. 2000;132:365-86.