REPORT

A Single-Nucleotide Deletion in the *POMP* 5′ UTR Causes a Transcriptional Switch and Altered Epidermal Proteasome Distribution in KLICK Genodermatosis

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KLICK syndrome is a rare autosomal-recessive skin disorder characterized by palmoplantar keratoderma, linear hyperkeratotic papules, and ichthyosiform scaling. In order to establish the genetic cause of this disorder, we collected DNA samples from eight European probands. Using high-density genome-wide SNP analysis, we identified a 1.5 Mb homozygous candidate region on chromosome 13q. Sequence analysis of the ten annotated genes in the candidate region revealed homozygosity for a single-nucleotide deletion at position c.−95 in the proteasome maturation protein (POMP) gene, in all probands. The deletion is included in *POMP* transcript variants with long 5′ untranslated regions (UTRs) and was associated with a marked increase of these transcript variants in keratinocytes from KLICK patients. POMP is a ubiquitously expressed protein and functions as a chaperone for proteasome maturation. Immunohistochemical analysis of skin biopsies from KLICK patients revealed an altered epidermal distribution of POMP, the proteasome subunit proteins α7 and β5, and the ER stress marker CHOP. Our results suggest that KLICK syndrome is caused by a single-nucleotide deletion in the 5′ UTR of *POMP* resulting in altered distribution of POMP in epidermis and a perturbed formation of the outermost layers of the skin. These findings imply that the proteasome has a prominent role in the terminal differentiation of human epidermis.

Keratosis linearis with ichthyosis congenita and sclerosing keratoderma syndrome (KLICK syndrome [MIM 601952]) is a rare disorder of keratinization of the skin. The disease is characterized by ichthyosis, palmoplantar keratoderma with constricting bands around fingers, flexural deformities of fingers, and keratotic papules in a linear distribution on the flexural side of large joints.[1–4] Histological examination of the skin of affected individuals shows hypertrophy and hyperplasia of the spinous, granular, and horny epidermal layers.[1,2] Ultrastructural examinations of the epidermis reveal abundant abnormal keratohyaline granules with enlarged and rounded shape in differentiated keratinocytes.[2] The disease is inherited as an autosomal-recessive trait, but the mutant gene and the molecular basis of the disease are unknown.[1] KLICK syndrome shares some clinical and histopathological features with several other skin disorders, such as the autosomal-recessive congenital ichthyosis (ARCI), keratitis-ichthyosis-deafness (KID) syndrome (MIM #148210) and Vohwinkel syndrome (MIM #604117, #124500). These phenotypically related disorders are caused by mutations in genes encoding proteins of importance for the formation of the horny epidermal layer; e.g., in lipid transporters, cornified cell envelopes, and gap junctions.[5–13]

In the present study, we included twelve individuals affected by KLICK syndrome and 13 healthy family members from eight families after obtaining informed consent. This project was approved by the Ethics committee, Upsala. The families are nonrelated and originate from Spain, Italy, The Netherlands, Sweden, and Norway (Figure S1, available online). All patients were thoroughly examined by dermatologists, and probands of four families have been described previously.[1–4] The patients share the clinical manifestations of mild ichthyosis, thickened horny layer of the epidermis on hands and feet, hyperkeratotic plaques on knees and wrists and in axillae, circular sclerotic constrictions around fingers, flexural deformities of fingers, and linear hyperkeratotic papules on flexural surfaces of wrists, elbows, and knees (Figure 1). There are no obvious extracutaneous manifestations.

We analyzed DNA samples from six affected individuals (three Spanish siblings, three Swedish sporadic cases) by whole-genome SNP analysis (Affymetrix SNP GeneChip Mapping 10K Array).[14,15] Homozygosity mapping in the three affected siblings revealed one candidate region of 12.7 Mb spanning 62 consecutive homozygous SNPs (probability 1.54×10^{-29}, LOD 24.82) on chromosome 13q (Figure 2A).[14] We then analyzed the array data of the three sporadic KLICK patients, with specific emphasis on the chromosome 13q region. Within this region, two sporadic patients were homozygous for a distinct haplotype over 39 consecutive SNPs (probability 1.50×10^{-24}, LOD 19.83) spanning 4.5 Mb (Figure 2B).[14] The third sporadic case was homozygous for four consecutive SNPs

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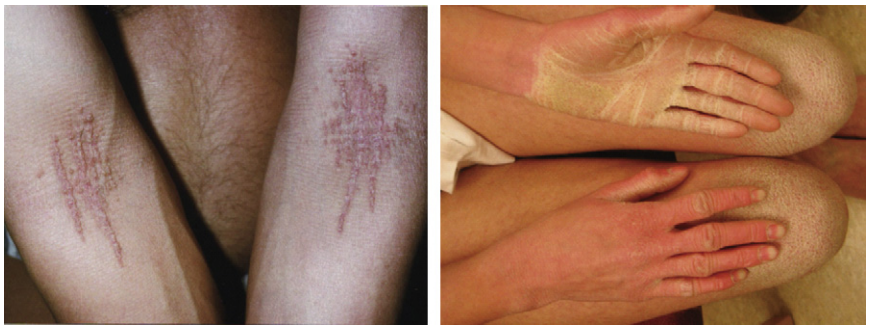

Figure 1. Clinical Symptoms of KLICK Syndrome
Pictures of a 32-year-old male showing typical features of KLICK syndrome, including mild ichthyosis, hyperkeratotic papules forming radiating lines in arm and knee folds, keratoderma of palms, sclerotic constrictions around fingers, and hyperkeratotic plaques on knees. Pictures published with the consent of Acta Dermato-Venereologica.

within this interval. This refined the critical region in these six patients to approximately 1.5 Mb (Figure 2C), which was further restricted to approximately 0.8 Mb with the use of microsatellite marker analysis. The candidate region spans ten annotated genes and two pseudogenes (NCBI build 36.3). All of the annotated gene exons and exon-intron boundaries were sequenced on genomic DNA from three affected individuals (primer sequences available upon request).[15] We identified one previously nonannotated sequence variant, c.−59C>T (ss161109886), located in *Flt1* (MIM *165070), in a homozygous state in one affected individual. We also identified a single-nucleotide deletion, c.−95delC (g.28,131,226, NCBI build 36.3; ss192545708), in the 5′ region of the *POMP* gene (Figures 2D and 2E) in a homozygous state in all 12 affected individuals. Segregation analysis showed that the six available parents were heterozygous and the five available healthy siblings were either heterozygous or noncarriers for the deletion. We performed further analysis of 280 Swedish control chromosomes, none of which carried the single-nucleotide deletion ($p = 9.10 \times 10^{-27}$, Fisher's exact test). Haplotype analysis using microsatellite markers flanking *POMP* in the eight affected probands formed at least five different haplotypes (Figure S1), suggesting that the c.−95delC variant is a recurrent rather than a founder mutation.

Position c.−95 of *POMP* is located 14 bp 5′ from the annotated transcription start site (TSS c.−81) according to the NCBI and Ensembl databases (transcripts NM_015932.4 and ENST00000380842, respectively). The deletion is, however, included in an alternative *POMP* transcript with an extended 5′ untranslated region (UTR) (TSS c.−181) presented by the AceView database (transcript POMP fApr07). To confirm that the deletion is included in a 5′ UTR variant of *POMP* mRNA, we performed 5′ and 3′ rapid amplification of cDNA ends (RACE). Primary keratinocytes were cultured from skin-punch biopsies,[15] and total RNA was isolated from keratinocytes harvested at passages 3–6 by trypsinization via TRIzol (Invitrogen). RNA from one affected individual was used to perform 5′ and 3′ RACE of the *POMP* mRNA with the GeneRacer kit (Invitrogen) according to the manufacturer's protocols, with gene-specific primers used (Figure S2). The PCR products were cloned into TA vectors (Invitrogen), and individual clones

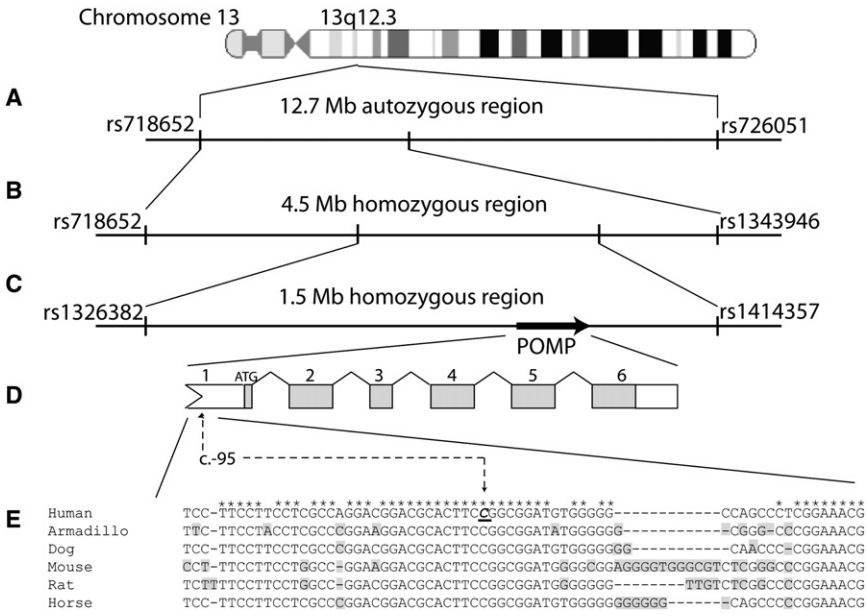

Figure 2. Genetic Mapping of KLICK Syndrome on Chromosome 13q and Sequence Alignment of the 5′ UTR Region of *POMP*
(A) The region of autozygosity shared among three affected siblings is located between rs718652 and rs726051.
(B) The region of homozygosity shared among the three affected siblings and two sporadic cases is located between rs718652 and rs1343946.
(C) The region of homozygosity shared among the three affected siblings and three sporadic cases is located between rs1326382 and rs1414357.
(D) The exon structure of *POMP* with the position of the single-nucleotide deletion (c.−95) identified in a homozygous state in all 12 cases with KLICK syndrome.
(E) DNA sequence alignment of the human *POMP* sequence c.−127 to c.−64 to orthologs of five mammalian genomes, with the use of the UCSC Genome Browser. Sequence similarities are indicated by an asterisk (*), showing a perfectly conserved motif of 19 nt, corresponding to c.−88 to c.−107 of the human *POMP*. Bases deviating from the human sequence are assigned in gray. The cytosine deleted in KLICK patients is indicated in italic, bold font and underlined.

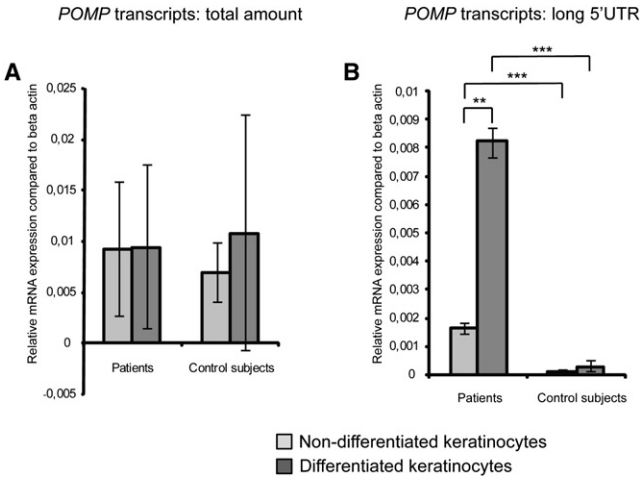

Figure 3. qPCR Analysis of *POMP* mRNA Levels in Primary Keratinocytes

(A) Quantification of POMP transcripts determined after amplification of a 164 bp fragment from exon 1 to exon 2 (c.−65 to c.+101) starting downstream of the common TSS of *POMP*. Quantification was performed in nondifferentiated and differentiated keratinocytes. No difference in levels of the total amount of *POMP* transcripts was observed between KLICK patients (n = 2) and control subjects (n = 4).

(B) Levels of *POMP* transcripts with a long 5′ UTR spanning c.−116 to c.−28 in keratinocytes from KLICK patients (n = 2) and control subjects (n = 4). *POMP* transcripts with a long 5′ UTR are increased in keratinocytes derived from KLICK patients. Keratinocytes (passages 3–6) were harvested by trypsinization, and total RNA was isolated with TRIzol (Invitrogen).[15,45] The RNA was treated with DNase I (Sigma), and 1 ug of total RNA was reverse transcribed by the iScript Select cDNA Synthesis Kit (Bio-Rad, Laboratories). Results are based on two separate RNA preparations from each cell culture and two qPCR:s for each cDNA synthesis. qPCR samples were run in triplicates and normalized to beta actin. Data are presented as mean ± standard deviation between individuals. Results were analyzed by Student's t test (Microsoft Excel) to give an indication of differences between groups. Significance levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

were sequenced with M13 primers. We identified five out of 31 capped 5′ RACE clones extending beyond c.−95 (endpoints between c.−100 and c.−218) and 26 clones that were shorter (endpoints between c.−59 and c.−71). This indicates that there are several alternative TSSs and that the deletion is included in a proportion of *POMP* transcripts derived from keratinocytes. The expression of *POMP* transcripts with 5′ UTRs spanning the locus c.−95 was also confirmed in other tissues (fibroblasts, testis, placenta, bone marrow, fetal brain) by RT-PCR (data not shown). To clarify whether *POMP* transcript variants with extended 5′ UTRs are alternatively spliced, we performed 3′ RACE. We sequenced one clone that contained a 704 bp transcript, spanning all six exons from c.−181 to the poly(A) tail, but no alternative splicing was found when compared to the reference variant.

Alignment of the human *POMP* 5′ UTR to that of other mammals shows a perfectly conserved 19 nt sequence spanning c.−95C (UCSC Genome Browser; Figure 2E). This suggests that the region is of functional importance.[16]

To investigate whether the c.−95delC alteration affects the mRNA levels of *POMP*, we performed quantitative real-time PCR (qPCR). A previous study of epidermal histology in KLICK syndrome has revealed abnormalities in differentiated layers but not in the basal layer of the epidermis,[2] and we analyzed mRNA from both nondifferentiated (cultured as described previously)[15] and differentiated keratinocytes. To obtain differentiated keratinocytes, we split cultured cells at passage two and then cultured them in cell medium supplemented with 2 mM calcium chloride for 3 days before harvesting. By this method, the cells start expressing markers of early differentiation, but they do not reach the level at which they express the markers of terminally differentiated cells.[17] Two distinct fragments of the *POMP* transcript were amplified by qPCR. One fragment spans c.−116 to c.−28, corresponding to transcripts with a TSS 5′ of the most frequently used TSSs for *POMP*, according to Ensembl and our results (Figure S2). The second fragment spans c.−65 to c.+101 (exon 1 to exon 2) and corresponds to the vast majority of *POMP* transcripts, as they share this region (Figure S2). Quantification of the downstream fragment, corresponding to the total amount of *POMP* transcripts, showed no significant differences between patients and control subjects, either in nondifferentiated or in differentiated keratinocytes ($p = 0.405$, $p = 0.890$; Figure 3A). However, analysis of the upstream amplicon, corresponding to transcripts with a longer 5′ UTR, revealed increased levels in patients compared to control subjects in both nondifferentiated and differentiated keratinocytes ($p = 1.70 \times 10^{-4}$, $p = 7.82 \times 10^{-6}$; Figure 3B). The difference was most striking in differentiated keratinocytes, in which the patients showed a 29-fold increase in expression of transcript variants detected by the upstream primer pair. Transcripts with a longer 5′ UTR constituted 18% of the total amount of *POMP* transcripts in nondifferentiated keratinocytes and 83% in differentiated cells in patients ($p = 0.00369$), whereas the proportions for control subjects were 1.3% and 2.6% ($p = 0.116$), respectively.

Alternative 5′ UTRs are known to regulate translation by different means; e.g., by translation of upstream open reading frames or by stable secondary structures that block ribosomes from reaching downstream start codons. In this respect, a longer 5′ UTR seems to be more repressive.[18–21] The sequence of a short (81 nt) *POMP* 5′ UTR was predicted with the use of UNAfold to form a moderately stable secondary structure (free energy value [ΔG] ~29 kcal/mol).[22] In contrast, a longer (181 nt) *POMP* 5′ UTR (with or without c.−95delC) was predicted to form a very stable structure (ΔG −79 to −85 kcal/mol), which is estimated to cause a significant decrease in translation efficiency.[23,24] We therefore investigated the effect of two different *POMP* 5′ UTRs on translation. Full-length *POMP* cDNAs with a 181 nt 5′ UTR and a 81 nt 5′ UTR, respectively, were cloned into pAcGFP vectors (Clonetech) and expressed in HeLa cells. Immunoblot analysis of fusion protein levels showed significantly higher POMP-GFP

protein levels in lysate of cells transfected with 81 nt 5′ UTR cDNA clones in comparison to lysate transfected with 181 nt 5′ UTR clones ($p = 0.0011$; Figure S5). These results confirm an inverse correlation between the length of *POMP* 5′ UTRs and the amount of translated POMP.

The marked increase of *POMP* transcripts with longer 5′ UTR in KLICK patients prompted us to investigate the levels of endogenous POMP protein and proteasome activity in primary keratinocytes from affected individuals. POMP is a ubiquitously expressed protein that interacts with immature proteasomes.[25–27] The proteasome is built up by two outer rings of seven α subunits and two inner rings of seven β subunits each, of which three beta subunits possess tryptic-like, chymotryptic-like, and peptidylglutamyl-peptidase enzymatic activities.[28] The first step during proteasome assembly is the formation of α subunit rings.[29] Next, POMP is thought to mediate binding of the α rings to the endoplasmatic reticulum and to recruit and incorporate β subunits to subsequently form a hemiproteasome. Finally, POMP mediates the dimerization of the hemiproteasomes to a mature proteasome, which degrades POMP as a first substrate.[30–32] In vitro overexpression of *POMP* results in increased proteasome activity, whereas POMP knockdown leads to decreased amount of mature proteasomes and thereby reduced proteolytic activity.[29,30,33]

The levels of POMP as well as the levels of the proteasome subunits α7 and β5 were quantified via immunoblot analysis. α7 has been shown to be associated with POMP in the immature proteasome, and the incorporation of β5 into the proteasome has proven to be dependent on POMP function.[29–31] The amounts of POMP, α7, and β5 did not differ significantly in either nondifferentiated or in vitro differentiated keratinocytes between patients and control subjects (Figures S3A–S3D). We also analyzed the chymotryptic-like activity of differentiated keratinocytes by using a 20S proteasome activity assay, but no significant difference in enzymatic activity was observed between patient and control lysates (Figure S3E).

The cultured nondifferentiated keratinocytes represent cells from the basal layer of epidermis, whereas the in vitro differentiated keratinocytes represent keratinocytes of the epidermal spinous layer, as the differentiation assay applied here does not result in terminally differentiated cells.[17] We hypothesized that an effect of c.−95delC on POMP protein levels may be noticeable only in terminally differentiated keratinocytes; i.e., the granular and horny layers, as suggested by previous histopathological studies of the epidermis of KLICK patients.[2] To investigate this, we studied POMP and the α7 and β5 proteins by using immunohistochemistry (IHC). We obtained frozen skin-punch biopsies of two healthy individuals and three KLICK patients. Basic histology of epidermal sections confirmed a thickened horny and granular layer, retention of nuclei in the horny layer, and numerous enlarged granules in the granular layer in KLICK patients.[2] Sections were stained with primary antibodies against POMP (Abcam) and proteasome subunits α7 and β5 (Biomol). POMP was strongly and consistently expressed from the basal layer to the granular layer of epidermis in sections from healthy subjects, whereas in KLICK patients the staining was strong in the basal layer with a gradual decrease toward the granular layer (Figures 4A and 4B). The proteasome subunit α7 staining was weak in the basal, spinous, and horny layers, with a very strong expression in the granular layer of normal skin (Figure 4C). KLICK patients showed an α7 expression equal to that of normal skin in the basal and spinous layers, but in the granular layer the expression was weaker, with a diffuse distribution as compared to sections from control subjects. A patchy staining of the horny layer was also seen (Figure 4D). The β5 expression appeared similar to that of POMP, showing a gradually decreased staining toward the granular layer in KLICK patients, not observed in control subjects (Figures 4E and 4F). The results suggest that α7 subunits are upregulated in the granular layer of normal skin and that KLICK syndrome is associated with an altered epidermal distribution of POMP and the proteasome subunits α7 and β5. For further investigation of the terminal epidermal differentiation in KLICK syndrome, skin sections were stained with antibodies against filaggrin (NCL-filaggrin, Novacastra), a skin-specific protein stored as a precursor variant in keratohyaline granules in the cells of the granular layer. At terminal differentiation, profilaggrin is degraded into filaggrin, which is important for aggregation of keratin filaments in the keratinocytes and for hydration of the horny layer.[34] In normal skin, the filaggrin staining was restricted to the outermost cells of the granular layer and the most basal cells of the horny layer (Figure 4G). In contrast, sections from KLICK patients were almost exclusively stained in the horny layer, in a broad and inconsistent manner (Figure 4H). This suggests a disturbed processing of filaggrin and consequently of the terminal cell differentiation in KLICK patients, which coincides with the altered distribution of POMP and the proteasome subunits. We also analyzed the POMP, α7, β5, and filaggrin skin-staining patterns in the two keratinizing disorders of ichthyosis, ARCI, due to ichthyin mutations, and KID syndrome, due to *Cx26* mutations. The staining of sections from a patient with ichthyin-deficient ichthyosis closely resembled that of the control sections, with the use of all four antibodies. However, sections from the KID syndrome patient, with a phenotype similar to KLICK, showed a wider and weaker staining in the granular layer with the use of α7 and filaggrin antibodies, as well as a patchy filaggrin staining of the horny layer as compared to normal skin (Figures S4A–S4P).

It is known that proteasome inhibition causes increased endoplasmic reticulum (ER) stress,[35] and we hypothesized that proteasome deficiency in KLICK patients, as suggested by the aberrant proteasome subunit distribution, may result in increased ER stress in the epidermis. To investigate this, we stained normal and KLICK affected skin sections with immunofluorescent antibodies against C/EBP homologous protein (CHOP), a transcription factor induced

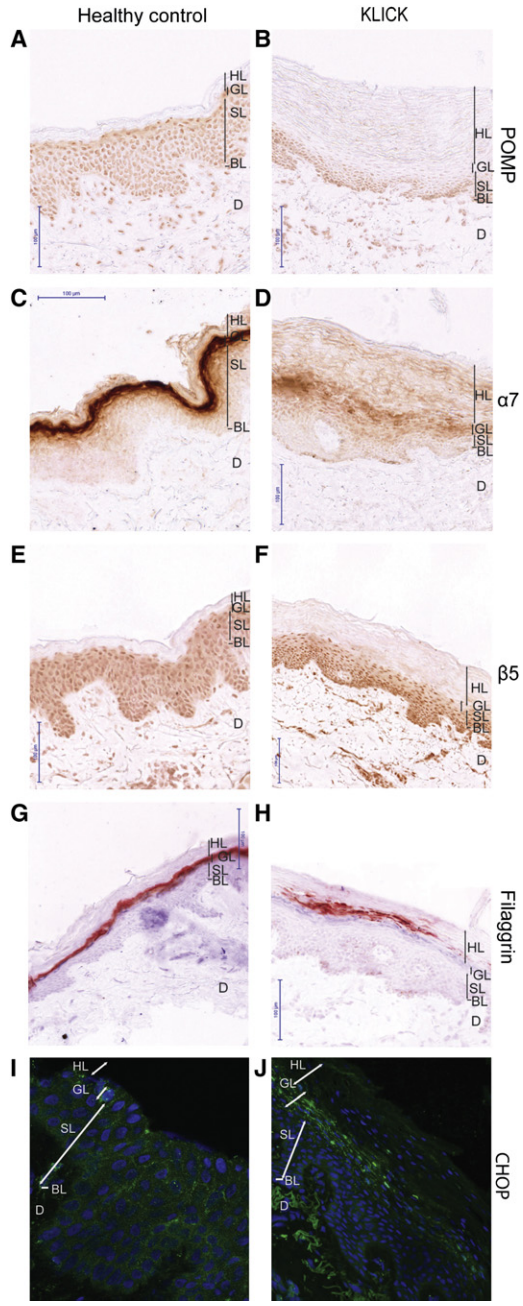

Figure 4. Immunohistochemical Analysis of POMP, α7, β5, Filaggrin, and CHOP

Shown are 6 μm skin-biopsy sections of a healthy subject (A, C, E, G, I) and a KLICK patient (B, D, F, H, J), stained with POMP antibodies (A and B), 20S proteasome subunit α7 antibodies (C and D), 20S proteasome subunit β5 antibodies (E and F), filaggrin antibodies (G and H), and antibodies against the UPR marker CHOP (I and J). Filaggrin sections were counterstained with hematoxylin, and CHOP sections were counterstained with DAPI (both blue). The KLICK-derived sections showed a weaker staining for POMP and β5 in the granular layer than in normal skin. The α7 and filaggrin staining is patchy, incoherent, and located mainly in the horny layer, compared to a consistent and strong staining of the granular layer in normal skin. The immunofluorescent staining of CHOP (green) is increased in the granular layer of KLICK patients when compared to the control. Skin sections were fixed in 100% acetone. For bright-field microscopy, endogenous peroxidase activity was blocked by Peroxidase1 and nonspecific staining was blocked by Background Sniper (Biocare Medical). Subsequently, the sections were incubated with primary antibodies for 1 hr at room temperature, followed by incubation with biotinylated secondary antibodies (anti-chicken, anti-mouse; Vector Laboratories) and thereafter with avidin-biotin complex. Peroxidase reactions were developed with DAB (Vector Laboratories). For immunofluorescence analysis, the skin sections were blocked with BSA 3% and then incubated with primary antibodies (Cell Signaling Technologies) for 1 hr and with secondary antibodies (rabbit anti-mouse, FITC conjugated; MuBio BV) for 1 hr. Tertiary antibody (swine anti-rabbit, FITC conjugated; MuBio BV) was added to boost the signal, and samples were then stained with DAPI. Staining was analyzed by confocal microscopy (Leica TCS SPE System). Colon carcinoma samples were used as positive controls. Magnification: normal skin, ×400; KLICK skin, ×200. HL, horny layer; GL, granular layer; SL, spinous layer; BL, basal layer; D, dermis.

by the unfolded protein response (UPR) and persistent elevated ER stress.[36] In normal skin, the CHOP staining showed a gradual, but moderate, increase from the spinous layer to the granular layer, in accordance with previous studies (Figure 4I).[37,38] In KLICK skin, the staining was clearly increased in the granular layer, and a weak staining was also seen in the horny layer (Figure 4J). This pattern is consistent with the abnormal distribution of POMP and the proteasome subunit α7 in the granular layer of individuals with KLICK. Thus, our combined results from IHC support the notion that ER stress due to a dysregulated proteasome function is a pathophysiological mechanism in KLICK.

Mutations in 5′ UTRs have previously been found to modulate translation and even to cause disease; e.g., in hereditary hypotrichosis and familial thrombocytosis.[39–41] We show herein that the autosomal-recessive skin disorder KLICK syndrome is associated with a single-nucleotide deletion located in the 5′ region of *POMP* and that the deletion is included in a proportion of *POMP* transcripts. Analysis of *POMP* mRNA in keratinocytes revealed that the deletion causes increased amounts of longer *POMP* transcripts, especially in in vitro differentiated keratinocytes. Possible explanations are that the single-nucleotide deletion stabilizes the long 5′ UTR *POMP* transcript by protecting it from degradation and/or that it increases the preferences of the preinitiation complex for an upstream TSS.[40–42] The observed lower translation efficiency of *POMP* transcripts with longer 5′ UTRs would result in reduced levels of POMP and subsequently in reduced levels of mature proteasomes. We also observed an aberrant expression of POMP and a deviant distribution of α7 and β5 subunits by IHC in the terminal epidermal differentiation of KLICK patients, which coincides with an increased expression of the ER stress marker CHOP. The normal protein levels and the normal proteasome activity in cultured keratinocytes may be explained by the fact that these cells correspond to earlier stages of keratinocyte differentiation, preceding the cells in the granular and horny layers in which proteasome function appears to be critical.

Our findings of an altered distribution of POMP and proteasome subunits during formation of the horny layer

in KLICK syndrome suggest that the disease is caused by proteasome insufficiency at a specific stage of epidermal differentiation. The proteasome is ubiquitously expressed, and the fact that the symptoms in KLICK syndrome are confined to the skin implies (1) that the differentiating keratinocytes are highly sensitive to reduced levels of POMP and (2) that the proteasome has a pivotal role in the formation of the epidermal water barrier. A previous study has shown that the UPR is activated during epidermal differentiation with a gradual increase of UPR proteins with keratinocyte differentiation.[37] The epidermal transition from the granular to the horny layer is an extraordinary phase in terms of the large amount of proteins that are synthesized and degraded. This requires well-functioning ER, UPR, and proteasomes. Proteasome deficiency results in increased epidermal ER stress, a process which is known to cause aggregations of misfolded proteins. These protein aggregates limit normal cellular functions[43] and can explain the disturbed terminal epidermal differentiation in KLICK syndrome. It was recently shown that the skin disorder erythrokeratoderma variabilis (EKV), due to *Cx31* mutations, is associated with increased epidermal ER stress and apoptosis.[44] In combination with our findings, this may suggest common disease mechanisms in EKV, KLICK, and possibly KID syndrome.

Our results imply that the proteasome plays a previously unknown, but important, role in terminal epidermal differentiation, and the precise mechanisms mediated by the proteasome for the formation of the horny layer will require further study.

Supplemental Data

Supplemental Data include five figures and can be found with this article online at http://www.ajhg.org.

Acknowledgments

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Web Resources

The URLs for the data and analytic approaches presented herein are as follows:

ACE view database, http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly
Ensembl, http://www.ensembl.org/index.html
Fisher's exact test, http://www.langsrud.com/fisher.htm
National Center for Biotechnology Information (NCBI), http://www.ncbi.nlm.nih.gov/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
UCSC Genome Browser, http://www.genome.ucsc.edu/
UNAfold, http://mfold.bioinfo.rpi.edu/

Accession Numbers

The NCBI dbSNP accession numbers for the variations c.−59C>T in *Flt1* and c.−95delC in *POMP* reported in this paper are ss161109886 and ss192545708, respectively. The NCBI GeneBank accession number for the long 5′ UTR *POMP* mRNA with the variation c.−95delC is GU045556.

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