

Identification of the first nonsense *CDSN* mutation with expression of a truncated protein causing peeling skin syndrome type B

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Summary

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Conflicts of interest

None declared.

A.M. and M.K. contributed equally to this study.

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Background Peeling skin disease (PSD), a generalized inflammatory form of peeling skin syndrome, is caused by autosomal recessive nonsense mutations in the corneodesmosin gene (*CDSN*).

Objectives To investigate a novel mutation in *CDSN*.

Methods A 50-year-old white woman showed widespread peeling with erythema and elevated serum IgE. DNA sequencing, immunohistochemistry, Western blot and real-time polymerase chain reaction analyses of skin biopsies were performed in order to study the genetics and to characterize the molecular profile of the disease.

Results Histology showed hyperkeratosis and acanthosis of the epidermis, and inflammatory infiltrates in the dermis. DNA sequencing revealed a homozygous mutation leading to a premature termination codon in *CDSN*: p.Gly142*. Protein analyses showed reduced expression of a 16-kDa corneodesmosin mutant in the upper epidermal layers, whereas the full-length protein was absent.

Conclusions These results are interesting regarding the genotype–phenotype correlations in diseases caused by *CDSN* mutations. The PSD-causing *CDSN* mutations identified heretofore result in total corneodesmosin loss, suggesting that PSD is due to full corneodesmosin deficiency. Here, we show for the first time that a mutant corneodesmosin can be stably expressed in some patients with PSD, and that this truncated protein is very probably nonfunctional.

What's already known about this topic?

- Peeling skin disease (PSD) is a generalized inflammatory genodermatosis.
- PSD is caused by autosomal recessive nonsense mutations in the human corneodesmosin gene (*CDSN*), which lead to total corneodesmosin deficiency.

What does this study add?

- This study reveals a new nonsense mutation in the human *CDSN* gene, which leads to a truncated form of corneodesmosin and is responsible for PSD.

Peeling skin syndrome (PSS) is a rare autosomal recessive genodermatosis, characterized by a lifelong peeling of the stratum corneum. Acral PSS (OMIM 609796), typically non-inflammatory, is confined to the distal extremities with localized exfoliation of the epidermis. It is caused by autosomal

recessive mutations in the *TGM5* gene, encoding transglutaminase 5,¹ or in the *CSTA* gene, encoding cystatin A.² Generalized PSS (OMIM 270300) affects the whole body and is subdivided into type A (noninflammatory) and type B (inflammatory). Type A, described as a continuous nonerythematous

exfoliation, is usually congenital or appears during childhood. A mutation in the *CHST8* gene, encoding a Golgi transmembrane *N*-acetylgalactosamine-4-*O*-sulfotransferase, was reported in one incidence of type A disease.³ Type B, synonymous with peeling skin disease (PSD), appears at birth or during early childhood and is characterized by generalized scaling with pruritic erythematous patches. It is caused by mutations in the *CDSN* gene, encoding corneodesmosin, a 52–56-kDa glycoprotein that is a component of corneodesmosomes. Corneodesmosin displays adhesive properties, mostly attributable to its *N*-terminal glycine-rich domain, and is sequentially proteolysed as corneocytes migrate towards the skin surface prior to desquamation.^{4,5} Mice lacking corneodesmosin die perinatally because of an impaired epidermal barrier and subsequent massive water loss.⁶ PSD shares clinical features with atopic diseases and Netherton syndrome, which is caused by mutations in the *SPINK5* gene, encoding LEKTI, a serine protease inhibitor that prevents corneodesmosome degradation.^{7,8}

Materials and methods

Our patient, a 50-year-old white woman, showed widespread peeling with erythema and elevated serum IgE. DNA sequencing, immunohistochemistry, Western blot and real-time polymerase chain reaction (PCR) analyses of skin biopsies were performed in order to study the genetics and to characterize

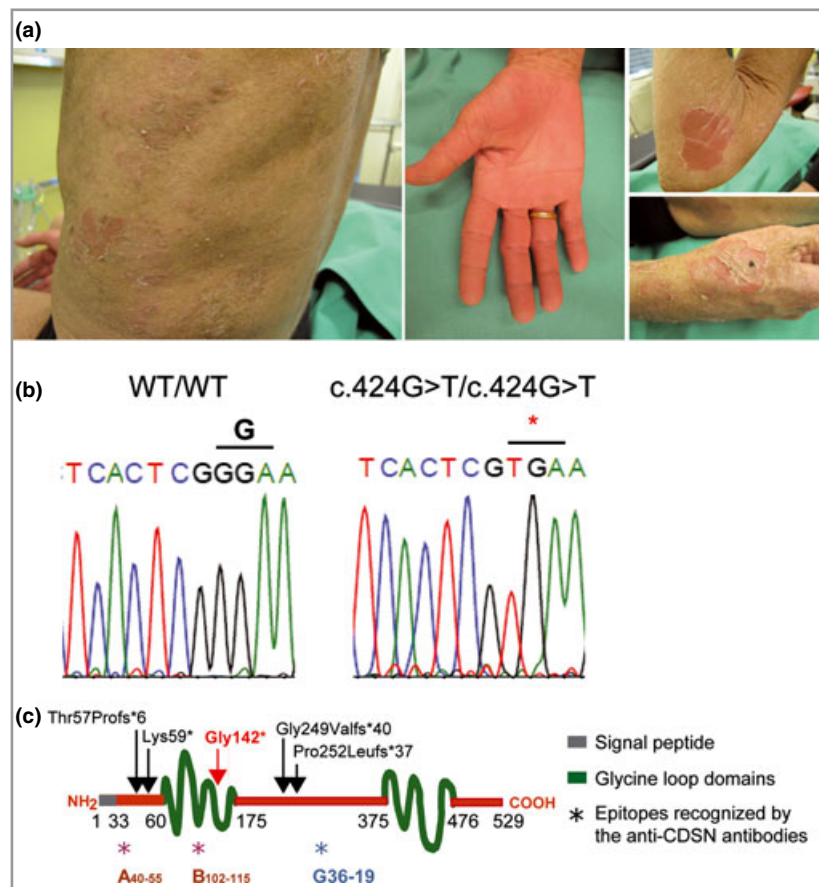
the molecular profile of the disease. Details of these procedures are provided as Supporting Information.

The patient has provided written and informed consent for the study (Geneskin protocol approved by the local ethics committee).

Results

Herein we report a case of generalized PSD, involving the palms and soles, caused by a new nonsense *CDSN* mutation. A 50-year old white woman was followed in our department since 1980.⁹ She was born from parents without known consanguinity. Both parents and the patient's two children had no history of skin disease. Nevertheless, she mentioned a brother who had a similar dermatosis and died of an unknown cause at the age of 4 months. Since birth she had shown initially grey-like patches of skin, which disappeared over time to give way to a widespread peeling with erythema (Fig. 1a). Her nails, hair, teeth and mucosae were macroscopically normal. Complete blood and urinary analysis showed results within standard limits. Fibrinogen, ferritin, IgG, IgA and IgM were normal, but serum IgEs were increased.⁹ A skin biopsy revealed marked hyperkeratosis, psoriasiform acanthosis of the epidermis and slight-to-moderate inflammatory infiltrates in the upper dermis (Fig. S1; see Supporting Information).⁹ Furthermore, real-time PCR analysis of skin mRNAs showed increased levels of the T helper (Th)2-related cytokines thymic stromal lymphopoietin

Fig 1. Clinical features and corneodesmosin (*CDSN*) sequencing analysis. (a) Peeling skin disease (PSD) with erythematous plaques over the trunk, elbow and hand. (b) Homozygous c.424G>T in exon 2 of *CDSN* (NM_001264.4), creating a termination codon. WT, wild-type. (c) Schematic representation of corneodesmosin with the peptide signal (grey line), the two glycine loop domains (green lines), the position of the four previously reported PSD-causing mutations and the fifth mutation identified in the present work. Amino acid residues delimiting the different protein domains are indicated by numbers, as are the positions of the epitopes recognized by the antibodies B_{102–115} and G36–19 produced and characterized in our laboratory (see Supporting Information).



and interleukin-13, and low expression of Th17- and Th1-related cytokines compared with healthy skin (Fig. S2; see Supporting Information). This supports the notion that PSD shares features with atopy and Netherton syndrome.^{7,8}

CDSN mutations were recently demonstrated to cause PSD. Sequencing analysis was thus performed to determine whether *CDSN* was mutated. A novel homozygous nonsense mutation, c.424G>T, was detected in exon 2, resulting in a premature stop codon at amino acid 142 (p.Gly142*) and leading to a predicted shortened protein ending inside the N-terminal glycine loop domain (Fig. 1b). Sequencing of *CDSN* from the genomic DNA of the patient's children confirmed the recessive inheritance of the mutation (Fig. S3; see Supporting Information). Interestingly, quantitative reverse-transcription PCR experiments revealed that mRNA encoding the mutated protein was not degraded in the patient's skin, and was even around twofold more abundant than *CDSN* mRNA in control skin (Fig. 2a). At the protein level, corneodesmosin expression was analysed by immunohistochemistry and Western blot (WB) using an anticorneodesmosin antibody recognizing epitopes located upstream (antipeptide antibodies A₄₀₋₅₅ and B₁₀₂₋₁₁₅) or downstream (monoclonal antibody G36-19) of p.Gly142* (Fig. 1c). In the patient's skin, the upper layers of the epidermis were labelled with B₁₀₂₋₁₁₅, revealing a truncated form of the protein, whereas the full-length protein was not detected (Fig. 2b). WB confirmed the presence of a ~16-kDa truncated corneodesmosin in protein extract from the patient's skin, which was detected using B₁₀₂₋₁₁₅ but not G36-19 (Fig. 2c). However, quantification analysis revealed that the mutated protein was ~22-fold less abundant in the patient's epidermis compared with the full-length corneodesmosin in a healthy epidermis. The discrepancy between the expression levels detected by the two methods could be due to difficulty in detecting the mutated protein by WB, because of modified biochemical properties (e.g. charge, conformation and size), which prevent its proper purification and/or revealing. Indeed, immunohistochemical experiments performed with the antibody A₄₀₋₅₅ gave a signal of similar intensity to that obtained with B₁₀₂₋₁₁₅ (Fig. S4; see Supporting Information). This supports our hypothesis that the mutant corneodesmosin is expressed in a significant amount in the patient's skin.

Discussion

Previous data concerning the four PSD-causing recessive *CDSN* mutations identified so far suggest that the disease is due to full deficiency of corneodesmosin.^{7,10-12} Indeed, the mutations p.Lys59* and p.Thr57Profs*6 could lead to very short peptides devoid of any glycine loop domain. Such proteins, if expressed, are most probably nonfunctional. Moreover, a reduction of *CDSN* mRNA by 75% was demonstrated for the former.⁷ The mutations p.Gly249Valfs*40 and p.Pro252Leufs*37 are very similar. Both lead to a frameshift coding for several amino acids before the occurrence of the premature stop codon. Immunohistochemical staining showed that the

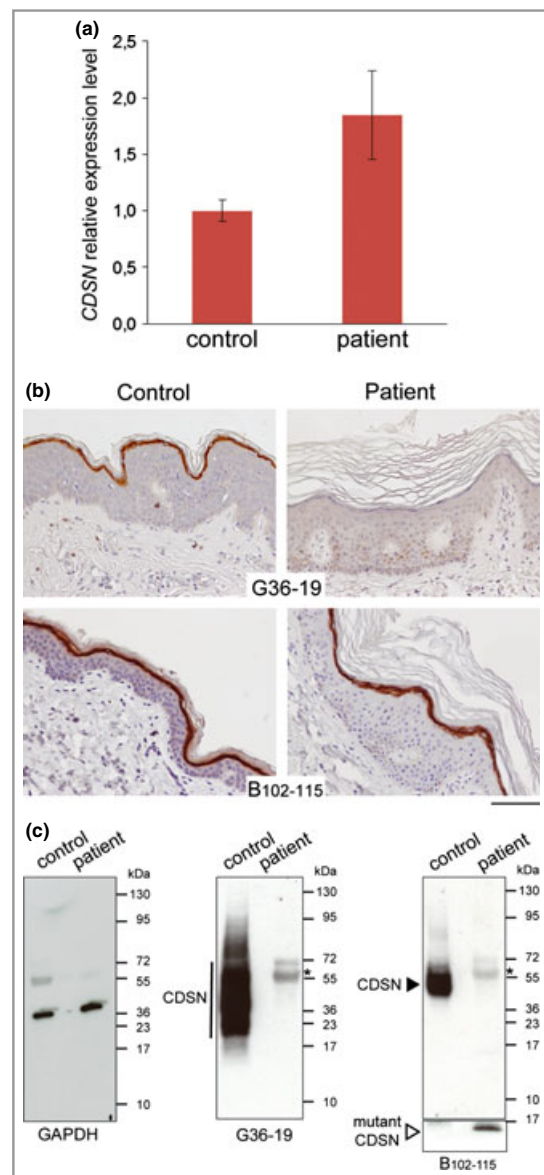


Fig 2. Corneodesmosin (*CDSN*) expression analysis. (a) Real-time polymerase chain reaction of cDNAs from control and patient's skin showing maintenance of *CDSN* mRNA in the patient's skin. The relative expression level of the protein in the control and patient's skin was normalized to that of RPL13A1 measured in the same total RNA preparation. (b) Antibodies B₁₀₂₋₁₁₅ and G36-19 reveal the expression of a mutant *CDSN* but absence of full-length *CDSN* in the upper epidermis of the patient. (c) Immunoblots realized with G36-19 and B₁₀₂₋₁₁₅ antibodies show the presence of full-length and proteolytically processed *CDSN* in protein extract from control but not patient's skin. In the patient sample only a ~16-kDa band was detected with B₁₀₂₋₁₁₅ (middle and right). Revelation of the immunoblot with enhanced chemiluminescent reagent (ECL) and 5-min exposure was sufficient to detect the full-length *CDSN* with both antibodies. Revelation with ECL Plus reagent and 5-min exposure was necessary to visualize clearly the 16-kDa band (open arrowhead). There was faint cross-reactivity of antibodies with keratins present in the extracts (asterisks). Immunoblot probed with an anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody indicates equal protein loading (left).

p.Pro252Leufs*37 mutant was not detectable in the epidermis of the patient.¹¹ Thus it is likely that both mutations influence the stability of the corresponding mRNA or protein. Here we show for the first time that a mutant corneodesmosin can be stably expressed in patients with PSD. This unexpected result is particularly interesting regarding genotype–phenotype correlations. The mutant protein has an interrupted N-terminal glycine loop domain, which could lead to a loss of function. However, an insufficient amount of a still-functional truncated protein cannot be excluded. This remains to be clarified.

Corneodesmosin also has a key role in the hair-anchoring structure and is strongly expressed in the inner root sheath of the human hair follicle.⁴ Autosomal dominant mutations in *CDSN* cause hypotrichosis simplex of the scalp.¹³ This disorder is characterized by progressive hair loss beginning in childhood and resulting in almost complete baldness of the scalp by the third decade. Three different autosomal dominant mutations in the *CDSN* gene have been reported (p.Gln200*, p.Gln215* and p.Tyr239*), and each led to a truncated protein with an N-terminal glycine loop domain.^{13,14} These mutant proteins abnormally accumulated as amorphous deposits at the periphery of hair follicles and in the papillary dermis.¹⁵ In our case, the patient has a slight hypotrichosis, which we consider age related and unusual in PSD. Accordingly, we did not detect deposits that were positive for the antibody B_{102–115} in the dermis, suggesting that the mutant corneodesmosin is unable to form amyloid-like aggregates. Also, the patient is not bald, contrary to patients of the same age suffering from hypotrichosis simplex of the scalp. These observations are not in favour of a link between the minor hair phenotype and corneodesmosin abnormalities.

This report reveals that homozygous expression of a truncated form of corneodesmosin is sufficient to lead to inflammatory PSS and loss of homeostasis in the epidermis. The paucity of clinical cases makes difficult the dissection of molecular mechanisms connecting corneodesmosin deficiency and PSD. Supplementary studies are necessary to understand the pathogenesis of PSD better.

Acknowledgments

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Supporting Information

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

Fig S1. Acanthosis, hyperkeratosis and moderate inflammation in the dermis (haematoxylin and eosin section).

Fig S2. Cytokine expression analysis.

Fig S3. Sequencing of *CDSN* from genomic DNA of the patient's healthy children.

Fig S4. Analysis of corneodesmosin expression by immunohistochemistry.

Data S1. Supplementary materials and methods.

Table S1. Primer sequences.

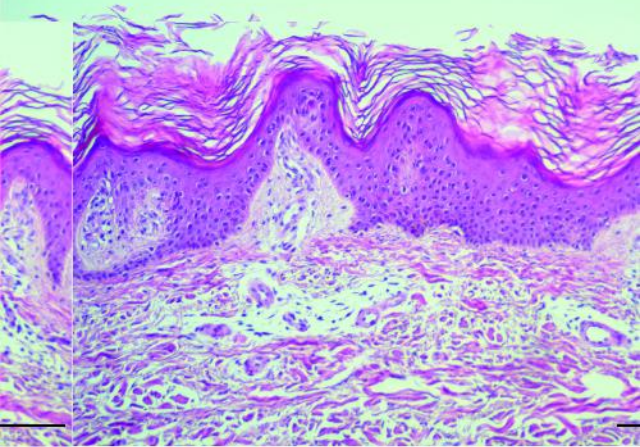


FIGURE S1

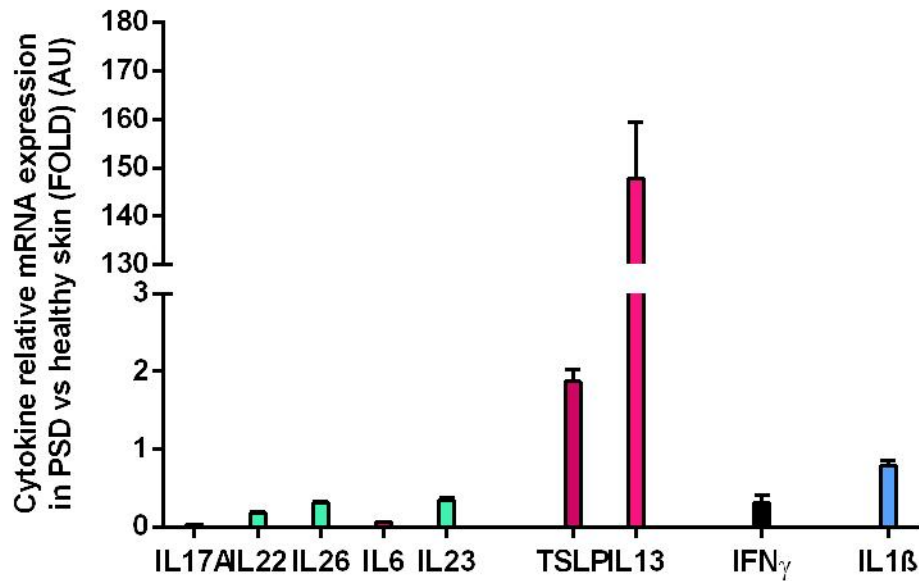
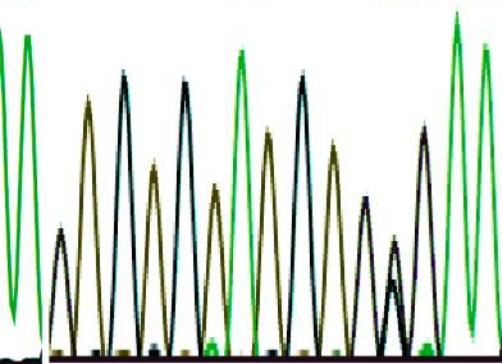


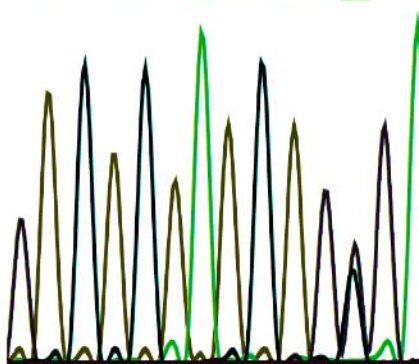
Figure S2

AA GCTCTCACTCGNGAA



offspring 1

GC TCTCACTCGNGA

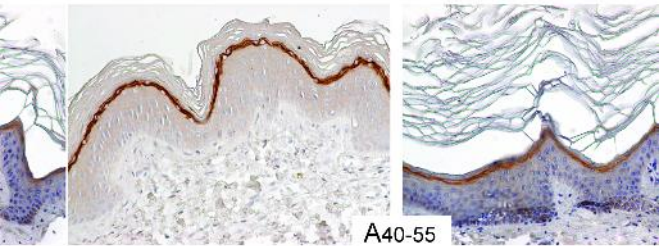


offspring 2

FIGURE S3

Control

Disease Patient



A40-55

Figure S4

SUPPLEMENTAL MATERIAL

Sequencing Analysis: Genomic DNA was extracted from the whole peripheral blood of our patient and of her children (Illustra DNA, Extraction kit BACC1 for blood and cell cultures, RPN-8501, GE healthcare). The entire *CDSN* gene (NM_001264.4) including all intron-exon boundaries was amplified by polymerase chain reaction (PCR), using specific primers¹ and sequenced by the Sanger method (3130 XL Applied Biosystems apparatus). We verified by genomic sequencing that the new mutation we identified was absent from 50 unrelated healthy subjects.

Histology: Skin biopsies from a healthy individual and the PSS patient were fixed overnight in freshly prepared PFA 4% (paraformaldehyde) at 4°C, then washed in TBS and embedded in paraffin. Skin specimens of 5µm thickness were deparaffinized, rehydrated and stained with hematoxylin and eosin as described². Stained sections were analyzed under the light microscope (Nikon Ellipse E400, Switzerland) coupled to a CCD camera.

Immunohistochemistry and immunoblot analysis: Affinity-purified anti-peptide antibodies B₁₀₂₋₁₁₅ and the anti-CDSN mAb G36-19 were produced and characterized in our laboratory³⁻⁵. A mouse monoclonal primary antibody recognizing human GAPDH was used (sc-32233, SantaCruz Biotechnology, TX). For immunohistochemistry, 5µm-thick paraffin sections of healthy skin (control) and patient's skin were labeled with primary antibodies. Detection was performed with the appropriate ImmPRESS anti-rabbit or anti-mouse Ig (peroxidase) kit (Vector Laboratories). Images were taken using a Nikon eclipse 80i microscope equipped with a Nikon DXM 1200C digital camera and NIS image analysis software. For western blot, proteins from total skin were extracted by incubation in extraction buffer (100 mM Tris-HCl, 20 mM DTT, 5 mM EDTA, 2% SDS, pH8.5) for 20 min. at 95°C under vigorous shaking. Equal quantities of proteins were separated by 12.5% SDS-PAGE and transferred to nitrocellulose membrane. The blots were probed with primary antibodies and HRP-conjugated secondary antibodies (Invitrogen). Detection was performed with ECL reagent or ECL Plus (GE Healthcare, Sweden). The NIH ImageJ free software (Bethesda, MD) was used to quantify

immunoreactive bands on Western blot films after scanning. Signals were normalized according to the signal obtained on immunoblots probed with the anti-GAPDH antibody.

RNA extraction and real time PCR: Total RNA from total skin biopsies was extracted using the RNeasy Fibrous Tissue Mini Kit (Qiagen, Germany) according to the manufacturer's guidelines. RNA (2 µg) was reverse-transcribed into cDNA using MMLV-reverse transcriptase (New England Biolabs, UK) and using the following conditions: 10 min at 25° C – 60 min at 42° C – 5 min at 95°C. Real-time PCR analysis was performed on a StepOne™ PCR apparatus (Applied Biosystems, UK) using a Power SYBR Green Master Mix (Applied Biosystems, UK) and specific primers. Samples were amplified as described². Primers were designed using the Roche software (Universal Probe Library, Assay Design Center) (Table I). Analysis of relative gene expression was performed using the $2^{-\Delta\Delta CT}$ method⁶. Ribosomal protein L13A (RPL13A) mRNA was used as internal control (Quanti-Tect Primer Assays, Qiagen, Germany).

Table 1: Primer sequences

Gene Name	Forward	Reverse	Accession Number
CDSN	ACTGCTGCTGGCTGGTCT	AGAGCTTCTGGCACTGGAAA	NM_001264
IL1 β	TACCTGTCCTGCGTGTTGAA	TCTTTGGGTAATTTTTGGGAT CT	NM_000576
IL6	GATGAGTACAAAAGTCCTGATCCA	CTGCAGCCACTGGTTCTGT	NM_000600.3
IL13	AGCCCTCAGGGAGCTCAT	TGATGCTCCATACCATGCTG	NM_002188.2
IL17A	TGGGAAGACCTCATTGGTGT	GGATTTCTGTGGGATTGTGAT	NM_002190.2
IL22	CAACAGGCTAAGCACATGTCA	ACTGTGTCCTTCAGCTTTTGC	NM_020525.4
IL23	AGCTTCATGCCTCCCTACTG	CTGCTGAGTCTCCCAGTGGT	NM_016584.2
IL26	TTTGAGGTGTGGGTTGCTGT	ATGCGGTCTTCTGGAATCGT	NM_018402.1
IFN γ	GGCATTTTGAAGAATTGGAAAG	TTTGGATGCTCTGGTCATCTT	NM_000619.2
TSLP	CCAGGCTATTCGGAAACTCA	TCCAGACATTTATTGGTTGTG ACT	NM_033035.4
RPL13A	Qiagen, QuantiTect Primer Assays QT02321333		NM_012423

SUPPLEMENTAL REFERENCES

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SUPPLEMENTAL FIGURES

Figure S1: Acanthosis, hyperkeratosis and moderate inflammation in the dermis (H&E section) Bar, 50 μ m.

Figure S2: Cytokine expression analysis.

Real-time PCRs of cDNAs from control and PSD, reveals increased expression of Th2-related cytokines mRNA, while Th17-related cytokines, and Th1-cytokine IFN γ mRNAs are reduced. (normalized with RPL13A). The experiment was realized in 3 control biopsies and 1 PSD biopsy collected from an inflamed area of the patient. Samples were analyzed in triplicates. The mean of PSD triplicates was normalized to the mean of the control samples, which is calibrated to 1.

Figure S3: Sequencing of *CDSN* from genomic DNA of the healthy patient's children shows heterozygous inheritance of the G>T transition at nucleotide 424 (from the translation start site) in exon 2.

Figure S4: Analysis of CDSN expression by immunohistochemistry

Immunohistochemical staining of control and patient's skin sections with antibody A40-55 reveals expression of a mutant CDSN in the upper epidermis of the patient. Note that the labeling is less intense in the case of the PSD patient. Bar, 100 μ m.