# Papillon–Lefèvre Syndrome: Mutations and Polymorphisms in the Cathepsin C Gene

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**The Papillon–Lefèvre syndrome, inherited in an autosomal recessive pattern, manifests with palmoplantar keratoderma and early, destructive periodontitis. Recently, mutations in the gene encoding cathepsin C have been disclosed in a limited number of families with Papillon–Lefèvre syndrome. We have examined two multiplex families with Papillon–Lefèvre syndrome, and evaluated the gene encoding cathepsin C for mutations. The mutation detection strategy consisted of polymerase chain reaction amplification of all seven exons and flanking intronic sequences, followed by direct nucleotide sequencing. This strategy identified two missense mutations, W39S and G301S, affecting highly conserved amino acid residues within the cathepsin C polypeptide. The affected individuals were homozygotes whereas heterozygous carriers of the mutations were clinically unaffected, confirming the recessive nature of the mutations. Addition of these cathepsin C gene mutations into the expanding Papillon–Lefèvre syndrome mutation database allows further development of genotype/phenotype correlations towards understanding this severe genodermatosis.**

***Key words: keratoderma, periodontitis, genodermatoses.***

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The Papillon–Lefèvre syndrome (PLS; OMIM no. 245000) is a relatively rare autosomal recessive condition manifesting with palmoplantar keratoderma, combined with a rapidly progressive periodontitis (Papillon and Lefèvre, 1924; for reviews see Haneke, 1979; Hart and Shapira, 1994; Siragusa *et al*, 2000). The estimated prevalence is one to four per $10^6$ (Verma *et al*, 1979). The initial clinical signs of skin involvement are usually evident during the first 4 y of life, and a histopathologic examination of the affected skin shows hyperkeratosis with psoriasiform parakeratosis. The gingival involvement may be noticeable as early as 3 or 4 y of age. Both the deciduous teeth and permanent teeth are lost prematurely, and in general the patients affected by PLS are edentulous by the age of 15 y. Calcification of the dura mater has been suggested to be the third component of the syndrome (Gorlin *et al*, 1964).

A number of suggestions for pathoetiology in PLS have been advanced over the years, including immune abnormalities and susceptibility to bacterial infections (see Hart and Shapira, 1994); however, the link between the cutaneous and gingival findings has not been clear. Originally, the PLS locus was placed on human chromosome 11q14 by homozygosity linkage mapping (Fischer *et al*, 1997; Laass *et al*, 1997; Hart *et al*, 1998, 2000c), and quite recently a number of mutations in patients with PLS have been reported in the gene encoding human cathepsin C (CTSC) (Toomes *et al*, 1999; Hart *et al*, 1999, 2000a, b). This gene consists of a total of seven exons and the corresponding mRNA, 1.8 kb, encodes a polypeptide of 463 amino acids. The deduced polypeptide consists of a 24 amino acid signal peptide, a 206 amino acid propeptide, and 233 amino acid mature enzyme (Rao *et al*, 1997). Cathepsin C, also known as dipeptidyl-peptidase I (EC 3.4.14.1), is a lysosomal cysteine proteinase, and apparently plays an important role in intracellular degradation of proteins and in activation of many serine proteinases within immune/inflammatory cells, including polymorphonuclear leukocytes, monocyte-macrophages, and mast cells.

In this study, we report CTSC mutations in two families with PLS, and the first polymorphisms in the gene.

## MATERIALS AND METHODS

### Clinical material and diagnostic features

*Family 1* The proband (I-2, Family 1 in **Fig 1**) was a 49-y-old female of Puerto Rican origin, with severe periodontitis and characteristic cutaneous findings diagnostic of PLS. Specifically, she had a history of bilateral scaly patches on the feet, palms, and elbows since childhood. She also had premature loss of both deciduous and permanent teeth, which required replacement with dentures at the age of 12 y. She denied recurrent skin or systemic infections.

The proband had 10 siblings, three of which (I-3, I-4, and I-5, Family 1 in **Fig 1**) were similarly affected. For example, the younger brother (I-5) was noted to have yellow, fissured hyperkeratotic palms and soles with slight pseudoainhum. Scaly hyperkeratotic plaques were present in elbows and knees.

Histopathology of the brother's (I-5) skin from right elbow and right palm revealed confluent parakeratosis, tortuous capillaries in the dermal

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Abbreviations: PLS, Papillon–Lefèvre syndrome; CTSC, cathepsin C gene.

papillae, and a sparse infiltrate of lymphocytes around venules of the superficial plexus, changes reported to be indistinguishable from psoriasis (Angel *et al*, 2001).

The proband's parents were dead but they were not known to have skin or dental findings similar to their affected children, and there was known parental consanguinity.

*Family 2* The proband at the time of initial examination was a 4-y-old female, the second child of consanguineous Japanese parents (II-2, Family 2 in **Fig 1**). She had an older, clinically unaffected brother (II-1), whereas a younger brother, 1 y of age (II-3), was clearly affected. The parents were clinically normal, and the inheritance was therefore consistent with an autosomal recessive pattern. The affected children had erythematous hyperkeratotic lesions, which were first noted on their feet at 4 mo of age, and the lesions subsequently developed on their hands, elbows, and knees (**Fig 2*a–d***). Histopathology of the skin from the proband's brother (II-3) at the age of 5 y revealed acanthosis with hyperkeratosis and parakeratosis (**Fig 3**). There was evidence of follicular hyperkeratosis. The proband also had a history of a liver abscess at the age of 5 y.

At the current age of 24 and 21 y, the two individuals with skin lesions (II-2 and II-3) have minimal dental complaints and do not display overt tooth loss. Panoramic X-ray examination of the teeth of the affected male (II-3), however, revealed extensive alveolar resorption (**Fig 2*e***). Examination of his older sister's (II-2) teeth revealed similar but less pronounced changes (not shown).

Leukocyte function studies, including neutrophil killer activity and neutrophil phagocytic activity, were essentially normal (87% and 92%–95% of the controls) in both patients at 24 and 21 y of age. Among the nine lymphocyte surface markers tested, the affected sister's (II-2) values were well within the normal range, whereas some of the affected brother's (II-3) values (and specifically those for CD56, CD11b, CD11c, CD 57, and CD 16) were at the lower end of the normal values or just below the lower limit.

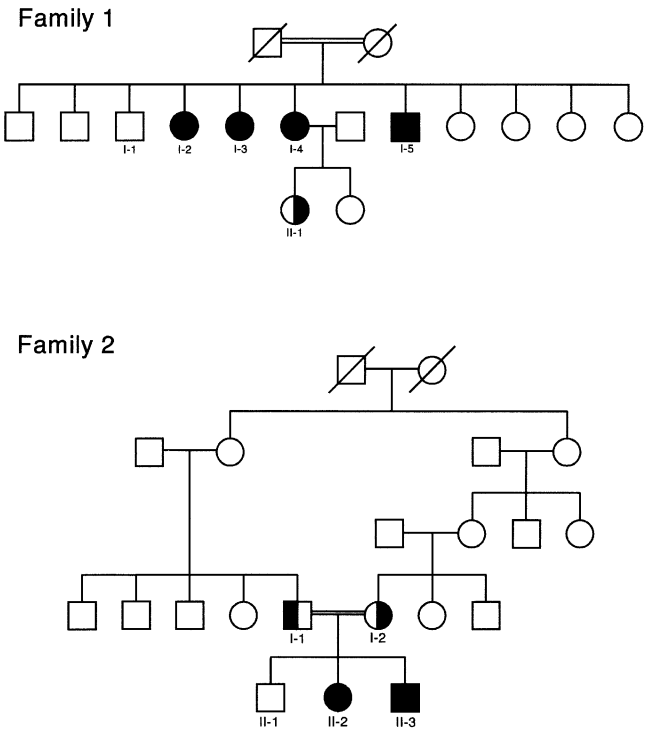


**Figure 1. Pedigrees of Family 1 and Family 2 with Papillon–Lefèvre syndrome.** The solid symbols refer to affected individuals, whereas the half-solid symbols refer to clinically unaffected individuals shown to be heterozygous carriers of a mutation. The open symbols refer to clinically unaffected individuals. DNA from individuals indicated by a number under their symbol were studied for mutations in the CTSC gene. Note that the parents of the affected individuals in the two families are related.

**DNA extraction** DNA was isolated from peripheral blood specimens from the probands and other available members of the nuclear families, as indicated in Fig 1, by phenol-chloroform extraction using standard procedures (Sambrook *et al*, 1989). Control DNA was obtained from 50 unrelated individuals with no evidence of a skin disease. DNA was used as a template for polymerase chain reaction (PCR) amplification of exons for mutation detection analysis.

**PCR** The PCR amplification of CTSC exons and of flanking intronic sequences was performed using primers as previously indicated (Toomes *et al*, 1999), except for the following newly developed primer pairs for exons 1 and the 5′ half of exon 7: exon 1F, 5′-TCTTCACC-TCTTTTCTCAGC-3′; exon 1R, 5′-GGTCCCCGAATCCAGTC-AAG-3′; exon 7-1F, 5′-TAAGCAGAGATACAGAGAAG-3′; exon 7-1R, 5′-GTAGTGGAGGAAGTCATCATATAC-3′.

The PCR reactions were performed in a total volume of 50 μl containing 1 × PCR buffer and 1.25 U of Amplitaq polymerase (Perkin-Elmer Cetus, Foster City, CA), in the presence of 4% dimethyl sulfoxide, 12.8 pmol of each primer, and 200 ng of genomic DNA. The amplification conditions were as follows: 5 min at 94°C for one cycle, followed by 38 cycles of 45 s at 94°C, 45 s at the annealing temperature of the primers (exon 1, 55°C; exon 7-1, 53°C), and 45 s at 72°C in a thermal cycler (Hybaid, Teddington, U.K.). Five microliter aliquots of the PCR products were analyzed by 2% agarose gel electrophoresis.

**DNA sequencing and verification of the mutations by restriction endonuclease digestion** PCR products were directly sequenced by using an ABI Prism 377 automated sequencing system (Perkin-Elmer

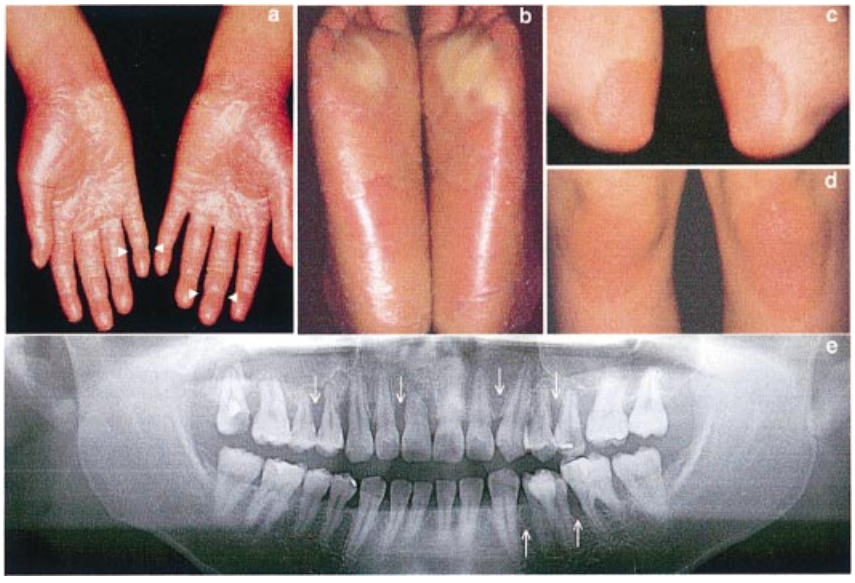


**Figure 2. Clinical findings in Family 2.** The palms and soles of individual II-3 at the age of 21 y (*a*, *b*) show severe erythema and hyperkeratosis. There was evidence of development of pseudoainhums on the fingers (*arrowhead* in *panel a*). The 24-y-old sister had well-defined erythematous plaques on her elbows and knees (*c*, *d*). Panozamic X-ray of the teeth of individual II-3 at the age of 21 y (*e*) shows general alveolar and tooth resorption (*arrows*).

Cetus). Verification of the mutation was performed by digestion of PCR products amplified from DNA obtained from the probands and his/her immediate family members by restriction endonucleases. The PCR products were digested at 37°C for *Aci*I or *Mnl*I for 15 h. The fragments were examined on 3% or 3.5% agarose gel.

## RESULTS

In Family 1, screening of CTSC for pathogenetic mutations revealed that the four affected individuals were homozygous for a G→C substitution at the nucleotide position 116 (**Fig 4**). A clinically unaffected child (II-1) of an affected individual (I-4) was a heterozygous carrier of this mutation (**Fig 4**). This novel nucleotide substitution resulted in a change from tryptophan (TGG) to serine (TCG) at the amino acid position 39, and this mutation was designated W39S (**Table I**). This novel amino acid substitution mutation affects an evolutionarily conserved tryptophan residue. The nucleotide substitution also creates a new restriction enzyme site for *Mnl*I, which was used for verification of the mutation in this family. *Mnl*I digestion also revealed that an unaffected older brother (I-1) is not a carrier of this mutation (**Fig 4**). Screening of 100 chromosomes in healthy, unrelated control subjects by *Mnl*I digestion demonstrated the absence of the W39S mutation, suggesting that it was indeed pathogenetic.

Screening of Family 2 for CTSC mutations revealed that the parents (I-1 and I-2) were heterozygous for a G→A substitution at nucleotide position 901, whereas the two affected children (II-2 and II-3) were homozygous for this mutation. This nucleotide substitution resulted in a change from glycine (GGC) to serine (AGC) at the amino acid position 301, and this mutation was designated G301S (**Table I**). This mutation has been previously noted in another unrelated family with PLS, and its presence has

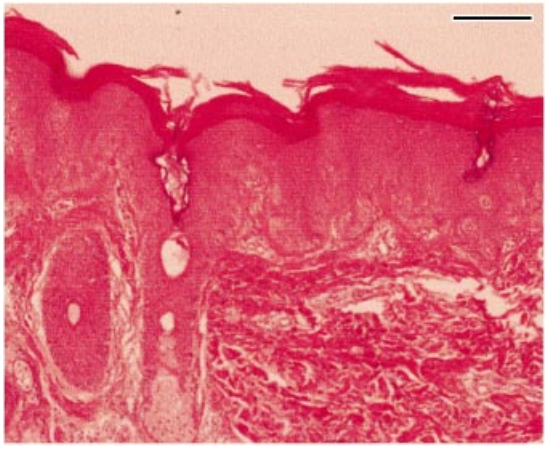

**Figure 3. Histopathologic findings in a skin biopsy from the knee of individual II-3 of Family 2 at the age of 5 y.** Note acanthosis and hyperkeratosis with follicular hyperkeratosis, parakeratosis, and perivascular inflammatory cell infiltrates (*scale bar*: 300 μm; hematoxylin and eosin stain).

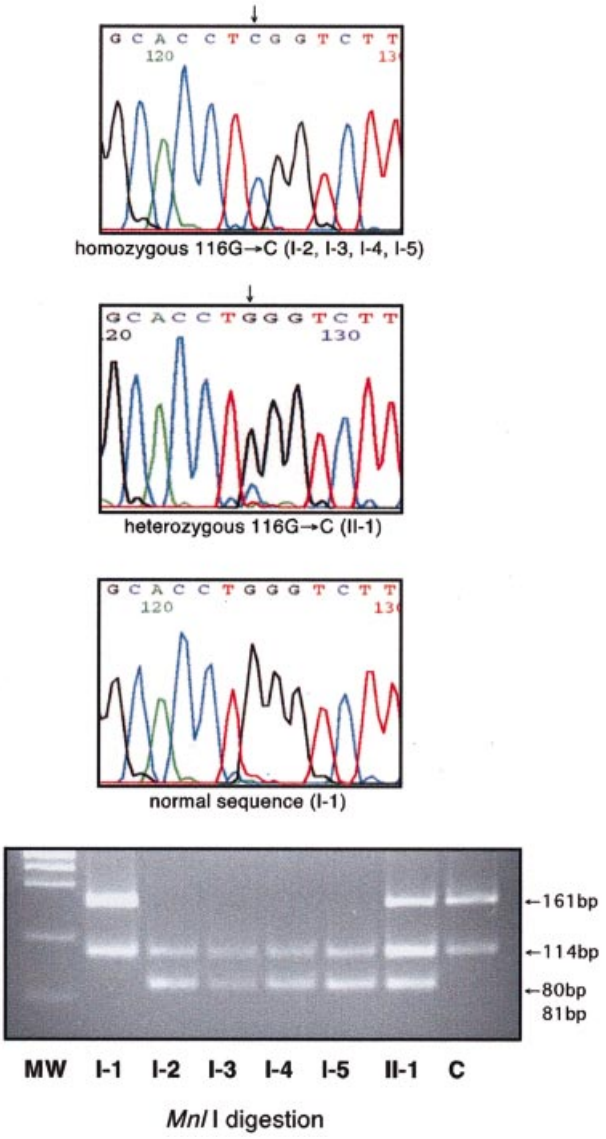


**Figure 4. Identification and verification of the homozygous missense mutation in Family 1.** Direct nucleotide sequencing of the PCR product spanning exon 1 revealed a homozygous 116G→C in the affected individuals (I-2, I-3, I-4, and I-5) (*top panel*), whereas an unaffected child (II-1) of the patient I-4 was heterozygous for this nucleotide change (*middle panel*), compared with the normal sequence also found in an unaffected brother (I-1) (*lower panel*). The mutation was designated W39S. Verification of the mutation by digestion with *Mnl*I (*bottom panel*). The mutation created a new restriction enzyme site for *Mnl*I, which cuts the 338 bp PCR product in the case of the normal allele to 161 bp, 114 bp, 37 bp, 19 bp, and 7 bp bands, and in case of the mutant allele to 114 bp, 81 bp, 80 bp, 37 bp, 19 bp, and 7 bp bands (the smaller bands are not visible). MW, molecular weight markers φX174/HaeIII; C, control.

**Table I. Nucleotide variations in the CTSC gene**

| Location | Nucleotide position[a] | Nucleotide change | Amino acid substitution | Restriction enzyme site | Allele frequencies |
|---|---|---|---|---|---|
| *Mutations* | | | | | |
| Exon 1 (Family 1) | 116 | G (Trp) → C (Ser) | W39S | *Mnl*I | – |
| Exon 7 (Family 2) | 901 | G (Gly) → A (Ser) | G301S | *Acl*I | – |
| *Polymorphisms* | | | | | |
| Intron 1 | 172 + 106 | G → A | – | *Hae*III | 0.714/0.286 |
| Exon 7 | 1173 | T (Thr) → G (Thr) | T391T | *Bsl*I | 0.953/0.047 |
| Exon 7 | 1357 | A (Ile) → G (Val) | I453V | *Msl*I | 0.953/0.047 |

[a]Nucleotide numbers refer to the open reading frame, the translation-initiation codon (ATG) being +1/+3 in CTSC cDNA sequence (GenBank accession no. NM001814).

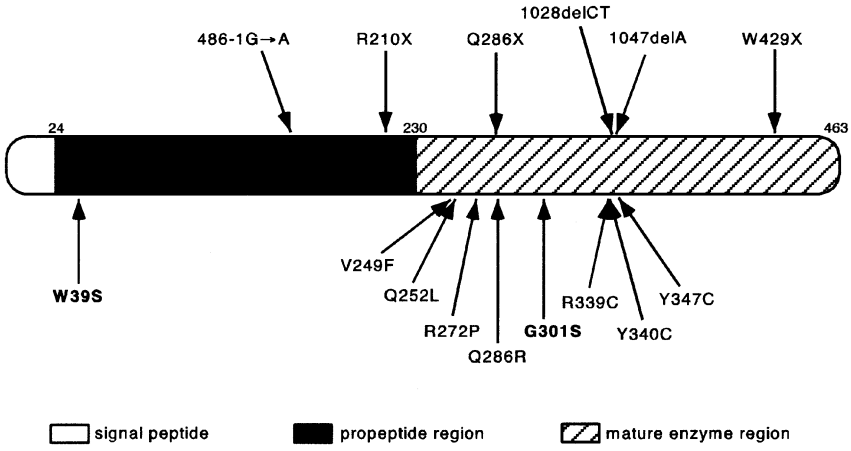


**Figure 5. Schematic illustration of the cathepsin C polypeptide, deduced from cDNA, with domain organization, and positions of all CTSC mutations disclosed thus far in patients with PLS.** The premature termination codon and the putative splice site mutation are shown above the molecule, whereas missense mutations are shown below. The mutations disclosed in this study are shown in bold. The amino acid positions at the domain borders are indicated above the polypeptide.

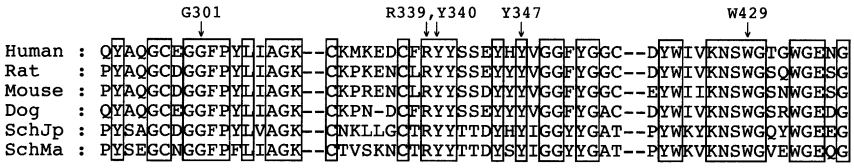


**Figure 6. Conservation of peptide segments encoded by exon 7 of the CTSC gene.** The partial human sequences are compared with those in other vertebrates indicated, as well as in Schistosoma japonicum (SchJp) and mansoni (SchMa). The conserved amino acids are boxed, and the positions of missense and nonsense mutations are indicated by arrows. The dashed lines indicated additional sequences not shown. A similar comparison of sequences encoded by exons 1–6 is presented elsewhere (Hart *et al*, 2000a).

been excluded from a control population of 200 unrelated healthy control individuals (Toomes *et al*, 1999). This nucleotide substitution also abolished a restriction enzyme site for *Aci*I, which was used for verification of the mutation. *Aci*I digestion confirmed that the parents were heterozygous carriers of the mutation whereas the affected individuals were homozygotes. The *Aci*I digestion also revealed that an unaffected brother (II-1) is not a carrier of this mutation.

During the search for pathogenetic mutations in these two families, three additional sequence variants were discovered (**Table I**). One of them was an intronic nucleotide substitution whereas another was a neutral polymorphism in exon 7 (T391T). The third one, 1357 A→G, substituted an isoleucine in position 543 by a valine (I453V). The allele frequencies of these apparent polymorphisms are indicated in **Table I**.

## DISCUSSION

In this study, we have identified homozygous missense mutations W39S and G301S in two families with PLS. Discovery of the novel CTSC mutation W39S brings the total number of distinct mutations found in different families with PLS to 15 (**Fig 5**). Nine of them are missense mutations, whereas five are nonsense mutations or small deletion mutations resulting in premature termination codon for translation. In addition, one family has a splicing mutation (486-1G→A) at the intron 2/exon 3 border.

The missense mutations, including W39S, affect critical, highly conserved amino acids (Hart *et al*, 2000a), and eight out of nine missense mutations reside within the mature enzyme domain of the polypeptides (**Fig 5**). Also, three of the amino acid substitutions lead to incorporation of a cysteine in the polypeptide. It is conceivable therefore that these missense mutations result in conformational changes that abolish the catalytic activity of the enzyme. In fact, the activity of this enzyme has been shown to be essentially undetectable in peripheral blood leukocytes of affected individuals in two families with PLS as a result of missense mutations V249F and Y347C (Toomes *et al*, 1999).

The novel missense mutation identified in this study, W39S, was found in homozygous state in all four affected individuals in Family 1, whereas a heterozygous carrier had no evidence of PLS. Furthermore, this mutation was not present in 100 alleles in unrelated control individuals, thus indicating that it is not a polymorphism. This tryptophan residue is precisely conserved in cathepsin C during evolution in various species between humans and Schistosoma japonicum (see **Fig 6**). Interestingly, however, the W39S mutation resides at the amino-terminal end of the propeptide (**Fig 5**), which is cleaved off during processing of the polypeptide to mature enzyme (Muno *et al*, 1993). It is conceivable then that the W39S mutation interferes either with the transport of the proform polypeptide from endoplasmic reticulum to lysosomes or impedes the subsequent proteolytic processing of the polypeptide to mature enzyme.

It is of interest that Family 2 with the G301S mutation had clinical features not entirely typical of PLS. Specifically, the two affected individuals in this family had retained their teeth up to their current age of 24 and 21 y whereas classic cases with PLS are edentulous by the age of 15 y. Such late onset of periodontitis has been noted before (Willett *et al*, 1985; Brown *et al*, 1993; Fardal *et al*, 1998). It is also of interest that the specific cathepsin C mutation, Y347C, is associated with severe periodontitis but the individuals homozygous with this mutation have no evidence of syndromic skin manifestations (Hart *et al*, 2000b). Finally, another autosomal recessive condition with palmoplantar keratoderma and

early periodontal destruction, the Haim–Munk syndrome (OMIM no. 245010), has been shown to be allelic with PLS (Hart *et al*, 2000a). A recurrent missense mutation, Q286R, in exon 6 of the CTSC gene has been identified in several nuclear families with the Haim–Munk syndrome, all of the same ancestry (Hart *et al*, 2000a). The same study reported a nonsense mutation, Q286X, in the same codon of CTSC in a family with classic features of PLS (Hart *et al*, 2000a). Collectively, this phenotypic variability of the CTSC mutations suggests either phenotype/genotype correlations or phenotypic modulation by associated genetic and/or epigenetic factors that are not yet evident from the relatively small cohort of patients.

The pathomechanistic implications of absent or markedly reduced cathepsin C activities in PLS are not entirely clear but it has been suggested that lack of functional CTSC may be associated with reduced host response against bacteria in dental plaque and possibly other sites (Oguzkurt *et al*, 1996; Czauderna *et al*, 1999). CTSC plays an essential role in activating serine proteinases expressed in the granules of bone marrow derived cells both of myeloid and lymphoid series (McGuire *et al*, 1993). These serine proteinases are implicated in a variety of inflammatory and immune processes, including phagocytic destruction of bacteria. In fact, previous leukocyte function studies have suggested depressed neutrophil phagocytic and lytic activity and depressed chemotactic response (Bullon *et al*, 1993; Ghaffar *et al*, 1999; Liu *et al*, 2000). Although these leukocyte functions were well within the normal limits in the two patients in Family 2, a number of lymphocyte surface markers were at the low end of normal values or slightly below the normal limits, suggesting immunologic deficiencies. Mechanistically, deficient activation of leukocyte serine proteinases due to lack of CTSC activity could possibly explain the severe periodontitis in PLS. The mechanisms leading to hyperkeratotic skin lesions are unclear, however. One could speculate that CTSC plays a role in epithelial differentiation leading to characteristic cutaneous findings in PLS.

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