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Functional *Cathepsin C* mutations cause different Papillon–Lefèvre syndrome phenotypes

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Abstract

Aim: The autosomal-recessive Papillon–Lefèvre syndrome (PLS) is characterized by severe aggressive periodontitis, combined with palmoplantar hyperkeratosis, and is caused by mutations in the *Cathepsin C* (*CTSC*) gene. This study aimed to identify *CTSC* mutations in different PLS phenotypes, including atypical forms and isolated pre-pubertal aggressive periodontitis (PAP).

Material and Methods: Thirteen families with different phenotypes were analysed by direct sequencing of the entire coding region and the regulatory regions of *CTSC*. The function of novel mutations was tested with enzyme activity measurements.

Results: In 11 of 13 families, 12 different pathogenic *CTSC* mutations were found in 10 typical PLS patients, three atypical cases and one PAP patient. Out of four novel mutations, three result in protein truncation and are thus considered to be pathogenic. The homozygous c.854C > T nucleotide exchange (p.P285L) was associated with an almost complete loss of enzyme activity. The observed phenotypic heterogeneity could not be associated with specific genotypes.

Conclusions: The phenotypic variability of the PLS associated with an identical genetic background may reflect the influence of additional genetic or environmental factors on disease characteristics. *CTSC* mutation analyses should be considered for differential diagnosis in all children suffering from severe aggressive periodontitis.

Key words: *Cathepsin C* gene mutation; Papillon–Lefèvre syndrome; pre-pubertal aggressive periodontitis

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Papillon–Lefèvre syndrome (PLS) is a rare autosomal-recessive disorder (one to four cases per million), described for the first time in 1924 in France (Papillon & Lefèvre 1924). It is characterized by severe aggressive periodontitis (AgP), combined with palmoplantar hyperkeratosis (PPK), and symptoms arise during the first years of life. PPK is often transgradient, also affecting the dorsa of the fingers and toes. Additionally, the elbows, knees or trunk can be affected by hyperkeratosis. Further sporadic findings include calcification of falx cerebri, mental or somatic retardation, arachnodactyly and hyperhidrosis (Gorlin et al. 1964, Haneke 1979). Approximately 20–25% of PLS cases suffer from an increased susceptibility to infections other than periodontitis; most show a predisposition to mild skin infections such as furunculosis or pyodermas. Occasionally, severe infections such as liver abscesses or pneumonia occur (Haneke et al. 1975, Almuneef et al. 2003).

AgP affects the primary dentition and extends to the permanent dentition. While successful therapy approaches have been reported, PLS results in premature tooth loss in most cases. Recent data suggest that mechanical debridement associated with antibiotics and followed by a consequent maintenance therapy can be successful in periodontal management in single cases (Rüdiger et al. 1999, De Vree et al. 2000,

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Eickholz et al. 2001, Ullbro et al. 2005, Schacher et al. 2006).

The increasing number of reported atypical PLS cases, including those with isolated keratosis or periodontitis, prompts the establishment of the correct diagnosis in this rare disorder. Atypical cases include late-onset or mild forms of periodontitis as well as mild or missing dermatological findings (Brown et al. 1993, Bullon et al. 1993, Soskolne et al. 1996, Fardal et al. 1998, Nakano et al. 2001, de Haar et al. 2004). Since the gene locus for PLS has been mapped to chromosome 11q14 in 1999, and the disease-associated gene was identified as the *Cathepsin C* (*CTSC*) gene (Hart et al. 1999, Toomes et al. 1999), more than 60 different homozygous or compound heterozygous mutations in typical PLS families as well as in atypical cases have been reported. Heterozygous carriers of a mutation are clinically unaffected, although one patient with plantar hyperkeratosis without periodontitis has been reported (Cury et al. 2002). Additionally, mutations in the *CTSC* gene have been identified in two closely related conditions: in the Haim–Munk syndrome (HMS) and in an isolated form of pre-pubertal aggressive periodontitis (PAP) (Hart et al. 2000b, Hart et al. 2000c, Hewitt et al. 2004, Noack et al. 2004, Cury et al. 2005). The latter is a rare form of aggressive periodontitis affecting the primary dentition and can extend to the permanent dentition, resulting in premature tooth loss in children and young adults. In most cases, PAP is considered to be a manifestation of certain systemic disorders, whereas in rare cases PAP is an isolated finding in apparently otherwise healthy patients (Tonetti & Mombelli 1999). However, it is likely that most of these children have an unknown systemic disease that increases their susceptibility to bacterial infections. The existence of *CTSC* mutations in these patients supports the hypothesis that a subgroup of PAP can be a phenotypic variant of PLS.

Biallelic mutations in the *CTSC* gene causing PLS result in an almost total loss of enzyme function. CTSC has been implicated in the processing of serine proteases, including neutrophil-derived Cathepsin G (CTSG) or neutrophil elastase (NE) (Adkison et al. 2002). It has been shown that reduced CTSC activity results in diminished activity and stability of polymorphonuclear leucocyte (PMN)-derived proteases (Pham & Ley 1999, de Haar et al. 2004, Pham et al. 2004). These proteases may play a crucial role in the regulation of innate immune responses against invading bacteria (Nuckolls & Slavkin 1999, Pham et al. 2004, de Haar et al. 2006). Recent studies have analysed the pathogenic role of CTSC deficiency in periodontitis, showing that PMN's capacity to neutralize leucotoxin and eliminate *Aggregatibacter actinomycetemcomitans* seems to be reduced (de Haar et al. 2006). Further studies have highlighted the importance of CTSC in human natural killer (NK)-cell function in controlling infection, and an impaired NK-cell cytotoxicity in PLS-associated periodontitis is being considered (Lundgren et al. 2005, Meade et al. 2006).

The aim of this study was to apply direct *CTSC* mutation analysis in differential diagnostics of different PLS phenotypes. We report the identification of four novel pathogenic mutations and eight known mutations in the *CTSC* gene in 11 of 13 families with PLS, atypical PLS or isolated PAP.

Table 1. Characteristics of analysed families

Family # (number of cases)	Origin	Consanguinity	Phenotype
1 (1)	Turkey	Unknown	PLS (typical findings†)
2 (1*)	Germany	No	PLS (typical findings)
3 (2)	Morocco	Yes	PLS (one case with typical findings, one case with mild skin findings)
4 (1)	Iran	Yes	PLS (typical findings)
5 (2*)	Eritrea	No	PLS (typical findings)
6 (1)	Russia	Adopted	PLS (typical findings)
7 (1)	Sri Lanka	Yes	PLS (mild skin findings)
8 (1)	Germany	No	PLS (late onset of AgP in permanent dentition at the age of 22 years†)
9 (2)	Germany	Yes	PLS (typical findings†)
10 (1)	Germany	No	PLS (typical findings)
11 (1*)	Germany	Yes	PAP
12 (1)	Germany	No	PAP
13 (1)	Germany	No	PAP

*Previously reported families (Eickholz et al. 2001, Noack et al. 2004, Schacher et al. 2006); typical findings: palmoplantar hyperkeratosis, aggressive periodontitis resulting in premature tooth loss in deciduous and permanent dentition (if already erupted).

†Adult patients that are edentulous or have only very few remaining permanent teeth now. All have reported premature tooth loss also of deciduous teeth due to aggressive periodontitis.

PLS, Papillon–Lefèvre syndrome; AgP, aggressive periodontitis; PAP, pre-pubertal aggressive periodontitis.

Material and Methods

Selection of families for mutation analysis

Typical PLS families were ascertained through PPK and AgP at least in deciduous dentition, and, if already present, in second dentition. Atypical PLS families were defined as those having at least one member showing mild dermatological findings, combined with AgP or palmoplantar keratosis, combined with later onset or a mild form of periodontitis. PAP families were selected if at least one apparently otherwise healthy family member showed severe aggressive periodontitis affecting both deciduous and, if already erupted, permanent dentition.

Patients and available relatives underwent complete oral examinations as well as inspection of the skin of the palms and soles. Each adult patient or parent received clinical and genetic counselling, and signed a consent form, approved by the ethics committee of the University of Dresden. A total of 10 families suspected of having PLS as well as three PAP families were available for mutation analyses, including two PLS families (#2 and #5, Table 1) and one PAP family (#11), which has been reported previously (Eickholz et al. 2001, Noack et al. 2004, Schacher et al. 2006).

CTSC mutation analysis

Peripheral blood was obtained from all patients and available family members for DNA isolation. Peripheral blood leucocyte DNA from 100 unrelated

individuals with no evidence of skin disease and/or AgP was used as a control.

Genomic DNA was purified from whole blood using the QiaAmp blood DNA purification kit (Qiagen, Hilden, Germany). All seven exons of the *CTSC* gene including exon/intron boundary regions were amplified and sequenced using PCR primers as described previously (Noack et al., 2004). PCR primer sets for amplifying and sequencing the promoter (three segments) as well as 3′UTR have been designed. The promotor was amplified using 5′TGTGCCC CCATTGAAGAGTATTTT3′, 5′GGA GGCACCTGGAAATAG3′and 5′AGG GGAAGAATAGCAAGTAAAACA3′ as the forward primers as well as 5′CCAG GGTGAGAAGGGTGAGGA3′, 5′GGG GGAATAAGGGAGTGG3′ and 5′GAG GGCGGCGAGCAGCAAGGAG3′ as the reverse primers. The forward primer 5′CCACTACAAAAAGGGGATCTAC CACCACA3′and the reverse primer 5′TGAAGACTAAGCAATACTCCCA AATACAAGATACA3′ were used to amplify 3′UTR. Purified PCR products were directly sequenced with the PCR primer pairs using the ABI PRISM® BigDye® Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase v3.0 and v3.1 (Applied Biosystems, Forster City, CA, USA) and capillary sequencing devices (ABI 3730 DNA Analyzer, Applied Biosystems). The sequences were generated by the Sequencher 4.7 software (Gene Codes Corp., Ann Arbor, MI, USA) and aligned with the published *CTSC* gene sequence (GenBank accession no. NT_008984). Identified DNA sequence variants were confirmed by sequencing at least two independent PCR products.

To confirm whether novel missense mutations were functionally relevant, enzyme activities of CTSC and of the two downstream serine proteinases CTSG and NE were analysed. PMNs were isolated from whole peripheral blood from the patients and three healthy controls by dextran sedimentation, followed by hypotonic lysis of erythrocytes. In each sample, PMNs were enumerated and cell concentration was adjusted to 3.3×10^6/ml.

CTSC enzyme activity was determined in the cell lysates using H-glycyl-L-arginine-7-amido-4-methylcoumarin (H-Gly-Arg-AMC) (Bachem, Weil, Germany) as a substrate at a final concentration of 500 μM in 25 mM 2-(N-morpholino)ethanesulphonic acid (MES, Sigma, Munich, Germany), 50 mM NaCl and 5 mM dithiothreitol (DTT) at pH = 6.0. The enzymatic substrate turnover was measured as the increase of fluorescence (excitation and emission wavelengths at 380 and 460 nm, respectively) for 60 min. using a Spectramax GEMINI XS (Molecular Devices Corp., Sunnyvale, CA, USA).

The CTSG and NE activities were determined by measuring the rate of release of *p*-nitroanilide (*p*Na) from *N*-succinyl-Ala-Ala-Pro-Phe-*p*-Nitroanilide (Suc-AAPF-*p*NA) and *N*-methoxysuccinyl-Ala-Ala-Pro-Val-*p*-nitroanilide (MeSuc-AAPV-*p*NA) used as substrates (both Sigma GmbH, Munich, Germany) for CTSG and elastase, respectively. Cells were lysed with 0.1% hexadecyltrimethyl ammonium bromide (CTAB) mixed with the PMN suspension at a 1:1 ratio and incubated at 37°C for 15 min.

A total of 75 μl of substrate solution [500 μM Suc-AAPF-*p*NA in 50 mM *N*-(2-hydroxyethyl)piperazine-N′-(2-ethanesulfphonic acid) (HEPES), pH 7.6, or 1 mM MeSuc-AAPV-*p*NA in 50 mM Tris-HCl, pH 7.5] and 75 μl of cell lysate were mixed and the rate of *p*NA released was monitored at 405 nm using a Spectromax 250 (Molecular Devices Corp.) for 30 min.

All functional assays in patients and controls were performed conjointly and simultaneously under identical conditions.

Results

Phenotype of the PLS and PAP families

Family characteristics are summarized in Table 1. In seven of 10 PLS families, only one member was affected. Two siblings have shown signs of PLS in three families each. A total of 13 PLS cases and 11 unaffected relatives were available for analyses. Four families were consanguineous, four families were non-consanguineous, one patient was an adopted child and data of one family were unavailable. All adult patients (family # one, eight and nine) have reported premature tooth loss of deciduous and permanent teeth caused by severe periodontitis. They were edentulous or had only very few remaining permanent teeth at the time point of examination.

Two atypical cases in two different families showed severe periodontitis accompanied by an increased susceptibility to systemic infections, but only mild hyperkeratosis. One patient in a third family suffered from severe palmoplantar keratosis but exhibited a late onset of AgP. First signs of periodontitis were observed in the permanent dentition at the age of 22 years. Rapid progression of periodontal destruction resulted in complete tooth loss at the age of 26. This patient had undergone partial liver resection due to recurrent liver abscesses.

Microbiological data were available for eight PLS patients before periodontal treatment was started. All examined patients were positively tested for *A. actinomycetemcomitans*. Most patients were also positive for other periodontal pathogens.

In addition, we analysed three affected PAP children in one consanguineous family and in two non-consanguineous families. In one female PAP patient, the periodontitis resulted in generalized premature tooth loss, both in the primary and in the secondary dentition. The microbiological test was positive for *A. actinomycetemcomitans*. The two other children were below 6 years of age, and hence no permanent teeth were erupted at the time of examination. *A. actinomycetemcomitans* was also found in one of these two children. The other one showed solely a localized form of attachment loss at the lower incisors and at all the four canines. All three PAP patients were apparently otherwise healthy and did not show any signs of palmoplantar keratosis.

Results of mutation analysis

Homozygous or compound heterozygous *CTSC* mutations were identified in all 10 tested typical and atypical PLS families, and in one out of three PAP families. Overall, 12 different mutations have been found in 14 cases. The changes included seven missense mutations and five mutations resulting in a premature stop codon (including three nonsense mutations and two frameshift mutations). All affected family members carried two mutated alleles. In three families the affected individuals were compound heterozygous. Heterozygous parents or siblings have shown neither skin lesions nor signs of severe periodontitis. All detected mutations are summarized in Table 2. The four novel mutations (three protein-truncating mutations and one missense mutation) are highlighted. The homozygous c.854

Table 2. CTSC mutations

Mutation #	Family #	Nucleotide	Exon	Effect
1	8	**c.322A > T***	**3**	**K108X**
2	8	**c.436delT***	**3**	**S146fs153X**
3	11	c.566-572Del	4	T189FS199X
3	9	c.566-572Del	4	T189FS199X
3	6	c.566-572Del*	4	T189FS199X
4	6	c.628C > T*	4	R210X
5	5	c.755 A > T	5	Q252L
6	1	c.815 G > C	6	R272P
7	3	**c.854 C > T**	**6**	**P285L**†
8	10	c.901G > A	7	G301S
9	2	c.947 T > G*	7	L316R†
10	4	c.1214A > G	7	H405R
11	2	c.1268 G > C*	7	W423S†
12	7	**c.1269 G > A**	**7**	**W423X**

Novel mutations are highlighted.
*Compound heterozygous.
†Analysis of enzyme activities showed almost complete loss of CTSC, CTSG and NE activity compared to wild-type controls.
CTSC, Cathepsin C; CTSG, Cathepsin G; NE, neutrophil elastase.

[a]Human	aa 278:	SQTPILS**P**QEVVSCS
[b]Rat	aa 277:	SQTPILS**P**QEVVSCS
[c]Mouse	aa 277:	SQTPILS**P**QEVVSCS
[d]Dog	aa 251:	TQTPILS**P**QE I VSCS
[e]*SchJp*	aa 271:	RLQPILS**P**QD I I DCS
[f]*SchMa*	aa 267:	SEQPILS**P**QTVVDCP

Fig. 1. Alignment of the part of the *Cathepsin C* protein harbouring the novel mutation p.P285L. Amino acid sequence: [a]human (GenBank accession no. X87212), [b]rat (GenBank accession no. D90404), [c]mouse (GenBank accession no. U74683), [d]dog (GenBank accession no. AF06017), [e]*Schistosoma japonicum (SchJp*, GenBank accession no. U77932) and [f]*Schistosoma mansoni* (*SchMa*, GenBank accession no. Z32531). The amino acid affected by mutation is highlighted. aa, amino acid; numbers refer to the initiator methionine as codon 1.

C > T nucleotide exchange (resulting in the amino acid change p.P285L) was associated with almost complete loss of protein function, as demonstrated by the analysis of the CTSC, CTSG and NE enzymatic activity. CTSC activity in PMNs from the two subjects harbouring this mutation in a homozygous state was < 1% of the activity of healthy controls. The activities of serine proteases CTSG and NE were below the detection level. In addition, we demonstrated that our previously reported two missense mutations of family #2 (Noack et al. 2004) result in almost complete loss of CTSC, CTSG and NE activities.

A protein-truncating deletion of seven basepairs has been observed in two PLS families and one PAP family. The latter was the only PAP family harbouring *CTSC* mutations (family #11). All *CTSC* nucleotide exchanges were not present in 200 chromosomes of unrelated controls. Remarkably, when searching for mutations in the *CTSC* gene in controls, two additional novel mutations resulting in protein truncation (c.725delG and c.1299A > G) were discovered. Both control subjects were heterozygous carriers of only one mutated allele showing no clinical signs of PLS or isolated aggressive periodontitis.

Discussion

Our findings and those of other reports indicate that PLS is caused by *CTSC* mutations in the vast majority of cases, suggesting low genetic heterogeneity, combined with a high allelic heterogeneity (Hewitt et al. 2004). In 10 analysed families of our PLS population, every family member suspected of having PLS carried either a homozygous *CTSC* mutation or compound heterozygous mutations. Unaffected parents and siblings were heterozygous carriers. In addition, we identified a heterozygous protein-truncating mutation in two healthy controls.

Unlike PLS, *CTSC* mutations are not responsible for all PAP cases. In two PAP families, we could not detect *CTSC* mutations either in the coding or in the regulatory regions of the gene. These findings are in accordance with Hewitt et al. (2004) and suggest that isolated PAP is a genetically heterogeneous disease. Only a subgroup of this aggressive periodontitis form originates from an almost total loss of CTSC activity, and is a phenotypic variant of PLS.

All *CTSC* nucleotide changes reported here fulfilled the criteria of a disease-causing mutation, because they were not present in a sample of 200 control chromosomes. Five mutations were protein truncating, and were considered to be pathogenic for abolishing CTSC protein function (mutation #1–4 and 12, Table 2). The previously described compound heterozygous *CTSC* mutations in family #2 (p.L316R and p.W423S) alter amino acids located in the functional domain of the protein highly conserved in evolution (Noack et al. 2004). The same can be applied to the novel mutation in family #3 at nucleotide c.854. The C > T transition changes a proline into leucine (p.P285L). Proline285 is also highly conserved in CTSC in evolution in various species (Fig. 1). These findings suggest a pathogenic role of these three missense mutations, which was confirmed by an almost complete loss of CTSC activities in our functional study. Additionally, there were no detectable CTSG and NE activities in the PMNs of the three PLS patients of families #2 and 3. This agrees with the findings of recent studies, which demonstrated that human CTSC deficiency is associated with a severe reduction in the activities of the downstream neutrophil-derived serine proteases (de Haar et al. 2004, Pham et al. 2004, Cagli et al. 2005). One homozygous carrier of the p.P285L mutation as well as the compound heterozygous carrier of the p.L316R and p.W423S mutations were typical PLS cases affected by severe AgP of primary and permanent dentitions and by palmoplantar keratosis. One child in family #3 with mild skin lesions was reported to be suffering additionally from several systemic infections, supporting the pathogenic role of the discovered mutation.

All other detected missense mutations (p.Q252L, p.R272P, p.G301S and p.H405R) have been described previously in PLS families and have been shown to result in loss of CTSC enzyme function (Toomes et al. 1999, Hart et al. 2000a, Nakano et al. 2001, de Haar et al. 2004, Hewitt et al. 2004, Pham et al. 2004).

Several case studies have reported a growing number of families showing

clinical features not entirely typical of PLS (Brown et al. 1993, Bullon et al. 1993, Soskolne et al. 1996, Fardal et al. 1998, Inalöz et al. 2001, Nakano et al. 2001). These cases included mild or missing skin findings as well as later onset of AgP or mild periodontal destruction. The latter form could be a coincidental presence of hyperkeratosis with periodontitis due to the high prevalence of chronic periodontitis in the population. However, the observed phenotypic heterogeneity could not be associated with specific genotypes. For example, specific *CTSC* mutations such as the p.Y347C mutation have been identified in PLS as well as in PAP (Toomes et al. 1999, Hart et al. 2000c, Hewitt et al. 2004). Additionally, identical mutations were described in PLS and in the closely related HMS (Hart et al. 2000b, Allende et al. 2001, Cury et al. 2002, 2005). Thus, HMS may also represent a variable phenotypic spectrum of *CTSC* mutations (Taibjee et al. 2005). Our results confirm this phenotypic variability of identical *CTSC* mutations. The p.G301S and the p.R272P mutation, which we and others have discovered in typical PLS families (Toomes et al. 1999, Hart et al. 2000a, Lefevre et al. 2001, Zhang et al. 2002, Hewitt et al. 2004, Pham et al. 2004), have also been associated with atypical cases. Patients were characterized by both late onset of periodontitis (Nakano et al. 2001) and mild skin lesions (de Haar et al. 2004). Furthermore, we identified a seven basepair deletion (p.T189FS199X) in one PAP family, which was also found in two typical PLS families, and that has been reported previously in PLS (Hewitt et al. 2004). These results are confirmed by a recent study that failed to associate the severity of hyperkeratosis and periodontitis with different *CTSC* genotypes in 39 PLS patients (Ullbro et al. 2006).

Besides a specific genotype/phenotype relationship, a potential association of the severity of hyperkeratosis and the type of mutation (i.e. missense mutations or protein-truncating mutations) could not be confirmed (Hart et al. 2000a, Selvaraju et al. 2003). Our study is in accordance with these results. In addition to typical PLS, atypical cases with mild or missing skin lesions as well as late-onset periodontitis patients harboured both missense and protein-truncating mutations. Furthermore, the locus of the protein alteration or truncation had no influence on the specific phenotype, i.e. CTSC can be altered or truncated both in the propeptide region and in the mature enzyme region, regardless of the phenotype. In summary, the phenotypic variability of CTSC mutations suggests a modulating influence of other genetic or environmental factors that are still unknown.

In conclusion, the results of our studies imply that mutation analyses can provide support in the differential diagnosis in suspected cases of PLS, especially with atypical findings such as isolated PAP cases. Therefore, PLS should be considered in all children suffering from severe aggressive periodontitis, particularly in the deciduous dentition, although the type and location of *CTSC* mutations do not predict the severity, progression or therapy outcome of the disease. This suggests additional genetic or environmental factors modifying the phenotypic variability of PLS caused by biallelic *CTSC* mutations.

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References

Adkison, A. M., Raptis, S. Z., Kelley, D. G. & Pham, C. T. N. (2002) Dipeptidyl peptidase I activates neutrophil-derived serine proteases and regulates the development of acute experimental arthritis. *Journal of Clinical Investigation* **109**, 363–371.

Allende, L. M., Garcia-Perez, M. A., Moreno, A., Corell, A., Carasol, M., Martinez-Canut, P. & Arnaiz-Villena, A. (2001) Cathepsin C gene: first compound heterozygous patient with Papillon–Lefevre syndrome and a novel symptomless mutation. *Human Mutation* **17**, 152–153.

Almuneef, M., Al Khenaizan, S., Al Ajaji, S. & Al Anazi, A. (2003) Pyogenic liver abscess and Papillon–Lefevre syndrome: not a rare association. *Pediatrics* **111**, e85–e88.

Brown, R. S., Hays, G. L., Flaitz, C. M., O'Neill, P. A., Abramovitch, K. & White, R. R. (1993) A possible late onset variation of Papillon–Lefevre syndrome: report of 3 cases. *Journal of Periodontology* **64**, 379–386.

Bullon, P., Pascual, A., Fernandez-Novoa, M. C., Borobio, M. V., Muniain, M. A. & Camacho, F. (1993) Late onset Papillon–Lefevre syndrome? A chromosomic, neutrophil function and microbiological study. *Journal of Clinical Periodontology* **20**, 662–667.

Cagli, N. A., Hakki, S. S., Dursun, R., Toy, H., Gokalp, A., Ryu, O. H., Hart, P. S. & Hart, T. C. (2005) Clinical, genetic, and biochemical findings in two siblings with Papillon–Lefevre syndrome. *Journal of Periodontology* **76**, 2322–2329.

Cury, V. F., Costa, J. E., Gomez, R. S., Boson, W. L., Loures, C. G. & De, M. L. (2002) A novel mutation of the cathepsin C gene in Papillon–Lefevre syndrome. *Journal of Periodontology* **73**, 307–312.

Cury, V. F., Gomez, R. S., Costa, J. E., Friedman, E., Boson, W. & De Marco, L. (2005) A homozygous cathepsin C mutation associated with Haim–Munk syndrome. *British Journal of Dermatology* **152**, 353–356.

de Haar, S. F., Hiemstra, P. S., van Steenbergen, M. T., Everts, V. & Beertsen, W. (2006) Role of polymorphonuclear leukocyte-derived serine proteinases in defense against *Actinobacillus actinomycetemcomitans*. *Infection and Immunity* **74**, 5284–5291.

de Haar, S. F., Jansen, D. C., Schoenmaker, T., De Vree, H., Everts, V. & Beertsen, W. (2004) Loss-of-function mutations in cathepsin C in two families with Papillon–Lefevre syndrome are associated with deficiency of serine proteinases in PMNs. *Human Mutation* **23**, 524.

De Vree, H., Steenackers, K. & De Boever, J. A. (2000) Periodontal treatment of rapid progressive periodontitis in 2 siblings with Papillon–Lefevre syndrome: 15-year follow-up. *Journal of Clinical Periodontology* **27**, 354–360.

Eickholz, P., Kugel, B., Pohl, S., Naher, H. & Staehle, H. J. (2001) Combined mechanical and antibiotic periodontal therapy in a case of Papillon–Lefevre syndrome. *Journal of Periodontology* **72**, 542–549.

Fardal, O., Drangsholt, E. & Olsen, I. (1998) Palmar plantar keratosis and unusual periodontal findings. Observations from a family of 4 members. *Journal of Clinical Periodontology* **25**, 181–184.

Gorlin, R. J., Sedano, H. & Anderson, V. E. (1964) The syndrome of palmar-plantar hyperkeratosis and premature periodontal destruction of the teeth. A clinical and genetic analysis of the Papillon–Lefèvre syndrome. *The Journal of Pediatrics* **65**, 895–908.

Haneke, E. (1979) The Papillon–Lefevre syndrome: keratosis palmoplantaris with periodontopathy. Report of a case and review of the cases in the literature. *Human Genetics* **51**, 1–35.

Haneke, E., Hornstein, O. P. & Lex, C. (1975) Increased susceptibility to infections in the Papillon–Lefevre syndrome. *Dermatologica* **150**, 283–286.

Hart, P. S., Zhang, Y., Firatli, E., Uygur, C., Lotfazar, M., Michalec, M. D., Marks, J. J., Lu, X., Coates, B. J., Seow, W. K., Marshall, R., Williams, D., Reed, J. B., Wright, J. T. & Hart, T. C. (2000a) Identification of cathepsin C mutations in ethnically diverse Papillon–Lefevre syndrome patients. *Journal of Medical Genetics* **37**, 927–932.

Hart, T. C., Hart, P. S., Bowden, D. W., Michalec, M. D., Callison, S. A., Walker, S. J., Zhang, Y. & Firatli, E. (1999) Mutations of the cathepsin C gene are responsible for Papillon–Lefevre syndrome. *Journal of Medical Genetics* **36**, 881–887.

Hart, T. C., Hart, P. S., Michalec, M. D., Zhang, Y., Firatli, E., Van Dyke, T. E., Stabholz, A., Zlotogorski, A., Shapira, L. & Soskolne, W. A. (2000b) Haim–Munk syndrome and Papillon–Lefevre syndrome are allelic mutations in cathepsin C. *Journal of Medical Genetics* **37**, 88–94.

Hart, T. C., Hart, P. S., Michalec, M. D., Zhang, Y., Marazita, M. L., Cooper, M., Yassin, O. M., Nusier, M. & Walker, S. (2000c) Localisation of a gene for prepubertal periodontitis to chromosome 11q14 and identification of a cathepsin C gene mutation. *Journal of Medical Genetics* **37**, 95–101.

Hewitt, C., McCormick, D., Linden, G., Turk, D., Stern, I., Wallace, I., Southern, L., Zhang, L., Howard, R., Bullon, P., Wong, M., Widmer, R., Gaffar, K. A., Awawdeh, L., Briggs, J., Yaghmai, R., Jabs, E. W., Hoeger, P., Bleck, O., Rudiger, S. G., Petersilka, G., Battino, M., Brett, P., Hattab, F., Al Hamed, M., Sloan, P., Toomes, C., Dixon, M., James, J., Read, A. P. & Thakker, N. (2004) The role of cathepsin C in Papillon–Lefevre syndrome, prepubertal periodontitis, and aggressive periodontitis. *Human Mutation* **23**, 222–228.

Inalöz, H. S., Harman, M., Akdeniz, S., Inalöz, S. S. & Isik, A. G. (2001) Atypical familial Papillon–Lefevre syndrome. *Journal of the European Academy of Dermatology and Venereology* **15**, 48–50.

Lefevre, C., Blanchet-Bardon, C., Jobard, F., Bouadjar, B., Stalder, J. F., Cure, S., Hoffmann, A., Prud'Homme, J. F. & Fischer, J. (2001) Novel point mutations, deletions, and polymorphisms in the cathepsin C gene in nine families from Europe and North Africa with Papillon–Lefevre syndrome. *The Journal of Investigative Dermatology* **117**, 1657–1661.

Lundgren, T., Parhar, R. S., Renvert, S. & Tatakis, D. N. (2005) Impaired cytotoxicity in Papillon–Lefevre syndrome. *Journal of Dental Research* **84**, 414–417.

Meade, J. L., de Wynter, E. A., Brett, P., Sharif, S. M., Woods, C. G., Markham, A. F. & Cook, G. P. (2006) A family with Papillon–Lefevre syndrome reveals a requirement for cathepsin C in granzyme B activation and NK cell cytolytic activity. *Blood* **107**, 3665–3668.

Nakano, A., Nomura, K., Nakano, H., Ono, Y., LaForgia, S., Pulkkinen, L., Hashimoto, I. & Uitto, J. (2001) Papillon–Lefevre syndrome: mutations and polymorphisms in the cathepsin C gene. *The Journal of Investigative Dermatology* **116**, 339–343.

Noack, B., Görgens, H., Hoffmann, T., Fanghänel, J., Kocher, T., Eickholz, P. & Schackert, H. K. (2004) Novel mutations in the cathepsin C gene in patients with pre-pubertal aggressive periodontitis and Papillon–Lefevre syndrome. *Journal of Dental Research* **83**, 368–370.

Nuckolls, G. H. & Slavkin, H. C. (1999) Paths of glorious proteases. *Nature Genetics* **23**, 378–380.

Papillon, M. & Lefèvre, P. (1924) Dens cas de keratodermie palmaire et plantaire symetrique familale (maladie de meleda) chez le frere et al soeur: coexdistence dans les deux cas d'alterations dentaires groups. *Societé française de Dermatologie et de Syphiligraphie* **31**, 82–87.

Pham, C. T., Ivanovich, J. L., Raptis, S. Z., Zehnbauer, B. & Ley, T. J. (2004) Papillon–Lefevre syndrome: correlating the molecular, cellular, and clinical consequences of cathepsin C/dipeptidyl peptidase I deficiency in humans. *Journal of Immunology* **173**, 7277–7281.

Pham, C. T. & Ley, T. J. (1999) Dipeptidyl peptidase I is required for the processing and activation of granzymes A and B in vivo. *Proceedings of the National Academy of Sciences of the USA* **96**, 8627–8632.

Rüdiger, S., Petersilka, G. & Flemmig, T. F. (1999) Combined systemic and local antimicrobial therapy of periodontal disease in Papillon–Lefevre syndrome. A report of 4 cases. *Journal of Clinical Periodontology* **26**, 847–854.

Schacher, B., Baron, F., Ludwig, B., Valesky, E., Noack, B. & Eickholz, P. (2006) Periodontal therapy in siblings with Papillon–Lefevre syndrome and tinea capitis: a report of two cases. *Journal of Clinical Periodontology* **33**, 829–836.

Selvaraju, V., Markandaya, M., Prasad, P. V., Sathyan, P., Sethuraman, G., Srivastava, S. C., Thakker, N. & Kumar, A. (2003) Mutation analysis of the cathepsin C gene in Indian families with Papillon–Lefevre syndrome. *BMC Medical Genetics* **4**, 5.

Soskolne, W. A., Stabholz, A., Dyke, T. E., Hart, T. C. & Meyle, J. (1996) Partial expression of the Papillon–Lefevre syndrome in 2 unrelated families. *Journal of Clinical Periodontology* **23**, 764–769.

Taibjee, S. M., Zhang, L., Chapple, I. L., Thakkar, N. & Moss, C. (2005) Pyogenic skin infections as a presentation of Papillon–Lefèvre syndrome: phenotypic variability or under-reporting? *Periodontal Practice Today* **3**, 191–197.

Tonetti, M. S. & Mombelli, A. (1999) Early-onset periodontitis. *Annals of Periodontology* **4**, 39–53.

Toomes, C., James, J., Wood, A. J., Wu, C. L., McCormick, D., Lench, N., Hewitt, C., Moynihan, L., Roberts, E., Woods, C. G., Markham, A., Wong, M., Widmer, R., Ghaffar, K. A., Pemberton, M., Hussein, I. R., Temtamy, S. A., Davies, R., Read, A. P., Sloan, P., Dixon, M. J. & Thakker, N. S. (1999) Loss-of-function mutations in the cathepsin C gene result in periodontal disease and palmoplantar keratosis. *Nature Genetics* **23**, 421–424.

Ullbro, C., Brown, A. & Twetman, S. (2005) Preventive periodontal regimen in Papillon–Lefevre syndrome. *Pediatric Dentistry* **27**, 226–232.

Ullbro, C., El-Samadi, S., Boumah, C., Al-Yousef, N., Wakil, S., Twetman, S., Alfadley, A., Thestrup-Pedersen, K. & Meyer, B. (2006) Phenotypic variation and allelic heterogeneity in young patients with Papillon–Lefèvre syndrome. *Acta Dermato-Venereologica* **86**, 3–7.

Zhang, Y., Hart, P. S., Moretti, A. J., Bouwsma, O. J., Fisher, E. M., Dudlicek, L., Pettenati, M. J. & Hart, T. C. (2002) Biochemical and mutational analyses of the cathepsin c gene (CTSC) in three North American families with Papillon Lefevre syndrome. *Human Mutation* **20**, 75.

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Clinical Relevance

Scientific rationale for the study: The PLS is characterized by PPK, combined with aggressive periodontitis in both dentitions. Mutations in the *CTSC* gene cause PLS. Atypical clinical PLS signs, that include isolated PAP, and that complicate the differential diagnosis, have been reported. *Principal findings: CTSC* gene mutations have been found in 11 families with different phenotypes: typical PLS, late onset, mild hyperkeratosis or isolated PAP.

Practical implications: PLS should be considered in the differential diagnosis of children suffering from severe aggressive periodontitis. Mutation analyses can establish the diagnosis, especially in atypical cases.