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INVESTIGATIVE REPORT

Phenotypic Variation and Allelic Heterogeneity in Young Patients with Papillon-Lefèvre Syndrome

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Papillon-Lefèvre syndrome is an autosomal recessive disorder characterized by palmoplantar hyperkeratosis and aggressive periodontitis. The aim of the study was to identify underlying cathepsin C mutations in 39 subjects with Papillon-Lefèvre syndrome and to explore any phenotypic associations. Genotyping and mutation analyses were performed using standard molecular techniques, and dermatological and oral characteristics were assessed with a semiquantitative clinical score. Three genotypes were present at microsatellite marker D11S1780 and two underlying mutations were identified. The most common genotype (183/183) was associated with an 815G → C mutation in exon 6 resulting in an arginine to proline change at amino acid 272 (R272P). Patients with the 173/173 genotype revealed an exon 7 G300D mutation resulting in a glycine to aspartic acid change at amino acid 300. The mutation in a family with 189/189 genotype remained unknown. A significant difference in hyperkeratosis of the feet was found between the patients with mutations G300D and R272P ($p<0.05$), but not regarding hands or periodontal condition. Young girls displayed significantly less palmoplantar hyperkeratosis ($p<0.05$) than young boys. In conclusion, considerable phenotypic heterogeneity was observed within the two cardinal mutations and in the 189/189 genotype. ***Key words: cathepsin C gene mutation; hyperkeratosis; periodontal inflammation.***

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Papillon-Lefèvre syndrome (PLS) is an autosomal recessive disorder characterized by erythematous palmoplantar hyperkeratosis and severe early-onset periodontitis. The periodontal inflammation causes premature loss of all deciduous teeth and, in many cases, most of the permanent teeth (1). Several reports have indicated a causal connection between PLS and mutations of the cathepsin C gene resulting in a reduced or deficient activity of cathepsin C (2), (3). Cathepsin C is a lysosomal cysteine protease with an essential role in intracellular and extracellular cleavage of proteins and activation of granular serine proteases expressed in bone marrow-derived cells of myeloid and lymphoid lineage (4). Biochemical analysis has demonstrated almost no cathepsin C activity in leukocytes of patients with PLS (5). Today more than 41 different cathepsin C mutations have been found in these patients (6), most of them homozygous for a given mutation (3), although compound heterozygous patients with PLS as well as a 'symptomless mutation' in the cathepsin C gene in three homozygous individuals have been described (7), (8). Heterozygous carriers of the mutations are clinically unaffected (9), although one heterozygous patient presented with plantar hyperkeratosis without periodontal disease (10). Mutations of the cathepsin C gene have also been confirmed in patients with Haim-Munk syndrome, a condition with signs similar to PLS (11), as well as in patients with prepubertal periodontitis (12).

The clinical expression of PLS varies considerably between patients and the signs seem to some extent to decrease with increasing age (13, 14). Although the genetic background of the disease has been characterized, a correlation between the mutations of the cathepsin C gene and the dermatological and the periodontal affections has not yet been studied. The aim of this study was to identify types of mutations in a cohort of subjects with PLS and to explore if there was a linkage between the phenotypic expression of the erythematous hyperkeratosis, the periodontal inflammation and the genotype of the patients.

MATERIALS AND METHODS

Patients

The cohort included a total of 52 patients; 47 of the patients had been examined earlier and clustered on the basis of a semiquantitative scoring system (15) and 5 patients were new additions. Thirteen of the patients were edentulous and thereby excluded from the study group. The genetic study enrolled 39 patients of Saudi nationality, 17 males and 22 females, from 25 families. All patients had the clinical diagnosis of PLS confirmed through their oral and dermatological conditions, and they had all or part of their deciduous or permanent dentition present. Their age ranged from 2 to 23 years, with a median age of 8 years. The study was approved by the Ethical Committee and the Research Advisory Council at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia. All patients or their guardians signed an informed consent.

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DNA extraction

Peripheral blood (10 ml) was drawn from the patients following confirmation of the diagnosis. DNA was extracted using the PUREGENE DNA Isolation Kit (Cat. No. D-40K; Gentra Systems Inc., Minneapolis, MN, USA). DNA was then spectrophotometrically quantitated and stored at 220°C in aliquots until required.

Genotyping

Genotyping was performed for a full set of 10 microsatellite markers (D11S901, D11S4187, D11S4147, D11S1795, AFM207ya5, D11S1354, D11S4197, D11S4082, D11S1780, D11S931) closely linked to the cathepsin C gene located at HSA 11q14.1-q14.3. PCR was performed using a standard protocol with Hot Star Taq DNA polymerase (Cat. No. 203205; Qiagen, Valencia, CA, USA). Briefly, 30 ng of genomic DNA was used in a standard 25 µl PCR containing 1 unit of Hot Star Taq DNA polymerase. PCR products were precipitated to remove unincorporated primers and run on a MegaBace 1000 (Molecular Dynamics, Sunnyvale, CA, USA) capillary sequencer for fragment analysis. Sizing and allele calling were performed using the Genetic Profiler fragment analysis software package (Molecular Dynamics).

Mutation analysis

The cathepsin C gene was screened for mutations by direct sequencing of PCR amplicons covering the entire coding region. Briefly, the seven exons of cathepsin C were amplified using primers positioned to allow quality reads of the coding regions. DNA from affected individuals who were representative of each identified haplotypic group was used for mutation analysis. PCR products were cleaned using exonuclease and shrimp alkaline phosphatase treatment to provide template for direct sequencing. Sequencing was performed in both the forward and reverse direction using the same primers as for amplification. Sequencing reactions were performed using an Amersham ET Dye Terminator sequencing kit (Amersham Life Sciences, code # 81090, Arlington Heights, IL, USA) according to the manufacturer's instructions. Sequencing reactions were cleaned up using ethanol precipitation and resuspended in a formamide EDTA solution for injection on a Megabace 1000 capillary electrophoresis system. Sequence analysis was performed using the SeqMan module of the DNA Star software package (DNA Star Inc., Madison, WI, USA) by comparing alignments of the affected individual with that of a normal reference sequence. Sequence variations were further assessed by Blast analyses against public genome databases.

Clinical examination

The patients had their dermatological and oral signs recorded according to a previously described scoring system (15). Every individual had the dermatologic and oral examination performed on the same day and by the same two clinicians (C.U. and K.T.P.). The dermatologic examination included hands and feet and a semi-quantitative scoring of hyperkeratosis was performed with 0 meaning 'no clinical findings' and 3 'pronounced hyperkeratosis' (maximum score 24). The oral examination included registration of number of teeth, gingival bleeding on probing and periodontal pocket depths. Pockets were measured using a periodontal probe (WHO CPITN-E, Henry Schein, Melville, NY, USA) and registrations were made mesially, distally, buccally and lingually for every tooth. The periodontal score was added up, giving the patient a total score between 0 and 4 (15). The number of permanent teeth extracted due to periodontal disease was recorded, as well as the number of permanent teeth present.

Statistical methods

Differences in dermatological and periodontal scores were subjected to a chi-squared test using the SPSS (11.5) software (Chicago, IL, USA). A p value<0.05 was considered as statistically significant.

RESULTS

Haplotype analysis of Saudi patients with Papillon-Lefèvre syndrome

Microsatellite-based genotyping was performed to identify common haplotypes indicative of founder mutations present in the study population. Data from 22 of the patients for the most informative markers (D11S1354, D11S4082 and D11S1780) are presented in Table I. Haplotypes coalesced around the marker D11S1780 at which three genotypes (173/173), (183/183) and (189/189) were present. Haplotype analysis and the genotypes observed at D11S1780 predicted the presence of three underlying mutations in the cathepsin C gene. In total the most common genotypes 183/183 and 173/173 were present in 30 (75%) and 6 (15 %) of the patients, respectively. The 189/189 and 187/189 genotypes were observed in three patients (8%) and one patient (2%), respectively, and may represent private familial mutations.

Identification of cathepsin C mutations

Sequencing exons of cathepsin C from individuals representing the three genotypes present at D11S1780 resulted in the identification of two underlying mutations. The most common genotype (183/183) was associated with an 815G→C mutation in exon 6 resulting in an arginine to proline change at amino acid 272 (R272P). Sequencing of the cathepsin C gene in patients with 173/173 genotype (D11S1780) revealed an exon 7 G300D mutation resulting in a glycine to aspartic acid change at amino acid 300. The mutation underlying PLS in the single family with 189/189 genotype (D11S1780) remains unknown despite analysis of the entire 'coding region'. The very different haplotype

Table I. Patients (n=22) have been clustered on the basis of genotypes for the markers D11S1354, D11S4082 and D11S1780

Patient	D11S1354	D11S4082	D11S1780
2, 11, 16, 17	176/176	124/124	173/173
14	169/169	124/124	173/173
10	176/176	124/124	183/183
1, 3, 4, 7, 8, 13, 20, 21, 22, 23	169/169	124/124	183/183
5, 6, 9, 19	169/169	122/122	183/183
12	169/169	122/124	183/183
15	169/169	124/124	189/189

observed in this Saudi family of Somalian descent (Table I) is likely to predict a novel mutation.

Relation between genotype and phenotype

There was a significant difference ($p<0.05$) between the mutations G300D and R272P in relation to the severity of hyperkeratosis of the feet (Table II). No significant differences were found between the two groups regarding hyperkeratosis of the hands or the periodontal condition. The average numbers of lost permanent teeth were 6.3 and 5.4, and the average number of present permanent teeth was 13.3 and 11.7 for the G300D and R272P groups, respectively.

There was a significant difference between males and females as regards the severity of hyperkeratosis of the hands and the feet ($p<0.05$). This difference involved the hyperkeratosis in the very young subjects with primary dentitions only. No difference between genders was found regarding the periodontal score in the deciduous or the permanent dentitions (Table III).

Table III. *Mean value (±SD) of dermatological and periodontal characteristics in relation to gender and age in patients with Papillon-Lefèvre syndrome*

Parameter	Deciduous dentition		Permanent dentition	
	Female (*n*=7)	Male (*n*=55)	Female (*n*=515)	Male (*n*=512)
Median age (years)	4	4	11	12
Genotype (n)				
173/183/189	0/5/2	0/4/1	3/12/0	3/9/0
Hand score	0.6*±0.9	5.3*±4.5	1.2±1.4	2.1±1.7
Foot score	1.9*±1.4	6.4*±3.7	3.0±2.6	3.5±2.0
Periodontal score	3.1±1.5	3.6±0.8	1.2±1.4	1.3±1.5

*Statistically significant difference, $p<0.05$ (χ^2 test).

DISCUSSION

The R272P mutation of the cathepsin C gene is the most frequently reported mutation in the PLS literature, including this study where 75% of the subjects had this mutation. The mutation was reported in one of the eight families studied by Toomes et al. (2) and was shown to result in loss of cathepsin C activity. Furthermore, the mutation has previously been described in a Saudi population and may provide the first direct evidence for a founder effect for cathepsin C mutations in PLS (16). Also the G300D mutation in cathepsin C has been reported previously (16). In the present study, no mutations could be assigned to the 189/189 and 187/189 genotypes (D11S11780). The new genotype (189/189) was observed in three children in a family of Somali descent which may predict a novel mutation. This will be subjected to further exploration. The 187/189 genotype was observed in one patient only and was not part of the comparative study.

Given the large number of patients with PLS, we opted for genotyping the samples prior to the sequencing of the cathepsin C gene, and the identification of underlying mutations (Table I). The basis for this was that identification of a limited number of haplotypes, i.e. establishment of a founder effect, would indicate the number of mutations likely to be found in the population being studied. It would also identify a subset of patients in which sequencing of the entire gene would be appropriate.

Our earlier study revealed no clear-cut correlation between the severity of dermatological changes and the level of periodontal inflammation within a group of subjects with PLS (15). Furthermore, the latter study showed that the phenotypic expression of the two cardinal features in PLS varied considerably between subjects. In the same study no correlation was found between age and the severity of the dermatological findings, although the periodontal inflammation was worse in young children with deciduous dentition. In the present study we investigated whether any of the variations in phenotypic expression were linked to a specific mutation. The result showed that patients with the R272P mutation had significantly more plantar hyperkeratosis than those with the G300D mutation, but there were no significant differences between the two groups regarding the level of palmar hyperkeratosis or periodontal infection (Table II). There was a significant difference regarding the severity of the dermatological changes between males and females, although this difference only

Table II. *Mean value (±SD) of dermatological and periodontal characteristics[1] in relation to genotypes around the marker D11S1780 in patients with Papillon-Lefèvre syndrome*

Genotype	*n*	Median age	Hand score	Foot score	Total dermatological score	Periodontal score
173/173	6	12	1.3±1.2	1.6*±1.1	2.9±1.8	1.1±1.5
183/183	30	8	2.0±2.8	3.8*±2.7	5.8±5.0	2.2±1.6
189/189	3	5	1.8±2.8	2.8±0.8	4.7±1.9	3.2±0.8

[1]Dermatological affections on hands and feet were measured in eight specific areas in accordance with a semi-quantitative score ranging from 0 (no clinical findings) to 3 (pronounced erythematous hyperkeratosis). The maximum dermatological score per patient was 24. The periodontal score included measurement of gingival inflammation and periodontal pocket formation in the permanent dentition. Maximum periodontal score per patient was 4 (15).
*$p<0.05$ (χ^2 test).

affected the very young patients with deciduous dentition. No such difference existed between subjects with permanent teeth. This diverse gender-related expression has never been reported before. The significant difference in hyperkeratosis within the confined group of siblings with the 189/189 genotype was an evident illustration of this finding. This genotype only affected three young siblings where the two girls had the classical signs of PLS, whereas the brother besides the palmoplantar hyperkeratosis had extensive xerosis of his skin on the extremities (Fig. 1a and b). No such gender variation could be detected in relation to their periodontal conditions.

PLS is characterized by periodontal disease affecting both deciduous and permanent dentitions (13). In accordance with earlier reports (13, 17) the expression of periodontal disease varies considerably in subjects with permanent dentitions. However, patients with deciduous dentitions showed a uniform expression with severe periodontal inflammation affecting every one of the very young patients. For the comparison of the number of present teeth, and teeth lost due to periodontal disease, only permanent teeth were accounted for. In subjects with the G300D and the R272P mutations periodontal conditions did not differ significantly either in respect of periodontal disease or in the number of teeth lost due to the disease. The subjects in this study are young and reports with late onset of periodontal inflammation in patients with PLS have been presented (18, 19). It was, however, our experience that in the permanent dentition, any sign of periodontal inflammation was diagnosed shortly after the eruption of the first permanent tooth.

Cathepsin C is important for the intracellular degradation of proteins and appears to coordinate the activation of many serine proteinases in inflammatory cells (20). These proteinases are implicated in a variety of immune and inflammatory processes, including cell-mediated cytotoxicity, phagocytic destruction of bacteria, local activation and deactivation of cytokines and other inflammatory mediators, and extracellular matrix degeneration (8). Subsequently, lack of cathepsin C might possibly explain the occurrence of the severe periodontal inflammation in patients with PLS. Cathepsin C is normally expressed in palmar, plantar and gingival epithelium (11), although its involvement in epidermal desquamation and its significance in the gingival tissue are unknown (21). It could be hypothesized that lack or deficiency of cathepsin C activity might interfere with normal shedding of epidermal cells in skin areas restricted to the phenotype of PLS and possibly also in the oral epithelium, as rapid turnover and desquamation of epithelial cells in the junctional epithelium is an important factor in the antimicrobial defence at the dento-gingival junction (22).

In conclusion, considerable phenotypic heterogeneity was observed in the patients with PLS in this study, although the heterogeneous expression of disease characteristics could not be associated with either of the two cardinal mutations. Similar phenotypic heterogeneity has been observed in other studies, albeit with much smaller patient numbers. Overall, the observations suggest that interaction of environmental factors and/or influence of other genes are important in shaping the PLS phenotype. Variation in the severity of signs such as the palmoplantar hyperkeratosis and periodontal inflammation also raises questions with respect to the role of regional cathepsin C expression patterns.

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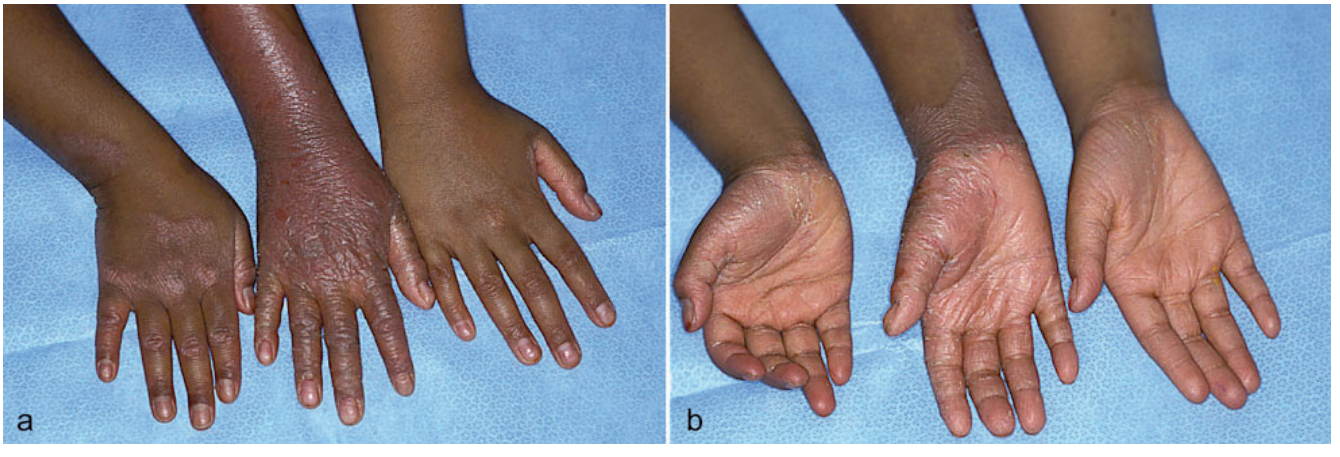

Fig. 1. (a, b) Palmar hyperkeratosis in three siblings with genotype 189/189. The hand of the boy is positioned in the middle, with the hands of the older girl to the left and the younger girl to the right.

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