Protein modeling of cathepsin C mutations found in Papillon–Lefèvre syndrome

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ABSTRACT

Background: Papillon–Lefèvre syndrome (PLS) is a rare autosomal recessive disorder characterized by hyperkeratosis involving the palms, soles, elbows, and knees followed by periodontitis, destruction of alveolar bone, and loss of primary and permanent teeth. Mutations of the lysosomal protease *cathepsin C* gene (*CTSC*) have been shown to be the genetic cause of PLS. This study analyzed *CTSC* mutations in five Iranian families with PLS and modeled the protein for mutations found in two of them.
Methods: DNA analysis was performed by direct automated sequencing of genomic DNA amplified from exonic regions and associated splice intron site junctions of *CTSC*. RFLP analyses were performed to investigate the presence of previously unidentified mutation(s) in control groups. Protein homology modeling of the deduced novel mutations (P35 delL and R272P) was performed using the online Swiss-Prot server for automated modeling and analyzed and tested with special bioinformatics tools to better understand the structural effects caused by mutations in cathepsin C protein (CTSC).
Results: Six Iranian patients with PLS experienced premature tooth loss and palm plantar hyperkeratosis. Sequence analysis of *CTSC* revealed a novel mutation (P35delL) in exon 1 of Patient 1, and four previously reported mutations; R210X in Patient 2, R272P in Patient 3, Q312R in two siblings of family 4 (Patients 4 and 5), and CS043636 in Patient 6. RFLP analyses revealed different restriction fragment patterns between 50 healthy controls and patients for the P35delL mutation. Modeling of the mutations found in CTSC, P35delL in Patient 1 and R272P in Patient 3 revealed structural effects, which caused the functional abnormalities of the mutated proteins.
Conclusions: The presence of this mutation in these patients provides evidence for founder *CTSC* mutations in PLS. This newly identified P35delL mutation leads to the loss of a leucine residue in the protein. The result of this study indicates that the phenotypes observed in these two patients are likely due to *CTSC* mutations. Also, structural analyses of the altered proteins identified changes in energy and stereochemistry that likely alter protein function.

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1. Introduction

Papillon–Lefèvre syndrome (PLS; OMIM245000) is a rare autosomal-recessive disorder characterized by early-onset periodontitis and palmoplantar hyperkeratosis with an estimated incidence of 1–4 per million (Gorlin et al., 1964), with males and females affected equally.

PLS was first described by two Frenchmen (Papillon and Lefèvre, 1924). The palmoplantar lesions are keratotic and sharply demarcated, and knees and elbows may be involved (Gorlin et al., 1964). PLS is also associated with premature loss of deciduous and permanent teeth and severe periodontitis. Gingival inflammation, pocket formation, and bleeding of the gums develop shortly after tooth eruption, but resolve after exfoliation (Haneke, 1979). Other clinical features not often reported include increased susceptibility to infections, especially furunculosis and pyoderma, pyogenic liver abscesses, and dura calcification (Cury et al., 2002).

In 1999, the PLS gene was localized to chromosome 11q14 (Fischer et al., 1997; Laass et al., 1997). At the end of 1999, two groups recognized the candidate gene cathepsin C (*CTSC*), whose mutations can result in PLS (Hart et al., 1999; Toomes et al., 1999). Hart's group then proposed that mutations in this gene could also cause prepubertal

periodontitis with no other PLS-type symptoms and Haim–Munk syndrome (HMS, MIM245010). The latter is characterized by hand deformities such as arachnodactyly, acroosteolysis, and onychogryphosis, in addition to typical PLS symptoms (P.S. Hart et al., 2000; T.C. Hart et al., 2000).

CTSC encodes cathepsin C (CTSC), a lysosomal protease that plays an important role in the activation of many serine proteinases of immune and inflammatory cells (de Haar et al., 2004; Toomes et al., 1999). Disturbances in CTSC function can result in abnormalities, especially where it is highly expressed, such as epithelial and immune cells (Meade et al., 2006). For example, natural killer cells and T lymphocytes require activated granzymes A and B to exert their cytolytic activities, and these granzymes are activated by the CTSC-mediated cleavage of their N-terminal peptides. Therefore, mutations in CTSC are expected to result in increased frequency of infections (Meade et al., 2006; Nakajima et al., 2008; Smyth et al., 1995). Similarly in the skin, mutations in CTSC might affect epithelial differentiation and lead to hyperkeratosis (Pilger et al., 2003).

It is likely that changes in coding sequences of CTSC may negatively affect protein function. According to the human genome mutation databank, 73 mutations in CTSC have been identified and 69 of these are associated with PLS. In the present study CTSC genes from five Iranian families with PLS from unrelated consanguineous nuclear pedigrees were analyzed; as a part of the in-silico study the deduced protein products of the novel mutations (P35delL) and a missense mutation (R272P) were modeled using online Swiss-Prot server for automated modeling and improved by recent bioinformatics software and techniques and also by the help of the solved structures of CTSC, 1K3Ba and 1K3Bb, to predict the tertiary structure and possible altered functions of the mutated proteins.

2. Materials & methods

2.1. Patients

The study was performed under the protocol approved by Mashhad University Health Science Center's Ethical Committee, under institutional approval and in adherence to the Declaration of Helsinki principles. The six patients with PLS were diagnosed at Mashhad University School of Dentistry, Periodontology Department, based on clinical examinations. All affected patients presented symptoms typical of PLS with no evidence of hand deformities or other symptoms reported in HMS. The patients were otherwise healthy.

Patients were from consanguineous nuclear pedigrees. The 50 healthy controls with no evidence of aggressive periodontitis and palmoplantar hyperkeratosis were also included in this study after providing informed consent.

2.2. PCR amplification and mutation analysis

Peripheral blood samples were collected from patients and controls by standard venipuncture into EDTA-containing tubes. Genomic DNA was isolated from leukocytes using conventional salting-out method. All exons of CTSC with adjacent sequences of exon and intron borders were amplified by PCR with the primers shown in Table 1 (Nakano et al., 2002; Toomes et al., 1999). Amplifications were carried out in 20 μL volumes containing 50 ng sample DNA, 0.2 mM dNTP (GenetBio, Korea), 500 nM of each primer, 2 μL of 10× buffer, 1.6 mmol/L MgCl$_2$, and 1 U Taq DNA Polymerase (GenetBio, Korea). After an initial denaturation step at 95 °C for 3 min, 35 cycles of amplification was performed consisting of 30 s at 95 °C, 30 s at the optimal annealing temperatures of 58 °C for exons 1, 2, and 6, and 56 °C for exons 3, 4, 5, and 7, and a 5 min terminal elongation step, in a TechGene thermal cycler (Germany). The amplified products were visualized by electrophoresis in an ethidium bromide-stained 2% agarose gel.

Table 1
Primers used to amplify the CTSC exon.

Region	Primer sequence (5′ → 3′)	Amplicon (bp)
Exon 1	F: TCTTCACCTCTTTTCTCAGC	337
	R: GGTCCCCGAATCCAGTCAAG	
Exon 2	F: GACTGTGCTCAAACTGGGTAG	338
	R: CTACTAATCAGAAGAGGTTTCAG	
Exon 3	F: GGGGCACATTTACTGTGAATG	285
	R: CGTATGTCTCATTTGTAGCAAC	
Exon 4	F: GTACCACTTTCCACTTAGGCA	313
	R: GGAGGATGGTATTCAGCATTC	
Exon 5	F: CCTAGCTAGTCTGGTAGCTG	305
	R: GTATCCCCGAAATCCATCACA	
Exon 6	F: CTCTGTGAGGCTTCAGATGTC	244
	R: CAACAGCCAGCTGCACACAG	
Exon 7	F: TAAGCAGAGATACAGAGAAG	574
	R: GTAGTGGAGGAAGTCATCATATAC	

2.3. Sequencing

The PCR products were directly sequenced in an ABI Prism 310 Automated Sequencer (Big Dye Terminator Cycle Sequencing Ready Reaction Kit, Applied Bio systems, and Foster City, CA, USA). To identify mutations, data were analyzed with the sequencing program Sequencher 4.10.1. DNA sequences were compared to published CTSC sequences [Ref sequences: NG_007952 (NCBI), C-001 ENST00000227266 (ensemble)].

2.4. RFLP

RFLP was used to identify previously unreported CTSC variants in 100 alleles of controls and compare them to patients' sequences. Ten microliters of PCR product was digested with 1 U of BseY1 (New England Biolabs, USA) in 0.2 mL 10× reaction buffer at 37 °C for 12 h. The BseY1 restriction fragments were visualized by electrophoresis in 15% polyacrylamide gel.

2.5. Structural analyses

To model the newly-identified P35delL and previously characterized R272P mutations, the mutated CTSC deduced amino acid sequences were compared to template databases using the online server Swiss-Prot Template Identification tool (Arnold et al., 2006; http://swissmodel.expasy.org).

The template most similar to the target mutated proteins, with 99% sequence identity to each, was human cathepsin C, which has been solved to 2.15 Å 1K3Ba and 1k3Bb (Zdobnov and Apweiler, 2001) that 1K3Ba includes the amino acids of the first chain of the protein and 1K3Bb includes the second chain respectively.

1K3Ba and 1K3Bb were submitted for the homology modeling using the online Swiss-Prot server for automated modeling (Arnold et al., 2006; Guex and Peitsch, 1997; Schwede et al., 2003; http://swissmodel.expasy.org). The result was set for the energy minimization job using ZMM software. The ZMM uses the Amber all-atom force field (Weiner et al., 1984) with a cut-off distance of 10 Å to minimize conformational energy in the space of generalized coordinates including torsion and bond angles. Low-energy conformation was reached by the Monte Carlo minimization method (Li and Scheraga, 1987).

The energy minimization was terminated after 100 sequential minimizations failed to improve the lowest-energy conformation.

The essential accuracy and correctness of the model were evaluated using the PROCHECK (laskowski et al., 1993) and WHAT-IF (Vriend, 1990) programs from the online server http://nihserver.mbi.ucla.edu/SAVES/.

The electrostatic potential of the molecule was computed using Coulomb's law and the Swiss-PdbViewer 4.02 (Guex and Peitsch, 1997), as well as the graphical representations presented here.