Studies on Clinical Expression, Genotype, and Gingival Crevicular Fluid Characteristics in Young Patients with Papillon-Lefèvre Syndrome.

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Abstract

Papillon-Lefèvre syndrome (PLS) is an autosomal recessive condition with palmoplantar hyperkeratosis and aggressive periodontitis as cardinal features. The disorder is linked to mutations of the gene for cathepsin C, a lysosomal protease essential in activation of serine proteases in immune and inflammatory cells. The genetic background of the disorder has been identified, but its relation to phenotypic expression is obscure.

The aims of the project were to explore phenotypic expression in young patients with PLS, and to investigate any correlation between clinical expression and identified genotype. Additionally, biochemical properties of gingival crevicular fluid (GCF) were investigated, and the result of an oral treatment protocol based on plaque control was evaluated. Major results and conclusions from the studies were:

- The severity of the skin lesions showed no correlation to patient’s age or level of periodontal disease, supporting the concept that the two major components of PLS are independent of each other.
- Genotyping revealed two cardinal genotypes, but no correlation between the identified genotypes and expression of phenotypes could be found, suggesting that it is the interaction with environmental factors and/or other genes that is important in shaping the phenotype.
- Analyses of gingival crevicular fluid (GCF) from patients with PLS did not show any clear-cut pathognomonic expressions with regard to content of cytokines, metalloproteinases or inhibitor of metalloproteinase-1.
- The level of plasminogen activator inhibitor in GCF was significantly higher in PLS patients than in controls, indicating atypical activity of the plasminogen activating system with, possibly, disturbed epithelial function. This may affect the epithelial barrier function and its role in the innate defence system.
- Evaluation of a PLS oral treatment protocol showed treatment from an early age and compliance to the program to be important in preserving permanent teeth in PLS.
Preface

This thesis is based on the following papers referred to in the text by their Roman numerals:


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# Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>A.a.</td>
<td><em>Actinobacillus actinomyctemcomitans</em></td>
</tr>
<tr>
<td>BOP</td>
<td>gingival bleeding on probing</td>
</tr>
<tr>
<td>CCE</td>
<td>cornified cell envelope</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme immunosorbent assay</td>
</tr>
<tr>
<td>GCF</td>
<td>gingival crevicular fluid</td>
</tr>
<tr>
<td>GBI</td>
<td>gingival bleeding</td>
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<tr>
<td>Ig</td>
<td>immunoglobulin</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>LJP</td>
<td>localized juvenile periodontitis</td>
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<tr>
<td>MMP</td>
<td>matrix metalloproteinases</td>
</tr>
<tr>
<td>PAI-1</td>
<td>placental plasminogen activator inhibitor - 1</td>
</tr>
<tr>
<td>PAI-2</td>
<td>placental plasminogen activator inhibitor-2</td>
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<tr>
<td>PA-system</td>
<td>plasminogen activator system</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PLS</td>
<td>Papillon-Lefèvre syndrome</td>
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<tr>
<td>PMN</td>
<td>polymorphonuclear neutrophil leukocyte</td>
</tr>
<tr>
<td>PPK</td>
<td>palmoplantar keratoses</td>
</tr>
<tr>
<td>TNF-α</td>
<td>tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>t-PA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>tissue inhibitor of matrix metalloproteinase-1</td>
</tr>
<tr>
<td>u-PA</td>
<td>urokinase plasminogen activator</td>
</tr>
<tr>
<td>µL</td>
<td>mikro liter</td>
</tr>
<tr>
<td>VPI</td>
<td>visible plaque index</td>
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</tbody>
</table>
Figure 1. Palmar hyperkeratosis. Note accentuation of hyperkeratosis in palmar creases.

Figure 2. Plantar hyperkeratosis in three siblings.

Figure 3. Severe periodontal inflammation in an eight year old boy with PLS.

Figure 4. Panoramic radiograph of a 15-year old girl with PLS.
Introduction

Papillon-Lefèvre syndrome (PLS) is an autosomal recessive disorder characterized by palmoplantar erythematous hyperkeratosis and early development of aggressive periodontal inflammation (Hart & Shapira 1994). The skin lesions (Figures 1 and 2) and a devastating periodontal inflammation (Figures 3 and 4) are cardinal signs of PLS and they emerge early in life, often during the first year. An increased susceptibility to infection has been reported in approximately 20% of patients with PLS (Bergman & Friedman-Birnbaum 1988, Hanek et al. 1975). Painful fissures and recurrent pyogenic infections of the skin seem to be the most common medical complications (Hanek et al. 1975), although a number of patients with abscesses or pseudotumors of the liver have been described (Czauderna et al. 1999, Almuneef et al. 2003). There have also been reports of PLS patients with other stigmata such as growth retardation, non-symptomatic intracranial calcifications, and mental retardation (Hanek 1979, Hart & Shapira 1994). The scaly erythematous lesions over knees, elbows and interphalangeal joints are sometimes misdiagnosed as psoriasis although the histological findings are different (Lucker et al. 1994).

The premature and extensive tooth loss has a severe impact on oral health resulting in functional as well as cosmetic handicaps. The oral signs and symptoms can, together with the dermatological lesions, instigate psychological and social problems in young children. Although knowledge about the disorder has increased during the last decades, the cause of the clinical expression in PLS is still not fully understood. A better understanding of the underlying genetic alterations and their correlation to the phenotypic expression would help in developing successful treatment strategies. The findings presented in this work were focused on expression of, and factors shaping, the clinical manifestation of PLS.

Genetics

PLS was first described by two French physicians, Papillon and Lefèvre, in 1924. The disorder was discussed in the dental literature early on (Woods & Wallace 1941), and autosomal recessive transmission was suggested (Dekker & Jansen 1956). Gorlin et al. (1964) confirmed this hypothesis after noting that parents were not affected, and similar
pathology was diagnosed in affected siblings. The observed rate of consanguinity in families with PLS was also noted to be far greater than that for the general population. Between 2 and 4 people per 1000 are heterozygous for the PLS gene and therefore, carriers of the disorder. This results in a population prevalence of 1 case per 1 - 4 million people (Gorlin et al. 1964). However, this rate is higher in isolated societies, and particularly those in which consanguineous marriages are common. It is calculated that 1/3 of all cases of PLS are the result of consanguinity (Gorlin et al. 1990). If both parents are carriers of the defective gene there is a 25% risk that their child will be born with the disorder.

By using homozygosity linkage mapping Fischer et al. (1997) and Laass et al. (1997) located the gene for PLS to chromosome 11q14. In 1999 two groups were able to identify the mutation linked to PLS as a lack-of-function mutation of the gene encoding cathepsin C (Hart et al. 1999, Toomes et al. 1999). Biochemical analysis has demonstrated almost no cathepsin C activity in leukocytes from patients with PLS (Zhang et al. 2002, de Haar et al. 2004). Cathepsin C is a lysosomal cysteine proteinase functioning as a central coordinator in degradation of proteins and as an activator of various serine proteases in immune and inflammatory cells (Rao et al. 1997). It activates the serine proteases granzymes A and B in cytotoxic lymphocytes and natural killer cells, which are required for the cytotoxic lymphocytes granule-mediated apoptosis of tumour and infected cells (Pham & Ley 1999). It also activates tryptase (Sheth et al. 2003) and chymase (Caughey 2002) in mast cells, and cathepsin G, elastase and proteinase 3 in neutrophils (Adkison et al. 2002). This activation is essential for the phagocytic destruction of bacteria (de Haar et al. 2004). Furthermore, activation of serine proteases is important for local activation and deactivation of cytokines and other inflammatory mediators, and for extracellular matrix degradation (Murphy et al. 1992, Turk et al. 2001, Hewitt et al. 2004). Cathepsin C functions by removing dipeptides from the amino terminus of the protein substrate, as well as having endopeptidase activity (Turk et al. 2001). The importance of cathepsin C in the defence of the organism has been shown on cell lines from cathepsin C-deficient mice, which fail to activate serine proteinases in immune and inflammatory cells (Wolters et al. 2001).

Today more than 41 different mutations of the cathepsin C gene have been identified (Selvaraju et al. 2003), all of them homozygous (Hart et al. 2000). However,
compound heterozygous mutations in patients with PLS as well as “symptomless mutations” in the cathepsin C gene in homozygous individuals have also been described (Allende et al. 2003, Hewitt et al. 2004, Noack et al. 2004). Heterozygous carriers of the mutation are clinically unaffected (Nakano et al. 2001) although one heterozygous patient presented with plantar hyperkeratosis without periodontal disease (Cury et al. 2002). Mutations of the cathepsin C gene have been confirmed in patients with Haim-Munk syndrome (Hart et al. 2000), a condition with phenotypic expression similar to PLS, plus in addition arachnodactyly, atrophic changes of the nails, and deformity of the phalanges of the hand (Haim & Munk 1965). Mutations have also been reported in patients with prepubertal periodontitis, which is characterized by a periodontal condition similar to PLS periodontitis, but without the dermatological effects. It has been suggested that prepubertal aggressive periodontitis could be an allelic variant of PLS (Hart et al. 2000, Noack et al. 2004) or a genetically heterogeneous disease that, in some families, manifests as partially penetrant PLS (Hewitt et al. 2004). However, conditions with aggressive periodontal disease are not always the result of cathepsin C mutations and there is no evidence that patients will suffer from aggressive periodontitis simply because they have a low activity cathepsin C variant (Hewitt et al. 2004).

**Palmoplantar hyperkeratosis – in general**

PLS belongs to a heterogeneous group of skin disorders called palmoplantar keratodermas or keratoses (PPKs). The conditions are characterized by hyperkeratotic lesions primarily affecting the palms of the hands and soles of the feet. Historically PPK classifications have been based on pattern of inheritance, clinical expression, histology and co-occurrence with associated clinical features (Itin 1992, Paller 1999). Application of modern molecular biology techniques has led to an increased understanding of the genetic basis of these disorders, and classifications based upon molecular pathology have been achieved (Ratnavel & Griffiths 1997). More recently, the specific gene defects responsible for many of the hereditary PPKs have been identified (Kimyai-Asadi et al. 2002).

The dermatological signs in PLS usually start with redness and thickening of the palms of the hands and soles of the feet during the first year of life. Hyperkeratosis develops in pressure areas, such as palms, soles, knuckles, ankles, elbows and knees. The keratotic plaques are symmetric, diffuse, thick and scaly and even though the lesions may
occur focally they usually involve the entire surfaces of the palms and soles, sometimes extending to the dorsal surfaces of the hands and feet (Siragusa et al. 2000). Frequently the feet are more severely involved although the hands occasionally have accentuation of hyperkeratosis in the palmar creases. The gravity of the hyperkeratosis varies between affected individuals even between those in the same family. It has been alleged that factors such as seasonal variations, the concurrent level of periodontal inflammation, as well as the individual’s age may influence the severity of the skin lesions (Gorlin et al. 1964, Hattab et al. 1995). Electron microscopy reveals non-specific histological changes in the affected skin of PLS patients, with lipid-like vacuoles in the corneocytes and granulocytes, a reduction in tonofilaments and irregular keratohyaline granules (Lucker et al. 1994).

**Palmoplantar hyperkeratosis - cathepsin C**

The cathepsin C gene is normally expressed in those epithelial regions frequently affected by PLS such as the palms, soles, knees, and keratinised oral gingiva (Rao et al. 1997). Nuckols and Slavkin (1999) suggested that it may be essential for establishing or maintaining the structural organization of the epidermis of the extremities and the integrity of the tissues surrounding the teeth, and that it might also participate indirectly in the processing of proteins such as keratins. The exact mechanism by which cathepsin C gene mutations cause or are involved in PPK is unclear since its role in the epidermis has not yet been studied in detail. However, the high cystein content of loricrin, the major protein component of the cornified cell envelope (CCE), makes it a potential target for degradation by cathepsin C. The CCE is an extremely durable, protein-lipid polymer, formed interior to the cytoplasmic membrane of differentiating keratinocytes. Eventually the CCE resides on the exterior of cornified cells, providing the mechanical and chemical barrier of the stratum corneum (Nemez & Steinert 1999). This suggests that cathepsin C might possibly enhance the desquamation of the terminal corneocytes (Kimyai-Asadi et al. 2002).
Palmoplantar hyperkeratosis – treatment of skin lesions

Mild to moderate palmoplantar hyperkeratosis can be treated with lubricants and topical agents like 20% urea cream, or 12% lactic acid, with or without 6% salicylic acid, in petroleum jelly. PLS patients with severe palmoplantar hyperkeratosis may, in addition to topical treatment, receive systemic medication with synthetic retinoids (acitretin 0.5 mg/kg/day, Neo-Tigason, Roche, Basel, Switzerland), which have been proven effective (Nazzaro et al. 1988). The retinoids down-regulate the expression of metalloproteinases, cytokines and skin-derived anti-leukoproteinase (Nagpal et al. 1996). However, case reports have indicated that long-term retinoid medication may increase the risk of adverse side effects such as hyperostoses, severe skeletal changes, teratogenicity, and liver toxicity (Sillevis Smitt & de Mari 1984, DiGiovanna 1986, Halkier-Sorensen & Andresen 1989). Use of the drugs has, consequently, been restricted.

Periodontal disease - the periodontal pocket

The gingival tissue is lined with three types of mucous membranes, the oral gingival epithelium, which is keratinised, and the oral sulcular and junctional epithelia, both of which are non-keratinised (Figure 5). At the interface, where healthy gingiva meets the tooth surface, the structural continuity is secured by the junctional epithelium, which is attached to the tooth surface by the epithelium attachment apparatus (Schroeder & Listgarten 1997). The epithelium attachment apparatus consists of hemidesmosomes at the plasma membrane of the cells directly attached to the tooth and a basal lamina-like extracellular matrix, the internal basal lamina (Kobayashi et al. 1976).

The junctional epithelium is a stratified epithelium composed of two strata, the basal layer facing the connective tissue, and the suprabasal layer, extending to the tooth surface. It forms an epithelial barrier of active cells with antimicrobial functions,
protecting the connective tissue against microbial invasion. At the same time it allows access of gingival crevicular fluid (GCF), inflammatory cells and components of the immunological host defence to the gingival margin. Junctional epithelium cells exhibit a rapid turnover, which contributes to host-parasite equilibrium and rapid repair of damaged tissue (Genco 1996). However, a wide variety of bacterial species and their products, inflammatory cytokines and host-generated proteases, that are involved in the defence against microbes, seem to have the potential to initiate lateral and apical proliferation of the junctional epithelium into the connective tissue. Furthermore, they appear to instigate epithelial disintegration through degradation of the internal basal lamina and an increase in epithelial permeability (Pöllänen et al. 2003). These are the initial signs of destructive periodontal disease.

**Periodontal disease - in general**

While microbial and other environmental factors are believed to initiate and modulate periodontal disease progression, there is now strong evidence that genes also play a role in the predisposition to and progression of periodontal diseases (Hart 1996, Hodge & Michalowicz 2001). In some cases, the genetic disorder responsible for the clinical manifestation is known. Destructive periodontal disease is estimated to occur in between 0.5 and 3% of children (Albandar & Tinoco 2002). The prevalence of localized juvenile periodontitis (LJP) in a selected Saudi population was found to be slightly lower at 0.42%, with a female to male ratio of 1.88:1 (Nassar et al. 1994).

Diagnosis and classification of periodontal diseases are almost entirely based on clinical assessment, although attempts have been made to identify subclasses of periodontitis based on gene expression profiles (Papanaou et al. 2004). The classification of periodontal diseases in children and adolescents has undergone considerable modifications over the years. In 1989 the American Academy of Periodontology proposed ‘early-onset periodontitis’ as a collective designation for a group of dissimilar destructive periodontal diseases, including pre-pubertal and juvenile periodontitis, which affect patients under the age of 35 years. This group of diseases is characterised by advanced periodontal destruction at an early age (Baer 1971), along with a tendency to familial aggregation (Hassel & Harris 1995). Prepubertal periodontitis has its onset during or soon after the eruption of the deciduous teeth, while juvenile periodontitis starts
between puberty and 35 years of age. Both disorders exist in localized and generalized forms. The 1999 International Workshop for a Classification of Periodontal Diseases and Conditions devised a new classification in which the diagnosis ‘early-onset periodontitis’ was renamed ‘aggressive periodontitis’ (Armitage 1999). The diagnosis ‘pre-pubertal periodontitis’ was discarded and is now described as ‘localized or generalized aggressive periodontitis occurring before puberty’. The International Workshop agreed that certain systemic conditions, like PLS, could modify and cause periodontitis, and classified these as ‘periodontitis as a manifestation of systemic disease’. Other examples of disorders in this diagnostic group of genetic conditions are chronic familial neutropenia (Kostmann’s syndrome), leukocyte adhesion deficiency types I and II, and Chediak-Higashi syndrome types 4 and 8 (Armitage 2004).

**Periodontal disease - in PLS**

In young patients with PLS, the mucosal and gingival tissues appear normal prior to the eruption of the deciduous teeth. After eruption, the gingival tissues become inflamed, swollen and start to bleed. These changes are followed by rapid destruction of the periodontal tissues causing hypermobility of the teeth and periodontal abscesses (Haneke 1979, Lu et al. 1987). In the deciduous dentition, treatment options are restricted due to the very young patients inability to cooperate to meticulous oral hygiene measurements and to conservative periodontal treatment. In the majority of PLS patients most of the deciduous teeth are shed spontaneously by the age of 5, although some children require extractions in order to resolve their painful periodontal condition (Hart 1999). Following the loss of the deciduous teeth the inflammation subsides and the gingival tissues resume a normal appearance. After eruption of the permanent teeth, the inflammatory process often repeats itself leading to devastating periodontal destruction. In the permanent dentition the extent and the severity of the periodontal disease are more variable, but many patients with PLS will become edentulous by their early teens (Gorlin et al. 1964). On the other hand, a number of patients express a less severe periodontitis and these are able to preserve some or most of their teeth into their adult life (Preus 1988, Wiebe et al. 2001).

Considering the aggressive periodontitis (Hart & Shapira 1994) and reports of increased general susceptibility to infections in patients with PLS (Haneke 1979,
Almuneef et al. 2003), it is not unreasonable to assume that patients with this condition have underlying dysfunctions of the immune or inflammatory defence mechanisms. Polymorphonuclear neutrophil leuokocytes (PMN) play a crucial role in the cell-mediated immune response against bacterial plaque at the gingival margin (Page et al. 1997). The mechanism by which PMNs neutralize pathogenic microorganisms involves many different steps: adhesion to the capillary endothelium in the inflamed region, trans-endothelial migration, chemotaxis, phagocytosis, and bacterial killing by oxidative and non-oxidative mechanisms (Dennison & van Dyke 1997, Del Fabbro et al. 2000). A defect in just one of these steps leads to an altered neutrophil function and, hence, increased susceptibility to tissue infection. Depressed chemotaxis of PMNs (Liu et al. 2000, Firatli et al. 1996), significantly depressed phagocytic and lytic PMN activity (Ghaffer et al. 1999), defective leukocyte adhesion (Stalder et al. 1988), impaired production or increased release of superoxide radicals (Bullon et al. 1993, Bimstein et al. 1990), and impaired monocyte phagocytosis (Preus & Mörland 1987) have been reported in patients with PLS. Different investigative techniques could, in part, explain the inconsistent findings with regard to PMN function. Tinanoff et al. (1995) found normalized PMN function after successful conservative periodontal treatment of a PLS patient, and Preus (1988) discovered a normalized monocyte function once the periodontitis and Actinobacillus actinomycetemcomitans (A.a.) were eradicated. Crossner et al. (1990) report a defect phagocytic capacity of PMN cells in two young children associated with elevated IgG titers against A.a. These findings suggest that the dysfunction could be related to a toxic effect caused by extracellular toxins and lipopolysaccharide antigen from periopathogens and, consequently, secondary to the infection as suggested by Agarwal et al. (1996). Whether or not the dysfunction is caused by failed PMN activation due to lack of cathepsin C function has not been elucidated.

Lyberg (1982) reported normal lymphocyte transformation in two patients with PLS and recent investigations of lymphocyte population in peripheral blood did not reveal any significant alterations in the T and B lymphocyte distribution, although natural killer cells were significantly increased (Celentigil et al. 1992, Firatli et al. 1996). A few studies have explored the level of immunoglobulins in patients with PLS. These include reports of a normal concentration of salivary IgA (Lundgren et al. 1996) and increased serum IgG titers against A.a. (van Dyke et al. 1984, Wara-aswapati et al. 2001).
The relationship between periodontal disease in general and bacterial infection is well established and certain groups of predominantly gram-negative bacteria have been consistently found in periodontal lesions (Genco et al. 1988). However, to date no direct relationship has been proven to exist between any specific bacteria and type of periodontitis (Hart 1996, Kinane & Attström 2002). Various periodontal diseases affect children and *A.a.* is the pathogen most frequently associated, including patients with PLS (Van Dyke et al. 1984, Preus 1988, Kleinfelder et al. 1996, Velazco et al. 1999, De Vree et al. 2000). Antibody data provide strong evidence of previous or current infection with a periodontal pathogen and Wara-aswapati et al. (2001) found an increased serum IgG1, IgG2, and IgG3 titers against *A.a.* in a young patient with PLS. Other investigators have failed to link PLS periodontitis to *A.a.* (Lundgren et al. 1999, Robertsson et al. 2001). It has been suggested that other periopathogens (Clerehugh et al. 1996, Velazco et al. 1999) as well as viruses from the herpes group (Ting et al. 2000, Velazco et al. 1999) may be involved in the causation and/or progression of localized aggressive periodontitis and PLS periodontitis.

Periodontal disease – immune and inflammatory reactions

Both genetic and environmental factors are important in the development of aggressive periodontitis (Hart 1996). Even though specific bacteria are essential for the induction of periodontal infection, and for the progress and severity of the disease, the rate of breakdown of connective tissue and alveolar bone, are dependent on the host’s immune and inflammatory responses. Endotoxins and antigens from the cell walls of periodontal pathogens stimulate circulating mononuclear phagocytes and resident cells to produce cytokines that drive the inflammatory response and initiate destruction of periodontal tissue by formation, secretion and activation of matrix metalloproteinases (Meikle et al. 1986, Meikle et al. 1989).

Cytokines are messenger molecules transmitting signals to other cells. They possess multiple effects and not only enhance the inflammatory response but also increase matrix metalloproteinase (MMP) activity (Meikle et al. 1992). The interleukins are members of this cytokine group and involved primarily in communication between leukocytes and other cells implicated in the immune and inflammatory process, such as epithelial and endothelial cells, and fibroblasts.
Interleukin-1 is a potent proinflammatory cytokine produced in alpha- and beta-forms. It is an early mediator of the inflammatory and overall immune responses and can as such influence the host response in periodontal lesions. Interleukin-1beta (IL-1β) is the major inflammatory cytokine occurring in gingival tissues affected by periodontitis (Tokoro et al. 1996). It is produced primarily by monocytes, macrophages and polymorphonuclear phagocytes, as well as by epithelial cells, keratinocytes in the skin, gingival and dermal fibroblasts, B cells and osteocytes. By mediating the production of potent inflammatory molecules, such as prostaglandins and cytokines, IL-1β has a central role in tissue destruction (Delaleu & Bickel 2004). It also plays a crucial part in regulation of immune and inflammatory responses such as T and B-cell activation, and in collagenase release, and is one of the most potent proinflammatory cytokines stimulating bone resorption (Kornman et al. 1997). Mononuclear cells and macrophages producing IL-1β have been associated with the host’s susceptibility to early-onset periodontitis (Salvi et al. 1998). IL-1β increases degranulation of neutrophils by potentiating their response to other stimuli and by increasing the release of other proinflammatory substances, such as tumour necrosis factor-alpha (TNF-α) and interleukin-8 (Brandolini et al. 1997, Sfakianakis et al. 2001). It may also modulate periodontal ligament cell function allowing these cells to participate directly in the disease process by assuming responsiveness to lipopolysacharides at the expense of their normal structural properties and functions (Agarwal et al. 1998). IL-1β and TNF-α have been shown to be of particular importance in connective tissue resorption (Meikle et al. 1989), where TNF-α induces the secretion of collagenase by fibroblasts (Tokoro et al. 1996). An increased release of IL-1β and TNF-α has been demonstrated in mononuclear cells cultured from the peripheral blood of patients with chronic periodontal disease (McFarlane et al. 1990). TNF-α from monocytes and macrophages has synergistic effects with IL-1 cytokines, although it is somewhat less potent than IL-1 (Okada & Murakami 1998).

Interleukin-8 (IL-8) has a powerful chemotactic effect on leukocytes, especially neutrophils. PMNs respond to IL-8 by migration, release of granule enzymes, respiratory burst of the PMN cells, and other intra- and extra-cellular changes. Neutrophil enzymes, released upon activation, effectively degrade connective tissue constituents (Bickel 1993, Baggiolini et al. 1989).
Matrix metalloproteinases (MMPs) are proteolytic enzymes that contribute to the tissue breakdown in periodontal disease (Birkedal-Hansen 1993). They are generally secreted in latent proforms later activated by other MMPs or plasmin (Murphy & Reynolds 2002). MMPs are mainly produced by infiltrating mononuclear leukocytes (PMNs), other haematopoietic cells and resident gingival cells (Kornman et al. 1997). Each of the major cell types in gingival tissue is capable of expressing a unique complement of MMPs when stimulated (Birkedal-Hansen 1995). The MMPs are key enzymes in tissue degradative processes since protein components are the predominant determinants of tissue structure and function in most extra cellular matrices. The MMP family has three major subgroups: the interstitial collagenases (MMP-1 and -8), the gelatinases (MMP-2 and -9) and the stromelysins (MMP-3, -10 and -11). There is a high degree of similarity between the enzymes in each group. MMPs can synergistically digest all the macromolecules of connective tissue matrices (Reynolds & Meikle 1997). In health, a balance of MMP activity along with naturally occurring tissue inhibitors of metalloproteinases (TIMP) facilitates normal tissue turnover. In disease there is an imbalance between MMPs and TIMP leading to breakdown of the extracellular matrix (Seguier et al. 2001).

The plasminogen activator (PA) system plays many roles in the inflammatory process, in tissue remodelling and repair, and is considered to have a significant role in periodontal tissue destruction and healing (Buduneli et al. 2004). Through the action of the specific plasminogen activators t-PA and u-PA, the inactive precursor plasminogen is converted into the aggressive broad-spectrum serine proteinase, plasmin. These activators are balanced by specific inhibitors PAI-1 and PAI-2, thus maintaining the integrity of healthy tissues. Plasmin acts directly on connective tissue components, and also indirectly by activating the proforms of metalloproteinases (Birkedal-Hansen 1995). Plasminogen is present in blood and other body fluids and the activators and inhibitors are produced locally. In the gingival area t-PA and PAI-2 have been found to be the dominating components of the PA system (Kinnby et al. 1994). The concentration of t-PA and PAI-2 in gingival crevicular fluid (GCF) increases during inflammation (Kinnby et al. 1994) and their expression in inflamed gingival tissues is also enhanced (Lindberg et al. 2001). In the gingiva, by far the strongest expression of t-PA and PAI-2 has been found in epithelial tissues (Lindberg et al. 2001). Its strong expression
in the sulcular and junctional epithelia indicates an important role of the PA-system in these barrier tissues. Cathepsin C has been shown to activate serine proteases extracellularly thereby possibly affecting the PA system (Turk et al. 2001).

Progressive periodontal disease may be caused by either an increased production of cytokines and/or MMPs or a decrease in the production of the balancing TIMP. It is possible to study the enzymes released by stromal, epithelial and inflammatory cells by examining the GCF a mixture of serum transudate and inflammatory exudates which originate in the periodontal tissue. Collection of GCF and biochemical analyses of the fluid is a non-invasive way of assessing host responses in periodontal diseases. The advantages of using GCF instead of serum are ease of access, atraumatic sampling, rapid equilibrium with the whole intracrevicular pool and capacity for repeated sampling (Lamster 1997).

**Periodontal disease - treatment of PLS periodontitis**

Early on, treatment of PLS periodontitis was mainly restricted to extraction of severely affected teeth (Rosenthal 1951). Later traditional mechanical scaling and chemotherapy were tried but proved ineffective (Haneke 1979, Hathway 1982). In order to create an infection-free edentulous period prior to eruption of the permanent teeth, Baer & McDonald (1981) extracted all primary teeth at a young age followed by treatment with systemic tetracycline while the permanent teeth were erupting. This treatment approach has successfully been used by others as well (Tinnanoff et al. 1986, Preus & Gjermo 1987). Since *A.a.* seems to be an important periopathogen in PLS periodontitis it would seem valid to employ antimicrobials that target this microorganism in the treatment of the periodontal disease. The antimicrobial combination systemic amoxicillin and metronidazole taken concurrently has proven to be effective against *A.a.* (Pavicic et al. 1994) and is now commonly used in the treatment of PLS periodontitis (Kleinfelder et al. 1996, Rüdiger et al. 1999, Eickholz et al. 2001). However, Kleinfelder et al. (1996) report post-treatment improvement of clinical and radiological conditions in one patient treated with amoxicillin/metronidazole in spite of recurrent findings of *A.a.* which suggests that other microbes might be implicated. Furthermore, de Vree et al. (2000) describe another case where this combined antimicrobial treatment failed because the pathogen *A.a.* showed resistance to metronidazole. Other treatment modalities reported in PLS patients
are rinsing or subgingival irrigation with chlorhexidine solutions (Rudiger et al. 1999, Wiebe et al. 2001, Lundgren & Renvert 2004), and frequent professional prophylaxis including scaling and rootplaning (Kressin et al. 1995). Some authors have found that synthetic retinoids improve the periodontal condition (Kressin et al. 1995), while others find them ineffective (Lundgren et al. 1996).
Aims of the study

Numerous articles have described the clinical manifestations of patients with Papillon-Lefèvre syndrome, investigated the genetics and speculated about the mechanism behind the phenotypic expression. Most studies involve very few individuals and even though new information has been accumulated, the biology behind the clinical expression is still unclear. This work has been focused on the phenotype of young patients with PLS, seeking to quantify clinical expressions and identify genotypes, and to investigate the correlation between them. Biochemical properties of the gingival crevicular fluid were analysed with the intention of isolating factors that might be involved in the etiology of the devastating periodontal disease. Finally a systematic oral treatment protocol based on plaque control was evaluated.

The specific objectives of this study were:

- To rank the severity of the dermatological and oral affections and to evaluate whether the severity of the dermatological changes was related to age and/or periodontal condition.
- To identify types of mutation in subjects with PLS and to explore any linkage to phenotypic expressions.
- To investigate the presence of cytokines, matrix metalloproteinases, and tissue inhibitor of metalloproteinase-1 in gingival crevicular fluid, and how they reflect the periodontal tissue’s immunological and inflammatory response to gingival infection.
- To investigate the presence of tissue plasminogen activator, and placental plasminogen activator inhibitor-2 in gingival crevicular fluid.
- To evaluate the outcome of a systematic oral treatment protocol being used for young patients with PLS.
Overview of the methodology

The methodology used in paper I to V is briefly outlined here. Detailed descriptions can be found in the respective papers.

Clinical examination

The patients in this study were referred to the Department of Dentistry at King Faisal Specialist Hospital & Research Centre. The referrals came from other departments within the hospital and from various hospitals within the kingdom of Saudi Arabia. Forty-seven patients with a confirmed diagnosis of PLS were examined clinically, and the severity of their skin lesions and periodontal condition ranked according to a newly developed, semi-quantitative scoring system (Paper I). Both examinations were performed on the same day. A dermatologist graded the skin lesions on both the hands and the feet. The periodontal examination were performed by the author and included registration of visible dental plaque (VPI), gingival bleeding on probing (BOP) and, in the permanent dentition, depth of periodontal pockets. Due to variable cooperation in the very young children, tooth mobility and presence of granulation tissue were used as signs of advanced periodontal disease in the deciduous dentition in lieu of measuring periodontal pockets. The correlations between age and dermatological scores, between age and periodontal score, and between periodontal and dermatological scores were analysed by means of bivariate correlation. The Pearson correlation coefficient and the regression analysis of variance (ANOVA) were computed. Differences in scores between groups were tested with chi-square ($X^2$) test, and differences within individuals by means of Student’s paired 2-tailed $t$ test.

Genetic examination

Genotyping and mutation analyses were performed with polymerase chain reaction (PCR) after DNA extraction from peripheral blood samples (Paper II). Genotyping was performed on a full set of 10 microsatellite markers closely linked to the cathepsin C gene using a standard PCR protocol with Hot Star Taq DNA polymerase. The cathepsin C gene was screened for mutations by direct sequencing of PCR amplicons covering the entire coding region. The identified mutations were later related to the recorded
dermatological and periodontal scores, reported in Paper I. Differences in clinical scores were subjected to the $X^2$-test.

**Investigation of gingival crevicular fluid (GCF)**

Uncooperative patients, edentulous patients, and patients with severe periodontal disease involving all erupted teeth were excluded from study III and IV. GCF was collected by means of small discs, 3 mm in diameter and made of Millipore© filter (Paper III and IV). Areas of gingival inflammation in the upper incisor or cuspid areas were the primary test sites. The site was cleaned with a cotton pellet, isolated with cotton rolls and the discs sequentially and gently inserted into the gingival crevice until resistance was felt. They were left in the pocket until saturated, which was indicated by a clearly visible change in the colour of the disc. The discs were transferred to small plastic tubes containing 25 µL of a buffer solution and kept frozen at -70°C until analysed.

In Paper III, commercial enzyme-linked immunosorbent assay (ELISA) kits (Amersham Pharmacia Biotech) were used for analyses of the presence of cytokines (IL-1α, IL-1β, TNF-α, IL-8), matrix metalloproteinases (MMP-1, MMP-3, MMP-8, MMP-9) and tissue inhibitor of metalloproteinases (TIMP-1). Differences between PLS individuals and paired controls were analysed by means of Student’s paired two-sided t test or Wilcoxon’s paired sign test when indicated.

In paper IV, t-PA was analysed with an ELISA kit (Imulyse™ t-PA, Biopool) and PAI-2 by the ELISA technique using monoclonal and polyclonal antibodies (Lecander & Åstedt 1987). Values below the detection level were set to 0, and values above the upper limit of the standards were set to 10 mg/L. Due to these upper and lower limit adjustments, median values were used in the presentation of data. Wilcoxon’s signed rank sum test was used for paired comparison of the PLS patients and controls. For tests of gender differences or differences between PLS patients with and without signs of active periodontitis, Wilcoxon-Mann-Whitney rank sum test was applied. Speerman’s test of correlation was used for studying the influence of age.

**Evaluation of the dental treatment protocol**

Every patient with PLS was treated according to a systematic dental treatment protocol with oral hygiene instruction and prophylaxis every three months (Paper V). Each
appointment included registration of VPI, BOP, and depth of periodontal pockets. Radiological examinations with bitewing and panoramic radiographs were performed at baseline and then yearly thereafter. The patients were sub-grouped according to dental age when started on the treatment program. Group 1 contained patients followed since their eruption of the first permanent tooth and Group 2 included patients started on the treatment program after permanent teeth had started to erupt. VPI, BOP, permanent teeth lost and periodontal pocket depths were recorded at each visit from the start to the end of the study. The patients’ adherence to the treatment protocol was independently scored as ‘complier’ or ‘non-complier’ by two examiners. Data were presented descriptively and dichotomised data were used for odds-ratio calculations.
Results

Dermatological and oral examination (Paper I)

With no exception, skin changes were reported to develop during the child’s first year of life. Diffuse erythematous hyperkeratosis, with or without fissuring, was the most common lesion. It was found strictly in pressure areas and there was a strong correlation between the scores for the feet and the hands ($r=0.749; p<0.0001$), even though the scores for the feet were significantly higher than those for the hands ($p<0.0001$). A total of 23 of the 47 patients had well demarcated hyperkeratosis of the knees and 12 of them had similar lesions on the elbows. Ichthyosis was found in 2 patients, and extensive psoriasiform plaques over scalp, trunk, and extremities were observed in 3 individuals, of whom 2 were clinically suspected of having psoriasis or PLS with extensive skin involvement. These 2 patients had pronounced nail changes and another 11 had slight thickening of their nails, especially on the toes. Scalps were normal, except for two, who were found to have psoriasiform scaly plaque. No correlation was found between the severity of the dermatological lesions, the patients’ age or the severity of their periodontal condition.

At the initial examination 11 individuals had deciduous teeth, 28 had permanent teeth and 8 were edentulous. The periodontal score was significantly higher in the deciduous dentition ($p<0.05$) but there was no significant correlation between dermatological and oral scores ($r=0.013, p=0.929$) in this age group.

Genetic study (Paper II)

Haplotype analysis was performed on 39 patients and showed that the most common genotypes were 183/183 and 173/173. They represented 75% and 15% respectively of the total material of . Genotypes 189/189 and 187/189 were observed in 8% and 2% of the material, possibly representing mutations arising in single families. Sequencing exons of cathepsin C from individuals representing the three genotypes 183/183, 173/173 and 189/189 resulted in identification of 2 underlying mutations. The 183/183 genotype was associated with a mutation in exon 6 (R272P), and the 173/173 genotype with a mutation in exon 7 (G300D). The mutation underlying the 189/189 genotype remained unknown despite analysis of the entire coding region.
There was a significant difference (p<0.05) between the R272P and G300D mutations in relation to the severity of the hyperkeratosis of the feet (dermatological score 3.8 vs 1.6). However, no significant differences were found with regard to the hyperkeratosis of the hands or periodontal condition. The average number of permanent teeth lost by patients with the R272P and the G300D mutations was 5.4 and 6.3 respectively, and the average number of teeth present was 11.7 and 13.3 for the two mutations.

There was a significant gender difference in the severity of palmoplantar hyperkeratosis in children with primary dentitions, with the young males expressing more severe skin lesions (p<0.05). No such gender variation was found in periodontal scores in either deciduous or permanent dentitions.

**Analyses of gingival crevicular fluid (Papers III and IV)**

The analyses of cytokines, MMP, and TIMP showed significantly higher levels of IL-1β and MMP-8 (p<0.001 and p<0.05 respectively) and significantly lower levels of IL-8 and MMP-1 (p<0.05 and p<0.001 respectively) in PLS patients compared to controls. No disparities were found with regard to IL-1α, TNF-α, MMP-3, MMP-9 and TIMP-1. There were no gender differences identified for any of the variables but there was a tendency to higher levels of IL-1β and MMP-3 in the older age groups of both patients with PLS and controls. Individuals with PLS and presence of pathological periodontal pockets displayed lower levels of IL-1β than those who were periodontally healthy.

In Paper IV a significant difference (p<0.01) was shown between the median level of placental plasminogen activator inhibitor (PAI-2) in patients with PLS (1.38 mg/l) and the controls (0.33 mg/l). t-PA was slightly lower in the PLS patients than in the controls, although this was not statistically significant. No significant difference in levels of t-PA or PAI-2 were found vis-à-vis gender in either the PLS patients or the controls. A tendency towards lower levels of PAI-2 was found in both groups with increasing age. Patients with PLS, subgrouped according to presence or absence of periodontitis, showed no significant differences in levels of t-PA or PAI-2.
Evaluation of a preventive dental treatment protocol for PLS patients (Paper V)

As a natural consequence of the grouping system used, the median age in Group 1 was lower than that in Group 2. At baseline no patient in Group 1 had pathological periodontal pockets compared to 16 in Group 2. Oral hygiene and gingival health were better in Group 1 than in Group 2. Median levels for VPI, were 38% and 63%, and median levels of BOP were 17% and 41% respectively for the two groups. At the final examination the corresponding figures for VPI were 36 and 20%, and for BOP 10 and 19%, respectively.

Table 1. Median age, follow-up (FU) time and selected outcome measures in young patients with PLS and treated according to a preventive program

<table>
<thead>
<tr>
<th>group</th>
<th>median age, yr at FU (range)</th>
<th>FU time, y mean (range)</th>
<th>number of teeth present (range)</th>
<th>number of teeth lost during FU yes/no mean ±SD</th>
<th>number of periodontal pockets ≥5mm yes/no mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n=13)</td>
<td>9 (7-18)</td>
<td>5.1 (3-7)</td>
<td>11.8 (5-25)</td>
<td>2/11 0.6 ±1.7 (0-6)</td>
<td>1/12 0.3 ±1.1(0-4)</td>
</tr>
<tr>
<td>3 (n=22)</td>
<td>15 (9-23)</td>
<td>6.9 (3-7)</td>
<td>15.2 (0-28)</td>
<td>12/10 5.0 ±5.7 (0-17)</td>
<td>9/12* 3.4 ±5.4 (0-17)</td>
</tr>
</tbody>
</table>

n = 11 at the follow-up since one patient had all teeth extracted.

The patients were treated and followed for 3–7 years with a mean follow-up time of 5.3 years. During this period individuals in Group 1 lost a mean of 0.6 permanent teeth compared to 5.0 teeth for subjects in Group 2. The mean number of pathological periodontal pockets at the end of the study was 0.3 and 3.4 respectively for the two groups (Table 1).

Twenty-three patients were characterized as ‘compliers’ and 12 as ‘non-compliers’. Comparison at initial examination between the two groups showed higher values of both VPI and BOP for ‘non-compliers’, although the differences were not statistically significant. There were, however, significant differences (p<0.001) in mean values of VPI, BOP and number of pathological periodontal pockets at the end of the study (Table 2). Two of the 23 ‘compliers’ displayed pathological periodontal pockets as compared to 9 of the 12 ‘non-compliers’.
### Table 2
Mean values (±SD) of oral hygiene (Visible Plaque Index), gingival bleeding (GBI) and deep pockets in compliers and non-compliers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Compliers (n=23)</th>
<th>Non-compliers (n=12*)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible Plaque Index (VPI), %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial exam</td>
<td>40 ±30</td>
<td>60 ±22</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up</td>
<td>21 ±15</td>
<td>58 ±19</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>GINGIVAL BLEEDING INDEX (GBI), %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial exam</td>
<td>28 ±26</td>
<td>44 ±22</td>
<td>NS</td>
</tr>
<tr>
<td>Follow-up</td>
<td>10 ±8</td>
<td>43 ±21</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Pocket Depth, follow-up</td>
<td>0.1 ±0.5</td>
<td>6.5 ±6.1</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

* n=11 at the follow-up since one patient had all teeth extracted. NS = not significant
Discussion

Expression of genes can vary in different tissues, and mutations of a universally expressed gene can result in a tissue specific condition (Kinane & Hart, 2003). The mutations in PLS affect the cathespin C gene, and the expression of the mutated gene results in hyperkeratosis of skin and inflammation of periodontal tissues. A key question is whether more than one tissue is affected in PLS, that is, does the defect affect the epithelial tissue exclusively or, are other tissues involved for the full expression of PLS?

The methodology

The assessments of the skin lesions and periodontal inflammation were performed at the patient’s initial visit. The semi-quantitative scoring system was created in order to enable an easy and reproducible quantification of the gravity of the lesions. The scoring scales were chosen arbitrarily so the levels do not indicate an absolute value, but represent a relative ranking of affections. No repeated readings of the initial dermatological scoring were performed since it would have been unethical to delay implementation of any necessary local or systemic skin treatment.

In the course of both gingivitis and periodontitis, the GCF represents an inflammatory exudate containing a mixture of substances derived from serum, leukocytes, structural cells of the periodontium and oral bacteria (Uitto 2003). The selection of sites for GCF sampling is crucial in order to allow comparison between the GCF from the PLS patients and that from the healthy controls. Ranking of gingival inflammation was subjective and based on clinical criteria. Thus, on a histological level, the score given for the severity of the gingival affection might have differed within the groups and between individuals. The widespread age distribution of the patients and the various phases and degree of inflammation at the site of the GCF sampling, could also have been confounding factors. The quantity of exudate increases with the severity of the gingival inflammation (Ozkavaf et al. 2000) and, therefore, it could be assumed that the more severe the inflammation, the greater the dilution of the cytokines and MMPs. It has, however, been reported that the concentration of enzymes is positively associated with the volume of exudate (Golub et al. 1976). Villela et al. (1987) reported that GCF collagenase activity and GCF volume were correlated with the level of gingival inflammation, but not with one another. They suggest that crevicular fluid volume and
collagenolytic activity are two distinct and different parameters, although both are in some fashion related to inflammation. It would, therefore, have been interesting to measure the total production of cytokines and MMPs over time, even though the experimental method employed in this study did not allow estimation of the flow-rate of GCF.

Variation in phenotypic expression
The PLS phenotype is variable, and may occasionally present as either palmoplantar hyperkeratosis (Bullon et al. 1993, Brown et al. 1993) or prepubertal periodontitis alone (Soskolne et al. 1996). The result of this work confirmed the significant variation in expression of hyperkeratosis and periodontal disease in PLS patients, even between those from the same family. Variable clinical expression of single gene disorders is known to occur and may be due to interaction between the diseased gene and other modifying genes or the result of genetic imprinting (Soskolne et al. 1996). These genetic variances, together with variation in environmental exposure, are key determinants of the phenotypic difference between individuals (Kinane & Hart 2003). Two cardinal genotypes exhibiting a significant dissimilarity in plantar hyperkeratosis were found in this cohort of PLS patients, but no association between genotype and palmar hyperkeratosis, periodontal inflammation or number of permanent teeth lost could be found. Interestingly, a significant difference was noted in the severity of palmoplantar hyperkeratosis between young males and females, although there was none in the periodontal condition. The variations in palmar and plantar hyperkeratosis might be explained by individual differences in exposure of the skin to friction, although it is more likely that this is only one of several contributing factors. The evaluation of the oral treatment protocol showed that compliance to the preventive program had a strong impact on the presence of plaque, bleeding surfaces and pathological periodontal pockets. Patients with acceptable compliance had a significantly lesser expression of periodontal disease and lost fewer teeth than patients with poor compliance. These findings emphasize the importance of environmental factors in shaping the PLS phenotype.

Relation between hyperkeratosis and periodontal inflammation
This study showed no correlation between the severity of the skin lesions and level of periodontal inflammation, suggesting that the two cardinal features of PLS might be
expressed independently of each other. Kleinfelder et al. (1996) hypothesised that the periodontal component in PLS might be an add-on phenomenon and not a requisite symptom of the disorder. It is, however, likely that the periodontitis in PLS could be the result of the same epithelial defect as the skin lesions, a theory proposed by Lyberg (1982). He suggested that dyskeratosis of the gingival epithelium was a possible cause of the periodontal disease in PLS. Preus (1988) speculated that the hereditary defect causing periodontitis in PLS patients could be located at the epithelial surface barrier and that this would lead to reduced defence against virulent periopathogens when present. Mechanical tension and microbial strain, above a genetically determined threshold, could possibly interfere with the ability to prevent infiltration of periopathogens into the periodontal tissues (Kleinfelder et al. 1996). The presence and effect of cathepsin C in epithelial tissues have not been investigated, but there are indications that cathepsin C might be involved in the terminal differentiation of the epithelial cells (Kimyai-Asadi et al. 2002). Disturbed differentiation of the epithelial cells could lead to a decrease in epithelial cell turnover and cell termination, possibly resulting in hyperkeratosis. The junctional epithelial cells normally exhibit a rapid turnover, which contributes to the host-parasite equilibrium and speedy repair of damaged tissue (Genco 1996). Rapid shedding and effective removal of bacteria adhering to epithelial cells is an important part of the antimicrobial defence mechanism at the dentogingival junction. (Pöllänen et al. 2003). A decrease in the rate of turnover of junctional epithelial cells might, therefore, affect the epithelial barrier function.

**Function of oral epithelial tissues**

It is now recognized that the epithelium throughout the body does more than simply provide a physical barrier against pathogenic invasion (Marshall 2000). The epithelia of the skin and gingival tissue play an active role in innate host defence mechanism (Dale 2002). Epidermal keratinocytes probably contribute to inflammatory disease progression by secreting a number of pro-inflammatory cytokines and by expressing various adhesion molecules (Suchett-Kaye et al. 1998). Gingival epithelial cells respond actively to bacteria and are the major source of IL-1α and IL-1β in the periodontium (Sfakianakis et al. 2001). This response generates additional inflammatory mediators, such as IL-8, which in turn may enhance neutrophil recruitment to the site of infection (van Wetering et al. 1997). A
disturbed epithelial function could have an impact on the expression of these inflammatory mediators and on their function in innate host defence.

t-PA and PAI-2 are produced predominantly in the gingival epithelial tissues (Kinnby et al. 1999). The significantly higher level of plasminogen activator inhibitor (PAI-2) found in PLS patients could indicate a disturbance caused by an altered reaction pattern of the epithelium or simply an increased amount of epithelium. No difference in the PA system was seen between PLS patients with or those without signs of periodontal disease, further supporting the theory that the periodontal destruction in patients with PLS could be secondary to a defect of the epithelial barrier and may, therefore, not be an inevitable part of the syndrome. An altered activity of the PA system has been reported in other epidermal disorders like psoriasis, pemphigus, bullous pemphigoid, and lupus erythematosus (Baird et al. 1990, Jensen et al 1990, Gissler et al. 1993, Spiers et al. 1994, Lyons-Giordano et al. 1994, Bechtel et al. 1996). The results presented in this work indicate an altered epithelial activity of the PA system in this group of PLS patients as well. One can only speculate whether this is related to the lack of cathepsin C function. It is, however, a fact that cathepsin C has the ability to activate serine proteases extracellularly, which might possibly affect the PA system.

Periodontitis and age
The level of periodontal inflammation in the population studied was more severe in the younger patients, and in particular within the primary dentition. It has been reported in previous studies that the propensity of PLS patients to develop periodontal inflammation seems to decline with increasing age (Tinanoff et al. 1995, Ullbro et al. 1997). A possible explanation for this phenomenon has not been investigated in this study but a tendency towards higher IL-1β and MMP-3 levels, and lower levels of PAI-2 was noted with increasing age, both in patients with PLS and controls. The mechanism behind and the consequence of this age related effects are unknown. Maybe age-dependent presence of and susceptibility to A.a. (Asikainen et al. 1986, Mintz & Fives-Taylor 1994) might be the reason. Fransson et al. (1999) found differences in the inflammatory response in young and old subjects during the course of experimentally-induced gingivitis. Mombelli et al. (1989), studying the development of gingivitis during puberty, found a significant decrease in tendency to gingival bleeding after the age of 14 years, in spite of there being no
significant change in either the plaque or gingival indices. Puberty appears to have an affect on the composition of the periodontal flora (Darby & Curtis 2001) as well as there are changes in host response to bacterial stimulation. This is possibly related to physiological alterations in hormone levels during puberty (Morishita et al. 1988, Mascarenhas et al. 2003). A correlation between cytokine levels in GCF and estrogen status was demonstrated by Payne et al. (1993). In a clinical study Kinnby et al. (1996) found that the plasma level of progesterone in pregnant women was inversely related to the concentration of PAI-2 in GCF. Moreover, cathepsin C belongs to a group of cysteine proteases, with participation in precursor protein activation of proenzymes and prohormones (Berdowska 2004) and it could be hypothesized that the lack-of-function mutations of cathepsin C might affect disease development by influencing hormone levels.

Periodontitis and host response

The current understanding is that microorganisms within the dental plaque initiate periodontal diseases and that the development of periodontitis is governed by the subject's host response. The response itself is, to some extent, determined by previous experience, but predominantly influenced by the individual’s genetic make-up (Hart & Kinane 2003). Evidence for the role of cytokines and MMPs in the host response is well established (Murphy & Reynolds 2002). GCF cytokine and MMP profiles in patients with PLS have not been investigated in detail in the past, although the presence of IL-8, IL-1α and IL-1β positive cells were detected in inflamed gingival tissues from one PLS patient (Kabashima et al. 2002). It is tempting to hypothesize that the destructive periodontal disease in PLS is driven by an exaggerated inflammatory or immune reaction leading to an increased release of pro-inflammatory cytokines and/or tissue proteolytic enzymes. The present investigation showed significantly higher levels of IL-1β, and MMP-8, while levels of IL-8 and MMP-1 were significantly lower in PLS patients than in the controls. IL-1β is a multifunctional cytokine with a major role in acute and chronic inflammation and the IL-1β level in inflamed periodontal tissues has been reported to demonstrate a strong correlation with clinical parameters and degree of inflammation (Hou et al. 2003), as well as with collagen loss in inflamed gingival tissue (Ejeil et al. 2003). The GCF was sampled in areas with gingivitis and the key question is whether high levels of IL-1β in any
individual indicate future progression to periodontitis. When comparing PLS patients with and without signs of periodontitis, the level of IL-1β was surprisingly higher for the group without periodontal disease. However, statistical analyses disclosed a tendency to increasing levels of IL-1β with age, both in PLS and control patients. Most patients without periodontal disease belonged to the older age cohort, and the increased cytokine level seemed to be related to age more than periodontal condition.

IL-8 is an important chemo-attractant for PMN cells, affecting migration, chemotaxis and activation of these cells (Brandolini et al. 1997). In the present study the concentration of IL-8 was lower in the PLS patients than the controls, and no difference was found between PLS patients with healthy and diseased periodontium. The finding of a low IL-8 concentration is consistent with previous data from patients with established localized juvenile periodontitis and chronic periodontitis (Özmeric et al. 1998, Chung et al. 1997), but contrary to an earlier report of higher total amount of IL-8 in GCF from patients with PLS periodontitis (Liu et al. 2000). In those patients, Liu et al. (2000) noted normal PMN adherence and elastase activity, but depressed chemotactic response to IL-8. In a study of generalized aggressive periodontitis the strongest expression of IL-8, IL-1β and TNF-α was found in pocket epithelium and in adjacent connective tissue, with large numbers of infiltrating PMN cells. The enhanced accumulation of PMN cells was associated with up-regulation of IL-8, IL-1β and TNF-α expression (Liu et al. 2001). It would be reasonable to assume that low levels of IL-8, which in this study was found in both periodontally healthy and diseased PLS patients, would lead to poor recruitment and activation of PMN-cells, and subsequently increase the susceptibility to periodontal infection. However, further investigations of cytokine production and the involvement of epithelial cells are necessary before any definitive conclusion can be drawn from the present findings.

This study revealed significantly lower levels of MMP-1 and significantly higher levels of MMP-8 in the PLS patients. MMP-1 has been implicated as one of the MMPs involved in extracellular matrix degradation during periodontitis (Ejeil et al. 2003) and also as the major collagenase in GCF from individuals with juvenile periodontitis (Sorsa et al. 1995). Fibroblasts are the main producers of MMP-1 and in an in vitro study, fibroblasts from patients with LJP and slowly progressive periodontitis showed lower collagen-degrading activity than fibroblasts from healthy controls (Havemose-Poulsen et
al. 1998). Whether this represents a specific fibroblast phenotype or the result of decreased activity and reduced efficiency of the fibroblasts in healing and remodelling of the tissue is unclear. MMP-8 is an important mediator of tissue destruction in inflammatory disease and studies of anaerobic periodontal infections have reported that it is a major contributor to the collagenase activity in the matrix of diseased tissue (Romanelli et al. 1999). Active MMP-8 in gingival crevicular fluid is associated with periodontal tissue degradation in progressive stages, whereas the latent enzyme is predominant in gingivitis (Romanelli et al. 1999). The analysis of MMPs in this study reflected the total content of investigated proteases found in GCF as the method used did not allow the activated fraction of the MMPs to be identified. An important regulatory feature of MMPs is that they are generally secreted in latent proforms and require activation. Many MMPs share common activators that include other MMPs, leading to the concept of activation cascades where plasmin seems to be of particular importance in MMP-1 and MMP-8 activation (Murphy & Reynolds 2002). Considering the possible effect of increased levels of plasminogen activator inhibitor found in GCF, it would be of great interest to undertake an assessment of the activated fraction of the MMPs. Soell et al. (2002) examined the active and latent forms of MMP-1, -2, -3, and -9 in GCF and tissue supernatants from patients with severe periodontal disease and healthy controls. Although the quantity of all MMPs was significantly increased in GCF samples, in patients with periodontal disease the percentage of active forms of MMPs did not differ, between the diseased and healthy patients. Furthermore, the percentage of active MMP-3 and MMP-9 in GCF from both diseased and healthy patients was higher, while active forms of MMP-1 and MMP-2 were considerably reduced. In the same study the cathepsin C activity was found to be significantly lower in ‘diseased GCF samples’ than in ‘healthy GCF samples’ (Soell et al. 2002). The manner in which a decreased cathepsin C level in GCF influences the activation of MMPs or has an effect on the level of plasminogen activator inhibitor might provide an interesting focus for future investigations.

The importance of cytokines and tissue proteolytic substances in periodontal diseases are often discussed, particularly with regard to their role in pathological tissue destruction. However, degradation of connective tissue matrix is a normal event in physiological remodelling and associated with morphogenesis, growth and wound healing. Thus, since MMPs are essential for normal tissue remodelling and repair, it could be
hypothesised that failures in the regulation of degradative processes might adversely influence repair and wound healing. Plasminogen is involved in epithelial healing (Collen 2001) and the increased level of plasminogen activator inhibitor (PAI-2) found in the GCF of PLS patients points to an atypical function of the PA system with possible disturbances in the repair function of the epithelial cells.

It was not possible to demonstrate any clear-cut pathognomonic expression of cytokines or MMPs in this investigation, however, only a limited number of cytokines and MMPs were investigated. Further research could explore the role of other cytokines and MMPs involved in the immune and inflammatory processes. The ability of cytokines to induce MMP expression is well established (Havemose-Poulsen & Holmstrup 1997), but the feasibility of drawing conclusions from the results obtained may be limited since cytokines function as a network and individual elements of the network cannot be studied in isolation (Mosmann 1991). A similar limitation may apply to the investigation of the MMPs since it is the result of their combined presence that ought to be considered. When comparing the result of this work with that of others, it should be kept in mind that most other studies report findings in GCF samples collected in areas with established destructive periodontitis (Salvi et al. 1998, Özmeric et al. 1998, Liu et al. 2001) while in this study GCF was sampled in areas with gingivitis.

Treatment of PLS periodontitis

In spite of intense efforts, conservative periodontal treatment often fails to prevent the premature loss of most, if not all of the permanent teeth in individuals with PLS (Gorlin 1990, Hart & Shapira 1994). However, a number of reports of successful oral treatment in patients with PLS have been published (Rudiger et al. 1999, Eickholz et al. 2001). Given that individual host factors are of fundamental importance in the development of periodontal disease (Seymore 1991) and most of these reports contain very few patients, no definite conclusions can be drawn from them. In the study presented, the effect of a comprehensive preventive dental protocol was evaluated on 35 consecutive patients, the largest group of young patients with PLS to have been followed in a single institution. No control group could be included for ethical reasons, but the fact that a number of patients had problems keeping up with the program allowed the material to be sub-grouped according to compliance. All 35 patients had a history of severe periodontal disease.
affecting the deciduous dentition. In spite of this, by the end of the study period 11 of the 35 patients had not lost any permanent teeth or expressed any signs of pathological periodontal pocketing in the permanent dentition. Nine of these 11 patients had been treated according to the protocol since the eruption of their first permanent tooth while the other 2 had permanent teeth already erupted when the treatment was initiated. The patients who started on the protocol from an early age (Group 1) lost fewer teeth and showed less signs of periodontal disease than those who began their treatment later in life (Group 2). It could be speculated that the extraction of all deciduous teeth with the eradication of periodontal infection prior to eruption of the first permanent molar was critical for the preservation of permanent teeth. Such treatment allows the permanent teeth to erupt into an environment free of infection. The mean age of the patients in Group 1 was less than that in Group 2 and it is possible that these younger children may develop periodontal disease in the future, since late onset of periodontitis has been reported in isolated cases (Brown et al 1993, Bullon et al. 1993). Clinical experience has, however, been the opposite, that is that patients with PLS are less prone to develop periodontal disease after they reach the latter part of their teens (Ullbro et al. 1997). The result of this investigation provided further confirmation that amongst PLS patients, the majority of those who were going to develop periodontal disease in their permanent dentition did so before the age of nine and starting initially around one or more of the first permanent molars.

Several subjects developed early signs of periodontal inflammation during the follow-up period but this resolved provided the patients were able to attain and maintain good plaque control, and were given supportive systemic antibiotic treatment. Earlier studies report successful as well as unsuccessful use of systemic antibiotics (Tinanoff et al. 1986, Glenwright & Rock 1990, Bullon et al. 1993, Eronat et al. 1993, Van Dyke et al. 1984, Lu et al. 1987). Utilization of tetracyclines, such as low-dose doxycycline, minocycline and tetracycline, have been suggested as a possible treatment modality due to their potential to reduce the collagenase production, a mechanism independent of their antimicrobial properties (Ryan & Golub 2000). However, because systemic use of tetracycline is not recommended for children under the age of 12, this treatment protocol employed amoxicillin in combination with metronidazole, an antimicrobial combination with proven effectiveness against *A.a.* (Pavicic et al. 1994).
Compliance to the treatment program had a strong impact on presence of plaque, and number of bleeding surfaces and pathological periodontal pockets. Twenty-one of the 23 patients with acceptable compliance showed no clinical signs of on-going periodontal disease. This clearly indicates that in patients with single gene disorders, such as PLS, phenotypic expression can be successfully modified by altering environmental factors.
Conclusion

- The work presented here has shown a wide variation of phenotypic expression in patients with PLS.
- The two cardinal features, the palmoplantar hyperkeratosis and the periodontal inflammation, were expressed independently of each other. The palmoplantar hyperkeratosis demonstrated no correlation to patient’s age.
- The severity of periodontal inflammation was more pronounced in patients with deciduous dentition, but no correlation was found between dermatological and periodontal scores in this age group.
- The two mutations found in this cohort of PLS patients, exhibited a significant difference in plantar hyperkeratosis but no association between genotype and severity of palmar hyperkeratosis, periodontal inflammation or number of lost teeth.
- The analysis of the GCF in patients with PLS did not reveal any pathognomonic expression of cytokines, matrix metalloproteinases, or tissue inhibitor of metalloproteinase-1.
- The amount of GCF placental plasminogen activator inhibitor (PAI-2) was increased in individuals with PLS indicating an atypical function of the PA system, which might possibly be a sign of disturbed gingival epithelial function in these patients.
- The evaluation of the preventive oral treatment program showed that patients who started on the program at an early age lost fewer teeth and showed less signs of periodontal disease, than patients enlisted later on in life.
- The compliance with the treatment protocol was a significant determinant for a successful outcome and illustrates the importance of environmental factors in the shaping of the PLS phenotype.
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