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SHORT ROOT ANOMALY (SRA)
PREVALENCE AND PHENOTYPIC FEATURES IN FAMILIES

WITH EMPHASIS ON MATRIX METALLOPROTEINASES IN GINGIVAL CREVICULAR FLUID OF SRA AND ORTHODONTIC PATIENTS

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Academic Dissertation

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I Apajalahti S, Arte S, Pirinen S (1999). Short root anomaly in families and its association with other dental anomalies. *Eur J Oral Sci* 107:97–101.

II Apajalahti S, Hölttä P, Turtola L, Pirinen S (2002). Prevalence of short-root anomaly in healthy young adults. A*cta Odontol Scand* 60:56–59.

III Apajalahti S, Sorsa T, Ingman T (2003). Matrix metalloproteinase -2,-8, -9, and -13 in gingival crevicular fluid of short root anomaly patients. *Eur J Orthod* 25:365-369.

IV Apajalahti S, Sorsa T, Railavo S, Ingman T (2003). The *in vivo* levels of matrix metalloproteinase -1 and -8 in gingival crevicular fluid during initial orthodontic tooth movement. *J Dent Res* 82:1018-1022.

V Ingman T, Apajalahti S, Mäntylä P, Savolainen P, Sorsa T (2004). Matrix metalloproteinase -1 and -8 in gingival crevicular fluid in orthodontic tooth movement: a pilot daily follow-up study for one month after fixed appliance activation. *Eur J Orthod*. In press.

ABBREVIATIONS

α_1-PI	α_1-proteinase inhibitor
BMP	bone morphogenetic protein
Ca^{2+}	calcium ion
$CaCl_2$	calcium chloride
DAT	directly attached to the tooth
DD	dentin dysplasia
dH_2O	distilled water
DTPA	diethylenetriaminepentaacetic acid
ECM	extracellular matrix
EDA	anhidrotic ectodermal dysplasia
EGF	epidermal growth factor
FGF	fibroblast growth factor
FSHS	the Finnish Student Health Service
γ2-chain	gamma2-chain
GCF	gingival crevicular fluid
HCl	hydrogen chloride
HERS	Hertwig´s epithelial root sheath
IFMA	immunofluorometric assay
IgG	immunoglobulin
IL	interleukin
kD	kilodalton
$MgCl_2$	magnesium chloride
MMP	matrix metalloproteinase
MMP-1	collagenase-1/ fibroblast-type collagenase
MMP-2	72 kD gelatinase/ gelatinase A
MMP-8	collagenase-2/ neutrophil collagenase
MMP-9	92 kD gelatinase/ gelatinase B
MMP-13	collagenase-3
mRNA	messenger ribonucleic acid
MSX	homeobox transcription factor
MT1-MMP	membrane type matrix metalloproteinase
NaCl	sodium chloride
NaN_3	sodium azide
Nfic	gene encoding nuclear factor I proteins
PAGE	polyacrylamide gel electrophoresis
PAX	paired-box transcription factor

PDL	periodontal ligament
PG	prostaglandin
PMN	polymorphonuclear leukocyte
R/C	ratio between root length and crown length
SD	standard deviation
SDS	standard deviation score
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SRA	short root anomaly
TIMP	tissue inhibitor of matrix metalloproteinase
TGF	transforming growth factor
TNAP	tissue non-specific alkaline phosphatase
TNF	tumor necrosis factor
TBS	10 mM Tris-HCl, pH 7.5, 22 mM NaCl
TTBS	10 mM Tris-HCl, pH 7.5, 0.05% Triton X-100, 22 mM NaCl
Zn^{2+}	zinc ion
$ZnCl_2$	zinc chloride

ABSTRACT

Short root anomaly (SRA) is a rare developmental anomaly in the permanent dentition Its etiology is not well established, and no studies have reported the prevalence of SRA in the adult population with fully developed dentitions. In SRA dentitions, an increased tendency towards orthodontically induced root resorption is likely. Beneficial for successful treatment of SRA is therefore accurate pretreatment diagnosis.

Previous reports refer to familial occurrence; in this study it was hypothesized that the etiology of SRA is genetic. The specific aims of the first part of this thesis were to analyze the inheritance of SRA in a series of families, the phenotypic features of SRA, and its association with familial hypodontia and other dental anomalies. In addition, the purpose was to establish the prevalence of SRA in healthy Finnish young adults.

Previous studies indicate that mechanical stress induces the expression of mRNAs encoding for MMPs in human periodontal ligament (PDL) cells *in vitro*. In the second part of this thesis, the hypothesis was tested whether the levels, molecular forms, and degree of activation of MMPs in gingival crevicular fluid (GCF) reflect an early stage of orthodontic tooth movement in humans *in vivo*. Another hypothesis was that the pattern of MMPs in GCF of SRA teeth differs from that of control teeth, thus reflecting characteristic periodontal remodeling in physiological conditions. The specific aims were to address in more detail the role of MMPs in periodontal remodeling of short-rooted teeth in this rare anomaly and in teeth subjected to orthodontic force.

Although the family data supported the earlier suggestion of familial occurrence for SRA, the apparent genetic heterogeneity of the pedigrees did not permit definitive conclusions as to the type of inheritance. Based on these pedigrees with SRA, autosomal dominant or autosomal recessive modes of inheritance both were possible. An association was evident with hypodontia and ectopic canines.

The prevalence of SRA was at least 1.3%, affecting females significantly more often than males, and with a predisposition for the maxillary central incisors, premolars, and the mandibular second premolars to be affected. Therefore, in developing dentitions with bilateral occurrence of short-rooted maxillary central incisors, it is strongly advisable that root length be carefully analyzed in orthodontic treatment planning. This is particularly important in patients presenting with hypodontia.

A characteristic feature of SRA was an ongoing gelatinase (MMP-9) activity in the periodontium of short-rooted teeth, indicating the active periodontal remodeling in order to withstand stress loads under physiological conditions.

Significantly elevated levels of MMP-8 were found in the GCF of orthodontic patients after fixed appliance activation, implying that the cells within the periodontium when induced by the orthodontic force were up-regulated to produce MMP-8. According to molecular-weight, the majority of total MMP-8 immunoreactivity was presumed to correspond to a neutrophil-derived PMN-type enzyme. In addition, another form of MMP-8 found was presumed to represent the fibroblast-type enzyme produced by PDL resident cells such as fibroblasts. The results indicate that during initial orthodontic tooth movement both PMN-type and fibroblast-type MMP-8 contribute to periodontal remodeling, whereas the role of MMP-1 may be rather limited.

INTRODUCTION

Short root anomaly (SRA) is a rare developmental anomaly in the permanent dentition apparently unrelated to any systemic condition or syndrome. Its etiology is not known; however, familial occurrence has been suggested (Lind, 1972; Edwards and Roberts, 1990). The prevalences of SRA among populations range from 0.6% to 10% (Ando *et al*., 1967; Jakobsson and Lind, 1973; Bergström, 1977; Brook and Holt, 1978).

Root shortening as a result of apical root resorption is an unfavorable side-effect of orthodontic treatment. With SRA dentitions, this may present further problems. An increased tendency towards root resorption during orthodontic treatment and due to pressure from embedded canines has been reported in SRA dentitions (Lind, 1972; Newman, 1975). SRA has received little attention in the literature, without any relevant diagnostic criteria. The small number of reported cases may, at least in part, be due to lack of recognition of the condition, or to its misdiagnosis as resorption.

The specific aims of the present study were to analyze the phenotypic features of SRA and its inheritance in families, and its association with familial hypodontia and other dental anomalies (I). In addition, the purpose was to determine the prevalence of SRA in healthy Finnish young adults (II).

Tooth development is characterized by reciprocal interaction between the dental epithelium and mesenchyme, and many signal molecules and growth factors are involved in these interactions during crown morphogenesis (Thesleff and Mikkola, 2002a,b). Little is known, however, about the signaling pathways regulating root development (Thomas, 1995; Yamashiro *et al*., 2003). Root formation is initated by the downgrowth of an epithelial sheath, *i.e*., Hertwig´s epithelial root sheath (HERS), which directs root morphogenesis (Thomas, 1995). HERS cells appear to secrete enamel-related proteins, for instance, ameloblastin on the root surface prior to cementum formation (Fong *et al*., 1996; Fukae *et al*., 2001). One hypothesis is that these enamel matrix proteins may function as growth factors in cell-signaling events during root development (Fong *et al*., 1996; Hammarström *et al*., 1997).

Orthodontic tooth movement is based on the principle that prolonged pressure on teeth results in remodeling of periodontal structures, including the alveolar bone and periodontal ligament (PDL). Unlike the osteoclastic resorption of bone to provide the space for tooth movement, the corresponding remodeling process of the fibrous attachment is not clearly understood. Host-derived matrix metalloproteinases (MMPs) are the main proteinases involved in the remodeling of collagenous extracellular matrix. *In vitro* studies on human gingival and PDL

fibroblasts and animal studies have shown increased expression of mRNAs encoding for MMPs in response to mechanical force (Bolcato-Bellemin *et al*., 2000; Redlich *et al*., 2001; Takahashi *et al*., 2003).

In the present study, these enzymes were analyzed in gingival crevicular fluid (GCF) of orthodontic patients and SRA patients, in order to address in more detail the role of MMPs in periodontal remodeling in the periodontium of SRA teeth and in teeth undergoing orthodontic tooth movement (III-V).

REVIEW OF THE LITERATURE

1. TOOTH DEVELOPMENT

Tooth development starts with a local thickening of the dental lamina epithelium in order to make an ectodermal placode (Pispa and Thesleff, 2003). Subsequently, a condensation of the neural crest-derived mesenchymal cells (ectomesenchyme) forms under the placode. This is followed by budding of the placode into the mesenchyme (reviewed by Thesleff *et al*., 1995). The bud then undergoes folding morphogenesis, develops into a cap-like structure, and becomes known as the enamel organ. During the following bell stage, the hard tissue-forming cells of the tooth differentiate, and deposition of dentin and enamel begins (Pirinen and Thesleff, 1995). Enamel is formed by ameloblasts that differentiate from the inner enamel epithelium. The dental papilla mesenchymal cells surrounded by the enamel epithelium give rise to odontoblasts, which deposit dentin, and to the dental pulp. The condensed mesenchyme surrounding the enamel organ and dental papilla, the dental follicle, gives rise to the tooth-supporting tissues.

1.1. Signaling in tooth morphogenesis

Tooth development, including the number, size, and shape of the teeth, is under strict genetic control. Studies on transgenic mice that lack some functional genes, and molecular genetic studies of humans with dental aberrations all provide evidence for the roles of individual genes in tooth formation.

Tooth development is characterized by reciprocal interactions between the dental epithelium and mesenchyme. Several paracrine signaling molecules regulate these interactions throughout advancing morphogenesis and differentiation (reviewed by Thesleff and Mikkola, 2002a). Most of them belong to the fibroblast growth factor (FGF), bone morphogenetic protein (BMP), Hedgehog, Wingless, and tumor necrosis factor (TNF) families (Thesleff, 2003). These signaling molecules bind to specific cell surface receptors, which finally results in regulation of transcription in the nucleus by several transcription factors.

Recently, it has been shown that signaling by ectodysplasin, which belongs to the TNF family, and its receptor Edar are necessary for placode formation in all ectodermal organs including the teeth (Thesleff and Mikkola, 2002b). During tooth initiation, the placode functions as a transient epithelial signaling center (Pispa and Thesleff, 2003). The enamel knot, which forms at the bud-to-cap transition of tooth development, is another signaling center, in which more than ten signaling molecules are expressed regulating the morphogenesis and shape of the tooth crown

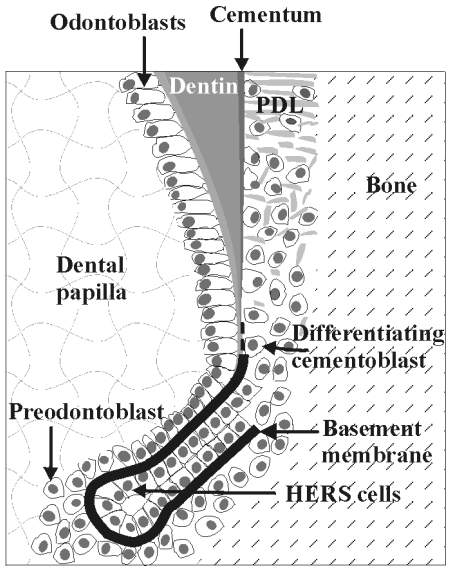


Figure 1. Schematic presentation of tooth root development. The double-layered Hertwig´s epithelial root sheath (HERS) directs root morphogenesis. Mesenchymal dental papilla cells differentiate into preodontoblasts along the inner surface of the HERS. As the HERS is disrupted, mesenchymal dental follicle cells come into contact with the dentin surface and differentiate into cementoblasts. (Illustration by courtesy of Pekka Nieminen).

(Jernvall *et al*., 1994; reviewed by Thesleff and Mikkola, 2002a). Early epithelial signals in the placodes include BMPs and FGFs, which induce the expression of transcription factors in the dental mesenchyme (Vainio *et al*., 1993). Some of these transcription factors are an absolute requirement for normal tooth development. *Msx1*- and *Pax9*-deficient mice lack all teeth (Satokata and Maas, 1994), and tooth development is arrested prior to enamel knot formation (Peters *et al*., 1998). In humans, mutations in these genes cause oligodontia (Vastardis *et al*., 1996; Nieminen *et al*., 2001, 2003; Lammi *et al*., 2003).

1.2. Root formation

Root formation begins when crown morphogenesis has been completed and continues even after the tooth has erupted into the oral cavity. The enamel organ epithelium forms a bilayer of cells called Hertwig´s epithelial root sheath (HERS), which directs root formation (Thomas and Kollar, 1989; Thomas, 1995) (Fig. 1). In its interior, the root sheath encloses the cells of the dental papilla, and exteriorly, it is surrounded by the cells of the dental follicle. The inner root sheath cells (preameloblasts) induce the adjacent mesenchymal dental papilla cells to differentiate into odontoblasts to form root dentin (Thomas and Kollar, 1989). Cementoblasts, which form cementum, are derived from the dental follicle (Thomas, 1995; Ten Cate, 1996).

The remnants of the fragmented HERS come to lie some distance from the root, in the PDL, throughout life and become known as the epithelial cell rests of

Malassez (Thomas, 1995). Cementoblast differentiation and cementogenesis are believed to be regulated by this fragmented epithelium (Thomas and Kollar, 1989; Thomas, 1995; reviewed by Hammarström *et al*., 1996). In humans, the root sheath is never seen as a continuous layer because it breaks down rapidly once odontoblast differentation begins, and its cells migrate away from the dentin surface, allowing the dental follicle cells to make contact with the root surface (Thomas, 1995). As root development proceeds in the apical third, cementum formation undergoes conversion from acellular cementum to cellular cementum (reviewed by Hammarström *et al*., 1996).

1.2.1. Molecular factors

Little is known about the molecular signals in root development, and no genes or signaling pathways are known to be expressed solely in the epithelial root sheath. Recently, expression patterns of several *Bmp*s, as well as *Msx1* and *Msx2*, have been analyzed during mouse root development (Yamashiro *et al*., 2003). In that study, no *Bmp* expression was detectable in the root sheath, but *Bmp3* was intensely expressed by the nearby cementoblasts (Yamashiro *et al*., 2003). Since *Bmp3* expression also exists in the root area by those dental follicle cells which give rise to cementoblasts (Åberg *et al*., 1997), it has been speculated that BMP3 may be involved in the formation of cementum (Åberg *et al*., 1997; Yamashiro *et al*., 2003). *Msx2* is continuously expressed in the root sheath and in the epithelial cell rests of Malassez (Yamashiro *et al*., 2003). Another study found that *Msx2* null mutant mice develop irregularly shaped molar roots (Oshima *et al*., 2002). These findings suggest that Msx2 is involved in root morphogenesis.

The ameloblastic cells of the inner layer of HERS apparently produce enamel-related proteins on the root surface prior to cementum formation (reviewed by Zeichner-David, 2001). One of these enamel matrix proteins, ameloblastin, is synthesized in rats by the cells of HERS during root formation (Fong *et al*., 1996). In addition, amelogenin, which is the major component of enamel matrix, is present in the area where cementogenesis is initiated in human teeth, but not in HERS *per se* (Fong and Hammarström, 2000). It has been hypothesized that these enamel matrix proteins are involved in cell signaling events during root development and induce cementoblast differentiation from dental follicle cells to form acellular cementum (Fong *et al*., 1996; Hammarström *et al*., 1997).

A mutation in the *Nfic* gene, which encodes the nuclear factor I family of transcription-replication proteins, affects mouse root development, loss of molar root formation´s being the most striking result (Steele-Perkins *et al*., 2003). Mice deficient in a membrane-type MMP, MT1-MMP (MMP-14), show arrested root

growth and failure to erupt (Beertsen *et al*., 2002). Moreover, mice lacking a functional tissue non-specific alkaline phosphatase (TNAP) gene show defective acellular cementum formation along the molar roots and delayed tooth eruption (Beertsen *et al*., 1999). In man, hypophosphatasia is an autosomally transmitted hereditary disease characterized by subnormal levels of TNAP in the blood, resulting in defective bone mineralization (Goseki *et al*., 1990). In such patients, the formation of acellular cementum is impaired, resulting in the cementum layers´ being thin or even being absent from the root surfaces, and in premature shedding of teeth (Bruckner *et al*., 1962).

1.2.2. Chronology of the development of permanent teeth

The development of permanent teeth begins prenatally. At birth, cusp mineralization has already started, beginning from the permanent first molars (Pirinen and Thesleff, 1995). The crowns of the permanent teeth (except third molars) are generally calcified by the age of six, followed by initation of root formation.

Marked differences occur in tooth development between the sexes, as well as between individual children. Variation in the timing of earlier-erupting permanent teeth is less variable than that of the later-erupting permanent teeth (Pirinen and Thesleff, 1995). Root formation and closure of the root apex has been analyzed in Finnish children by Haavikko (1970), according to whom, the median ages for root closure for the upper central incisor is 8.2 for girls and 8.7 for boys with a range (10^{th}-90^{th} percentile) of 1.7 years for girls and of 2.3 for boys. Correspondingly, for apex closure the median ages are 9.3 for girls and 9.8 for boys, ranges 0.9 and 2.6. For the upper second premolar, median age for root completion is 12.0 and for apex closure 14.0 years for boys, with ranges varying as much as from 3.3 to 4.0. Thus, for a normally developing boy, the latest age for root completion could even be 15.3 years and for apex closure 18.0 years.

Timing of permanent tooth eruption has been thoroughly examined in a longitudinal Finnish study of dental and craniofacial growth in a sample of 187 children (Nyström *et al*., 2001). The first permanent teeth to erupt into the oral cavity are the lower incisors and the first molars. The normal range for tooth emergence is usually considered to be two standard deviations (SD) above and below the mean age of emergence (Pirinen and Thesleff, 1995). Accordingly, within this normal range, the mean ages for the upper central incisor emergence are 6.7 ± 1.3 for girls and 7.0 ± 1.6 for boys. In the eruption of the second premolars the normal variation is largest, making the normal range (± 2 SD) of ages when these teeth emerge more than 6 years for boys, and more than 5 years in girls (Nyström *et al*.,

2001). Consequently, the mean ages for eruption of the upper and lower second premolars are 11.5 ± 2.7 and 11.4 ± 2.6 years for girls, and 12.0 ± 3 and 11.8 ± 3.1 for boys (Nyström *et al*., 2001).

The upper central incisors and first molars emerge with their roots half formed, whereas other teeth emerge when about three-quarters of their roots have formed, except for the canines, which erupt when the root length is almost complete (Haavikko, 1970).

1.2.3. Normal root-length variation

A special method exists for tooth-length measurements on radiographs, using relative root length defined as a ratio between root length and crown length (R/C) (Lind, 1972). In a sample of 1 038 Swedish schoolchildren aged 11 and over, the R/C ratio has shown a rather normal variation for the maxillary central incisors with the mean of 1.63 for the boys and 1.55 for the girls (Jakobsson and Lind, 1973). A similar figure, *i.e*., a mean R/C of 1.6 for the maxillary central incisors, has been reported in English schoolchildren aged 11 to 14 years (Brook and Holt, 1978).

2. STRUCTURE AND FUNCTION OF THE PERIODONTIUM

The periodontium is composed of gingiva, periodontal ligament, root cementum, and alveolar bone (Fig. 2). The gingiva comprises three different epithelia and their underlying connective tissue. The keratinized oral epithelium faces the oral cavity and extends from the free gingival margin to the mucogingival junction. The inner lining of the gingival sulcus wall is composed of the oral sulcular epithelium and junctional epithelium. Oral sulcular epithelium faces the tooth but is not directly in contact with tooth structure. It extends from the free gingival margin to the base of the histological sulcus.

The junctional epithelium extends from the cemento-enamel junction to the oral sulcular epithelium (Schroeder, 1996). It is a stratified fast-renewing epithelium composed of two strata, the basal layer facing the connective tissue and the suprabasal layer extending to the tooth surface. The free surface of the junctional epithelium forms the bottom of the gingival sulcus surrounding the tooth. Coronally, close to the sulcus, the junctional epithelium is about 15 cell layers thick, and narrows towards the apical part of the tissue. The junctional epithelium functions as a diffusion pathway for GCF and provides entry into the sulcus. In clinically healthy gingiva, the junctional epithelium is firmly attached to the enamel. This attachment of junctional epithelium to tooth is mediated through the epithelial attachment apparatus. This consists of hemidesmosomes at the plasma membrane of the cells directly attached to the tooth (DAT cells) plus a basal

lamina-like extracellular matrix (internal basal lamina) on the tooth surface (Kobayashi *et al*., 1976). A characteristic feature of the junctional epithelium is the relatively small number of desmosomes and gap junctions that connect the epithelial cells (Saito *et al*., 1981). Because of the low density of intercellular junctions, the intercellular spaces between junctional epithelial cells can vary considerably in size, and can be occupied by the inflammatory cells. Under non-inflammatory conditions, such cells occupy about 1 to 2% of the space, whereas with slight inflammation the percentage may rise to 30% and over (reviewed by Schroeder and Listgarten, 1997).

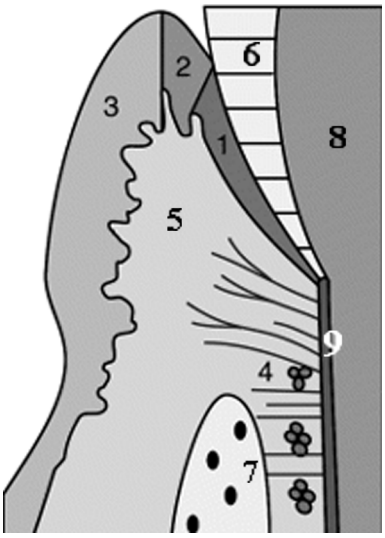


Figure 2. Schematic illustration of the structure of healthy periodontium. 1. Junctional epithelium, 2. Oral sulcular epithelium, 3. Oral epithelium, 4. Periodontal ligament, 5. Connective tissue of the gingiva, 6. Enamel, 7. Alveolar bone, 8. Dentin, 9. Root cementum. Modified from Pöllänen *et al*. (2003).

The gingival connective tissue comprises the collagen network, accounting for about 60% of the connective tissue volume. The predominant collagen is interstitial type I collagen, which forms 90% of the collagen fibers. Apart from the collagen fibers, the gingiva also contains a network of elastic, elaunin, and oxytalan fibers, which provides the gingiva with its elastic properties. These fibers differ in their relative microfibril and elastin contents, with the elastic fibers composed of the protein elastin (Chavrier *et al*., 1988). The gingiva also contains proteoglycans such as chondroitin sulphate, dermatan sulphate, and heparan sulphate. The proteoglycans consist of a central core protein to which polysaccharide chains called glycosaminoglycans are covalently attached. In addition, various glycoproteins are present such as fibronectin and laminin. Fibronectin is involved in the attachment of cells to the surrounding extracellular matrix and interacts with cell surface transmembrane receptors known as integrins.

The PDL is a highly specialized connective tissue, about 0.2 mm in width, connecting the alveolar bone to tooth roots. It mediates osseous remodeling during physiological and orthodontic tooth movement, participates in host defence, and provides nutrition for adjacent structures. In order to adapt to the positional changes of teeth, the collagen fibrils are continuously being remodeled. The collagen turnover rate in the PDL is higher than in any other tissue (Ten Cate and Deporter, 1974). The collagen fiber bundles in the ligament are mainly composed of interstitial collagen of types I and III. At each end, these fiber bundles are embedded in cementum or bone; the embedded portions of the fiber bundles are called Sharpey´s fibers. PDL also contains oxytalan fibers, representing an immature form of elastin (Ten Cate, 1997).

The principal cells of the PDL are fibroblasts, which are responsible for both synthesis and breakdown of collagen (Ten Cate and Deporter, 1974). In addition, the PDL cell population include osteoblasts, osteoclasts, cementoblasts, macrophages, undifferentiated mesenchymal cells, and epithelial rests of Malassez (Nojima *et al*., 1990; reviewed by MacNeil *et al*., 1998).

The cementum is a thin layer of mineralized extracellular matrix covering the surface of the roots. It is mainly composed of hydroxyapatite and type I collagen. Unlike bone, cementum is avascular, has no innervation, and does not normally undergo remodeling. Traditionally, cementum has been classified into cellular and acellular types, depending on the presence or absence of cementocytes, and these are further grouped into intrinsic and extrinsic fiber cementum based on the presence of collagen fibers originally formed by dental follicle-derived cementoblasts or by PDL fibroblasts, respectively (reviewed by Ten Cate, 1997).

Alveolar bone is a specialized part of the mandibular and maxillary bones that forms the primary support structure for the teeth. The alveolar processes consist of dense cortical bone that surrounds cancellous (trabecular) bone. Type I collagen comprises the major organic component in mineralized bone (Sodek and McKee, 2000).

Alveolar bone is a tooth-dependent bony structure: it fails to develop in the absence of teeth, as is the case in patients suffering from anhidrotic ectodermal dysplasia, a condition usually inherited as an X-linked disorder (Kere *et al*., 1996; Pirinen, 1998). Similarly, in patients with oligodontia or anodontia, alveolar bone is underdeveloped. Following tooth extraction, alveolar bone is gradually resorbed.

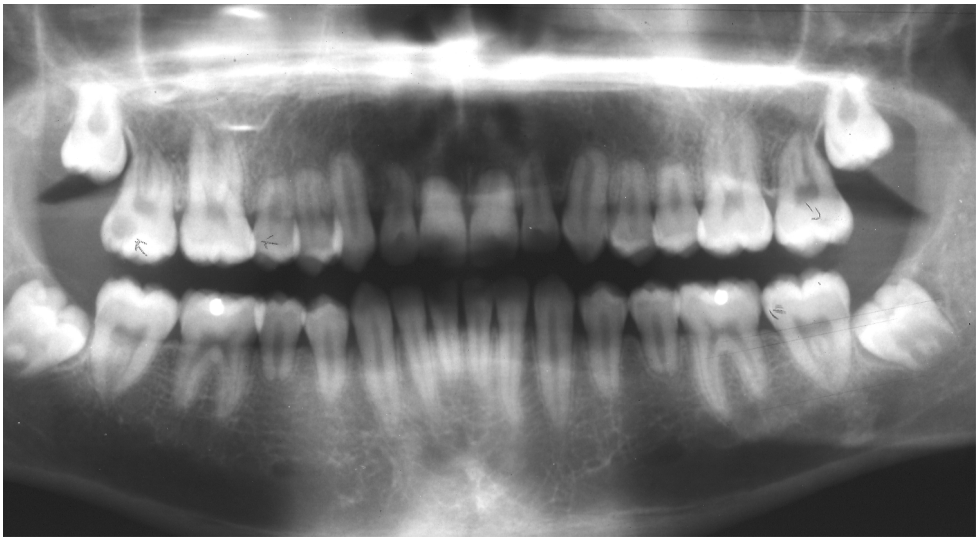


Figure 3. Characteristically short and blunt roots in a patient (16 years) with SRA. All the maxillary teeth except first molars are short rooted. In addition, mandibular second premolars have short roots.

3. SHORT ROOT ANOMALY (SRA)

3.1. Definition and characteristics

SRA was first documented by Lind (1972), who described this till-then-unclassified developmental dental anomaly with abnormally short roots always affecting both maxillary central incisors almost symmetrically.

In the present study, the term SRA describes a developmental anomaly in the permanent dentition affecting at least one tooth pair symmetrically. The condition is unrelated to any systemic condition or syndrome in which the short roots are due to resorption or to any developmental disturbance of exogenous origin. Since SRA gives no symptoms and the teeth look clinically normal, the diagnosis of SRA is based on radiographs. In everyday clinical work one pays attention to the anomaly only when the root length of several teeth is clearly reduced as seen in the panoramic radiograph. No relevant diagnostic criteria have been introduced for the anomaly.

SRA teeth have clearly visible short roots with a blunt radiographic appearance (Fig. 3). Root formation appears complete, with closed apices in the erupted teeth. Characteristic of SRA is its strong bilateral occurrence. In the SRA cases reported,

the maxillary central incisors seem always to be severely affected, usually with roots of about equal length or shorter than the crowns (Lind, 1972). Other teeth are less often involved, usually with premolars and canines affected (Ando *et al*., 1967; Lind, 1972). Newman (1975) found that "idiopathic" root shortness usually affects maxillary incisors, maxillary premolars, and mandibular second premolars, in that order, with maxillary teeth significantly more often affected than mandibular teeth.

Apart from root length, the teeth and their surrounding tissues are radiographically normal. In most cases, SRA does not present any symptoms, except that in severe cases the central incisors may have increased mobility. An increased tendency towards root resorption during orthodontic treatment and due to pressure from embedded canines has been reported in SRA dentitions (Lind, 1972; Newman, 1975). There have appeared occasional reports of an association with other dental anomalies such as developmentally missing teeth (hypodontia), peg-shaped lateral incisors, invaginations, generalized microdontia, and supernumerary teeth (Lerman and Gold, 1977; Brook and Holt, 1978; Edwards and Roberts, 1990), but no reports of SRA in the primary dentition.

3.2. Etiology

3.2.1. Genetic factors

The etiology of SRA is not known. Isolated cases of unknown origin have appeared (Leonard, 1972; Lerman and Gold, 1977), but pedigree data from both completely and incompletely ascertained families suggest a strong familial background (Lind, 1972; Newman, 1975; Edwards and Roberts, 1990). Furthermore, racial variation occurs; this racial variation, together with a strong familial occurrence, indicates that SRA has a genetic background, with an autosomal dominant pattern of inheritance suggested (Lind, 1972; Newman, 1975; Edwards and Roberts, 1990). Because of restricted family material, no definite conclusions have been established as to the mode of inheritance.

3.2.2. Environmental factors

In principle, many environmental factors such as trauma, periapical infection, or surgical procedures may cause arrest in root development or root shortening (resorption). Dental trauma is possibly the most important reason behind the shortening of the root of a single incisor. Among children aged 1 to 16 years, 35% experience injury to their primary or permanent dentition (Borssen and Holm, 1997). In the permanent dentition, 75% to 88% of the traumatized teeth are maxillary incisors (Borssen and Holm, 1997; Zaragoza *et al*., 1998). Permanent maxillary incisor root resorption related to the eruption of an ectopic canine is

reported in 47% cases of displaced canines (Ericson and Kurol, 2000). Radiographic examination of individuals who have undergone orthodontic treatment shows some loss of root length in 48% of cases (Remington *et al*., 1989). Radiographically clearly visible apical root resorption (> 2 mm to < 1/3 of root length) has been reported in 7% to 16% of patients treated with a fixed appliance (Hollender *et al*., 1980; Remington *et al*., 1989; Linge and Linge, 1991), whereas extreme root resorption leading to loss of more than one-third of the root length is seen in 1% to 0.4% (Remington *et al*., 1989; Janson *et al*., 2000). The etiology of root resorption during orthodontic treatment is multifactorial: type and duration of treatment, individual susceptibility, genetic predisposition, and systemic factors all may contribute. This resorptive potential varies between different patients and between different teeth in the same patient. Individual biologic factors, *e.g*., alveolar bone density, vascularity, and tooth structure may explain these variations (Melsen, 1999).

Maxillary incisors have been regarded as the most sensitive to root resorption (Newman, 1975; Remington *et al.*, 1989), in particular those with blunt or pipette-shaped roots (Levander and Malmgren, 1988). Mandibular incisors and mandibular first molars are also more likely to lose root length than are other teeth (Kennedy *et al*., 1983).

Some conditions of the dentition, dental anomalies, and morphological characteristics of the permanent dentition are mentioned as predisposing factors. These include hypodontia, invaginations, ectopic canines, atypical root resorption in connection with eruption of a permanent tooth, previous trauma, taurodontic molars, and SRA (Lind, 1972; Newman, 1975; Linge and Linge, 1991; Kjaer, 1995; Levander *et al*., 1998).

As for the type of treatment, intrusive force is considered most detrimental to the root, because of its stress concentration in the small area of the apical root (Beck and Harris, 1994). Time of treatment with elastics and time of treatment with rectangular archwires has been reported to contribute significantly to apical root resorption (Linge and Linge, 1991; Levander *et al*., 1998). Most studies have found no significant relationship with sex (reviewed by Brezniak and Wasserstein, 2002), although, controversally, studies have shown a greater prevalence of root resorption in girls (Newman, 1975; Kjaer, 1995).

Childhood anticancer therapy with chemotherapeutic agents and radiation therapy may cause premature apical closure and short V-shaped roots (Näsman *et al*., 1997). Irradiation causes more severe effects than chemotherapeutic agents do (Näsman *et al*., 1997).

Dioxins are ubiquitous environmental pollutants. In rats, lactational exposure to dioxin has been shown to cause prematurely closure of the root tips of the first and second molars (Lukinmaa *et al*., 2001). Interference of dioxin with root morphogenesis probably involves epithelial-mesenchymal signaling in the HERS, with a consequent arrest of root development (Lukinmaa *et al*., 2001). In humans, hypomineralized enamel defects of permanent first molars due to exposure to dioxins from mothers` milk have been reported (Alaluusua *et al*., 1999), but root lengths have not been studied.

3.3. Prevalence

Prevalence of SRA seems to vary with ethnic background. Populations studied differ in size and age distribution, and the diagnostic criteria for short-rooted teeth vary. In one study of Japanese schoolchildren, mean age 9 years, 10 months, the proportion with short-rooted central incisors was 10% (Ando *et al*., 1967). In Europe, prevalences of 2.4% and 2.7% for schoolchildren aged 11 and over have been reported, with a male:female ratio of 1:2.6 and 1:1.5 (Jakobsson and Lind, 1973; Brook and Holt, 1978). Bergström (1977) studied a sample of 2 589 school children aged 8 to 9, and found a prevalence of 0.6% for short-rooted maxillary central incisors. Girls were affected three times as often as were boys.

4. HUMAN CONDITIONS DISPLAYING ROOT-LENGTH VARIATION

In 47,XXY males with an extra X chromosome, root development is affected, leading to the development of taurodontism (Varrela and Alvesalo, 1988). X-chromosome deficiency also appears to influence root formation. In Turner syndrome (45,X), root length and crown height of incisors, canines, and premolars are significantly reduced, and a complex root morphology in premolars and molars is a common finding (Midtbo and Halse, 1994).

Defective root formation is a characteristic feature in type I dentin dysplasia (I DD), a rare autosomal dominant disorder of the dentin (Shields *et al*., 1973). An epithelial defect has been suggested to underlie type I DD (Witkop, 1988), however, the gene mutation behind type I DD is thus far unknown. The affected teeth are clinically normal, but because of their short or almost missing roots, the teeth move easily, and spontaneous exfoliation may occur. In addition to short tapering roots, characteristic dental features are periapical radiolucencies and pulpal obliteration with crescent-shaped radiolucent pulp remnants in the permanent teeth. Total pulpal obliteration is seen in the primary dentition (Steidler *et al*., 1984; Shankly *et al*., 1999). Histologically, the enamel and the outermost layer of the coronal dentin are normal, but the pulp chamber is obliterated by abnormal dentin.

In taurodontic molars, the bifurcation area is located more apically than in normal molars, resulting in a proportionately shortened root and enlargement of the pulp chamber. Different prevalence figures have been reported for taurodontism, due to differences in daignostic criteria. For taurodontic lower molars in one Dutch population, the prevalence was 10% (Schalk-van der Weide *et al*., 1993). Taurodontism has been associated with hypodontia (Seow and Lai, 1989; Arte *et al*., 2001) and oligodontia (Schalk-van der Weide *et al*., 1993).

Short roots appear along with rare underlying systemic conditions such as hypoparathyroidism (Jensen *et al*., 1981), Stevens-Johnson syndrome (de Man, 1979; Thornton and Worley, 1991), scleroderma (Foster and Fairburn, 1968), Down syndrome (Prahl-Andersen and Oerlemans, 1976), and Laurence-Moon-Bardet-Biedl syndrome (Borgström *et al*., 1996). Some short-stature syndromes, *e.g*., Aarskog syndrome (facial-digital-genital syndrome; Aarskog, 1970) and dwarfism of Seckel (Tsuchiya *et al*., 1981) have been associated with short roots. In addition, case reports describe patients with short stature or short roots or both, but with no recognized syndrome (Shaw, 1995; McNamara *et al*., 1998).

5. BIOLOGICAL MECHANISMS OF ORTHODONTIC TOOTH MOVEMENT

5.1. Tissue reactions in the periodontal ligament and bone

The tooth-supporting tissues are constantly remodeled under normal conditions, with physiological tooth migration. With orthodontic intervention, prolonged pressure on the teeth results in enhanced remodeling of periodontal structures, including supracrestal gingival and PDL fibers, as well as alveolar bone (Edwards, 1988).

Study of orthodontically induced reactions in the human PDL has been limited. Knowledge of histological responses in the supporting structures of teeth during orthodontic tooth movement is mainly based on animal studies. It has been suggested that the initial phase of orthodontic tooth movement involves many inflammatory-like reactions within the periodontal tissues, characterized by vascular changes and migration of leukocytes out of PDL capillaries (Rygh *et al*., 1986; Davidovitch et al., 1988; Vandevska-Radunovic *et al*., 1994; Rygh, 1995). The remodeling involves differentation of resident PDL cells into osteoblasts and fibroblasts (Nojima *et al*., 1990; reviewed by MacNeil *et al*., 1998). In addition, monocytes, originally derived from the blood-lymphatic system, are stimulated to develop into bone-resorbing cells, osteoclasts (Ohba *et al*., 2000; Rody *et al*., 2001). The mechanisms responsible for converting orthodontic force into cellular responses including cellular differentiation remain unexplained.

Osteoblasts control both the resorptive and formative phases of the bone remodeling cycle by regulating osteoclast recruitment and activity (Horowitz *et al*., 1989; reviewed by Sandy *et al*., 1993, Holliday *et al*., 2003a). In addition, osteoblasts produce collagenases, which degrade nonminerialized osteoid covering the resting bone surface, and thus enable the osteoclasts to gain access to the mineralized tissue (Everts *et al*., 1992). Prostaglandin (PG) functions as a mediator of the bone resorption induced by experimental tooth movement (Yamasaki *et al*., 1980; Saito *et al*., 1991).

On the pressure side, osteoclasts attack the alveolar bone from the PDL space in the process of direct resorption, allowing rapid tooth movement. On the tension side, new bone is deposited by the osteoblasts until the width of the PDL space has returned to its normal limits. Overcompression in limited areas of the PDL frequently totally occludes blood flow, causing cell death and the development of a cell-free necrotic area (Rygh, 1977). In light microscopy, the tissue reveals a glass-like appearance termed hyalinization. When hyalinization occurs, osteoclasts differentiate from the cells within the adjacent bone-marrow spaces to start undermining resorption from the underside of the lamina dura. This undermining resorption results in an inevitable delay in tooth movement and may increase root resorption (Rygh, 1974).

5.2. Orthodontically induced root resorption

Orthodontically induced root resorption is a part of the necrotic tissue elimination process preceded by hyalinization of the PDL. During the removal of the hyalinized zone by invading cells, the outer surface of the root with the cementoblast layer covering the cementoid is damaged, and resorption of the surface part of the root cementum may occur (Rygh, 1974). The first cells to be involved in this necrotic tissue removal are predominantly multi-nucleated macrophage-like cells activated from the adjacent bone marrow spaces by signals from the sterile necrotic tissue (Brudvik and Rygh, 1993). The root resorption process first starts in the periphery of the hyalinized zone, and only several days later on the root surface under the main hyaline zone. Once the resorption lacuna has been formed, root resorption will continue until the force is discontinued or falls below a certain level. Resorption lacunae developing on the root surface indirectly reduce the pressure exerted through force application, thus allowing the process to reverse, with a new deposition of cementum (Brudvik and Rygh, 1995). Recently it was, however, shown that some repair may occur in areas of previously resorbed cementum even without force decline (Bonafe-Oliveira *et al*., 2003).

5.3. Biochemical markers in orthodontic tooth movement

In order to monitor orthodontic tooth movement non-invasively in human beings, changes have been examined in the profile and levels of various cytokines, growth factors, and proteoglycans in GCF. Elevated levels of PG, interleukin (IL)-1β, IL-6, TNF-α, epidermal growth factor (EGF), and proteoglycans in the GCF have been well demonstrated as responsive to orthodontic force (Grieve *et al*., 1994; Uematsu *et al*., 1996; Waddington and Embery, 2001; Ren *et al*., 2002). Furthermore, in human GCF during orthodontic tooth movement, increased collagenase can appear (Sorsa *et al*., 1992a). Recently, significantly elevated levels have been found for cathepsin B, a lysosomal cystein protease, in GCF of teeth exposed to orthodontic force (Sugiyama *et al*., 2003).

6. MATRIX METALLOPROTEINASES (MMPs)

MMPs are a family of host-derived enzymes involved in degradation of extra-cellular matrix components during normal tissue remodeling in growth and development, wound healing, tooth morphogenesis, and tooth eruption (Sahlberg *et al*., 1999; Beertsen *et al*., 2002; Tsubota *et al*., 2002). MMPs not only degrade almost all extracellular matrix and basement membrane components but also growth factors, cell-surface receptors, and proinflammatory cytokines, thus affecting the regulation of cell behavior and signaling (reviewed by Uitto *et al*., 2003). In addition, MMPs are intimately involved in pathologic conditions such as rheumatoid arthritis (Hanemaaijer *et al*., 1997; Konttinen *et al*., 1998), tumor invasion and metastasis (Liotta *et al*., 1991; Kerkelä and Saarialho-Kere, 2003), chronic inflammatory airway disease (Prikk *et al*., 2001), periodontal disease (Sorsa *et al*., 1994, 1999; Ingman *et al*., 1996; Golub *et al*., 1997), and eye disease (Holopainen *et al*., 2003). Based on structure and substrate specificity, MMPs are divided into five subgroups: collagenases, gelatinases/type IV collagenases, stromelysins (including matrilysin and metalloelastase), membrane-type MMPs, and others. At least 25 different MMPs are known at present.

6.1. Collagenases

Collagenase-1 (MMP-1), collagenase-2 (MMP-8), and collagenase-3 (MMP-13) comprise the collagenase subfamily capable of initiating degradation of native fibrillar collagen types I, II, III, V, and IX (Weiss, 1989; Knäuper *et al*., 1996; Konttinen *et al*., 1998). The native triple helical collagens are cleaved at a specific site to yield N-terminal ¾ and C terminal ¼ fragments, which at body temperature denature spontaneously to gelatin (reviewed by Birkedal-Hansen *et al*., 1993).

MMP-1, -8, and -13 have three functionally important domains in their primary structure, namely, the propeptide domain that is lost upon activation, the catalytic domain containing the Zn^{2+} -binding site, and the C-terminal hemopexin domain. The latter appears to regulate substrate specificity, and is an absolute requirement for collagenases to cleave native collagen (Murphy *et al.*, 1992). In a secreted latent proform of the enzyme, the highly conserved cysteine residue in the propeptide domain binds covalently to the Zn^{2+} in the catalytic domain. Upon proteolytic activation, the covalent bond between the cysteine and the catalytic Zn^{2+} is disrupted, and the propeptide domain (about 10-20 kD in molecular weight) is cleaved off, resulting in a reduction in molecular mass (reviewed by Kähäri and Saarialho-Kere, 1999; Kerkelä and Saarialho-Kere, 2003) (Fig. 4).

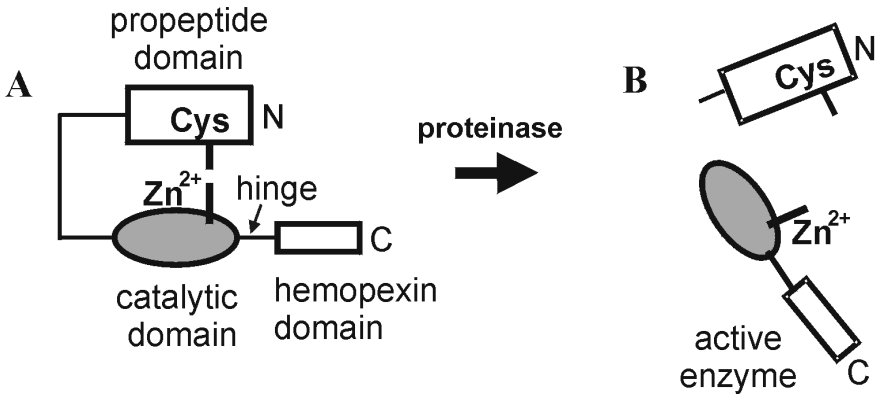


Figure 4. (A) Collagenase domain structure. The N-terminal propeptide domain (N) contains a free cysteine, which ligates to Zn^{2+} at the catalytic domain to maintain proMMP in an inactive form. The C-terminal hemopexin domain (C) is separated from the catalytic domain by a proline-rich hinge region. **(B)** Proteolytic activation of the enzyme involves the disruption of the Cys-Zn^{2+} interaction, exposing the catalytic site of the enzyme. The removal of the 10-20 kD propeptide proceeds often in a stepwise manner.

In normal conditions, the degradation and synthesis of ECM components is in balance, so that collagenases are expressed at very low levels, if at all, but their production and activation is rapidly induced whenever active tissue remodeling is required. MMP-1 and -13 are not stored in the cells, but the *de novo* synthesis and

subsequent release of the enzymes is regulated at the transcriptional level by hormones, by cell-cell and cell-matrix interactions, and by several growth factors such as transforming growth factor beta (TGF-β) and EGF as well as pro-inflammatory cytokines (IL-1α, -1β, TNF-α) (Salo *et al*., 1991; Birkedal-Hansen, 1993; reviewed by Nagase and Woessner, 1999; Tervahartiala *et al*., 2001; Domeij *et al*., 2002). All collagenases are secreted from cells as latent proenzymes and activated extracellularly *in vivo* mainly upon proteolytic cleavage by host-derived tissue and serine proteinases (other MMPs, PMN elastase, plasmin, capthepsin G, trypsin-2) or by bacterial proteinases (Sorsa *et al*., 1992b; Ding *et al*., 1995; Sorsa *et al*., 1997; Holopainen *et al*., 2003; Moilanen *et al*., 2003). In addition, non-proteolytic agents such as organomercurials and oxidants can activate MMPs *in vitro* (reviewed by Nagase and Woessner, 1999). MMP activity is further modulated through interactions with the specific tissue inhibitors of metalloproteinases (TIMPs) and non-specific serum proteins α-macroglobulins (reviewed by Birkedal-Hansen *et al*., 1993). Today, intense interest exists toward development of MMP inhibitors for therapeutic application in many human diseases (reviewed by Woessner, 1999).

6.1.1. Collagenase-1 (MMP-1)

MMP-1 most effectively cleaves collagen type III (Welgus *et al*., 1981). MMP-1 appears to be constitutively synthesized and secreted by fibroblasts and macrophages, and it is the collagenase most often associated with normal tissue remodeling. In addition, MMP-1 is currently shown to be produced by a variety of other cells such as osteoblasts and odontoclasts (Delaissé *et al*., 1993; Takiguchi *et al*., 1998). *In vitro* studies on human PDL and gingival fibroblasts have shown increased mRNA MMP-1 production under mechanical force (Carano and Siciliani, 1996; Bolcato-Bellemin *et al*., 2000). Elevated mRNA MMP-1 levels have been detected in gingival tissue of dogs during experimental tooth movement (Redlich *et al*., 2001). Whereas the effects of mechanically applied forces have been investigated in animals and tissue culture, no studies have focused on the expression of MMP-1 in the human PDL *in vivo*.

6.1.2. Collagenase-2 (MMP-8)

MMP-8 is the most effective collagenase in initiating type I collagen degradation (Hasty *et al*., 1987). Its main cellular source is polymorphonuclear leukocytes (PMNs), and the enzyme thus plays a key role in tissue destruction during inflammatory diseases. In PMNs, MMP-8 is stored in its latent form in specific granules, to be released upon degranulation from triggered PMNs by host-derived

or bacterial proteases (Sorsa *et al*., 1992b; Ding *et al*., 1995). PMN-type MMP-8 transcription is mostly completed before the cell emigrates from the bone marrow. MMP-8 activity is regulated by factors that affect the release of the enzyme by degranulation and not by its biosynthesis (Weiss, 1989). However, MMP-8 mRNA expression also occurs in circulating PMNs (Cole and Kuettner, 1995). PMN was once considered the only cellular source for MMP-8, but more recent studies have shown that multiple potential non-PMN lineage cellular sources for MMP-8 exist, such as chondrocytes (Chubinskaya *et al*., 1996; Cole *et al*., 1996), gingival and PDL fibroblasts (Kiili *et al*., 2002), gingival sulcular epithelial cells (Tervahartiala *et al*., 2000), odontoblasts and dental pulp cells (Palosaari *et al*., 2003), rheumatoid synovial fibroblasts and endothelial cells (Hanemaaijer *et al*., 1997), bronchial epithelial cells and monocytes/macrophages in bronchiectasis (Prikk *et al*., 2001), plasma cells (Wahlgren *et al*., 2001), and bone cells (Sasano *et al*., 2002; Tsubota *et al*., 2002). MMP-8 expression by at least some cell types other than PMNs is inducible (Hanemaaijer *et al*., 1997; Abe *et al*., 2001).

Highly glycosylated PMN-MMP-8 is secreted in a latent 75-80 kD form and converted to a 60 kD active enzyme upon selective PMN degranulation by host-derived or bacterial proteases (Sorsa *et al*., 1992b; Ding *et al*., 1995), whereas a 55 kD non-PMN-type MMP-8 proform is converted to a 45 kD active form upon activation (Cole *et al*., 1996; Moilanen *et al*., 2003).

6.1.3. Collagenase-3 (MMP-13)

MMP-13 expression was originally documented in human breast cancer (Freije *et al*., 1994), and MMP-13 prefers type II collagen and shows stronger gelatinolytic activity and broader substrate specificity than do other collagenases (Knäuper *et al*., 1996). In normal physiology, MMP-13 is highly expressed in developing bone and cartilage (Johansson *et al*., 1997a; Sasano *et al*., 2002). Moreover, MMP-13 is expressed during many pathological conditions associated with excessive degradation of the ECM, such as osteoarthritic cartilage and rheumatoid synovial membrane (Lindy *et al*., 1997), chronic cutaneous ulcers (Vaalamo *et al*., 1997), squamous cell carcinoma (Johansson *et al*., 1997b), oral mucosal epithelium during chronic inflammation (Uitto *et al*., 1998; Kiili *et al*., 2002), and odontogenic keratocysts (Wahlgren *et al*., 2003).

6.2. MMP-2 (72 kD gelatinase, gelatinase A) and MMP-9 (92 kD gelatinase, gelatinase B)

Gelatinases are distinguished by their fibronectin type II-like repeats, which allow binding to elastin and gelatin (Shipley *et al*., 1996). MMP-2 and MMP-9 degrade

type IV, V, VII, and X collagens, elastin, fibronectin, and proteoglycan (reviewed by Birkedal-Hansen *et al*., 1993). MMP-2 also shows collagenolytic activity against type I and II collagens and the laminin-5 γ2-chain (Aimes and Quigley, 1995; Pirilä *et al*., 2003). Both MMP-2 and MMP-9 are closely associated with tumor invasion, because they are able to degrade type IV collagen, a major structural component of the basement membrane (Robinson *et al*., 2003). Gelatinases are the primary matrix-degrading proteinases produced by the epithelial cells (Salo *et al*., 1994; Kähäri and Saarialho-Kere, 1997)

MMP-2 is constitutively expressed in a variety of normal and transformed cells. MMP-2 is predominantly produced by fibroblasts and other connective tissue resident cells, and odontoblasts and osteoblasts also produce MMP-2 (Rifas *et al*., 1989; Tjäderhane *et al*., 1998). Unlike other MMPs, MMP-2 synthesis in these cells is poorly inducible by cytokines and growth factors. Activation of pro-MMP-2 takes place primarily on the cell surface by the MMP-14/TIMP-2 complex (Sato *et al*., 1994).

Expression of MMP-9 is more restricted and is often low in normal tissues, but it can be induced during development, wound healing, bone resorption, and cancer invasion. MMP-9 is the best-established MMP in osteoclasts and is highly expressed by these cells (Okada *et al*., 1995; Rice *et al*., 1997; Sahlberg *et al*., 1999). In addition, MMP-9 is expressed by PMNs, odontoclasts, odontoblasts, keratinocytes, and macrophages, and by numerous malignant cells (Salo *et al*., 1991; reviewed by Birkedal-Hansen *et al*., 1993; Westerlund *et al*., 1996; Tjäderhane *et al*., 1998; Linsuwanont *et al*., 2002; Palosaari *et al*., 2003).

7. ROLE OF MMPs IN THE PERIODONTIUM

7.1. MMPs in periodontal disease

Numerous *in vivo* studies have demonstrated the relationship between MMPs and periodontal disease (Ingman *et al*., 1994a, b; Golub *et al*., 1997; Mancini *et al*., 1999; Romanelli *et al*., 1999; Sorsa *et al*., 1994, 1999; Tervahartiala *et al*., 2000; Kiili *et al*., 2002; Kinane *et al*., 2003; Mäntylä *et al*., 2003). The concerted action of several host-derived MMPs is involved in the pathophysiology of periodontitis. An imbalance between these enzymes and the TIMPs during periodontitis leads to irreversible connective tissue breakdown (Birkedal-Hansen, 1993; Ingman *et al*., 1996; Soell *et al*., 2002).

Both inflammatory cells (PMNs, macrophages) and resident PDL cells, especially epithelial cells, are involved in the tissue-destruction event in periodontitis. MMP-8 is mostly derived from PMNs, in which MMP-8 is stored in specific granules and released when triggered by the periodontopathogenic bacteria or their viru-

lence factors (Sorsa *et al*., 1992b, 1994, 1999; Ding *et al*., 1995). Active forms of PMN-derived MMPs (MMP-8, MMP-9) have been shown to be associated with the active phase of periodontitis (Westerlund *et al*., 1996; Tervahartiala *et al*., 2000). MMP-8 exists in elevated amounts in the GCF collected from inflamed periodontal pockets and is predominantly converted to the active 60 kDa form by the plaque host- and microbial-derived proteases (Sorsa *et al*., 1992b; Kiili *et al*., 2002; Mäntylä *et al*., 2003). After successful periodontal treatment, a significant reduction occurs in MMP-8 GCF levels (Sorsa *et al*., 1994; Ingman *et al*., 1996; Golub *et al*., 1997; Kinane *et al*., 2003), whereas in the GCF of healthy individuals, MMP-8 is virtually undetectable (Ingman *et al*., 1996; Mancini *et al*., 1999; Romanelli *et al*., 1999). In gingivitis, MMP-8 levels are slightly elevated, but the MMP-8 is mostly in its latent, inactive form (Sorsa *et al*., 1999; Mäntylä *et al*., 2003). Elevated MMP-8 and laminin-5 γ2-chain fragment levels in peri-implant sulcular fluid reflect the active phase of the inflammatory peri-implant disease (Kivelä-Rajamäki *et al*., 2003). Diagnostic test kits based on MMP-8 are under development for monitoring treatment of periodontitis (Sorsa *et al*., 1999; Mäntylä *et al*., 2003).

The 45-55 kD MMP-8 is produced in diseased periodontal tissues by certain non-PMN-lineage cells such as human gingival and PDL fibroblasts, plasma cells, odontoblasts, and endothelial cells (Hanemaaijer *et al*., 1997; Palosaari *et al*., 2003). In addition, human gingival sulcular epithelium affected by periodontitis expresses MMP-2, MMP-8, and MMP-13 *in vivo* (Tervahartiala *et al*., 2000). MMP-13 is also expressed in inflamed periodontal tissues by the basal cells of the gingival pocket epithelium (Uitto *et al*., 1998; Kiili *et al*., 2002). In these cells, MMP synthesis can be induced *de novo* by proinflammatory cytokines (TNF-α, IL-1β) and growth factors (TGF-β, EGF) (Ravanti *et al*., 1999; Abe *et al*., 2001; Domeij *et al*., 2002).

7.2. MMPs in SRA

MMPs have apparently never been studied in SRA. Mice deficient in MT1-MMP have roots showing underdevelopment and failure to erupt, indicating the important role of this enzyme in tooth root development; in addition, alveolar bone formation had ceased. The role of MT1-MMP in these developmental processes is thus likely to be accomplished through its participation in collagen remodeling, bone formation or both (Beertsen *et al*., 2002).

7.3. MMPs in orthodontic tooth movement

The PDL is constantly remodeled under physiologic conditions. A significant increase in collagen metabolism has appeared in the PDL during orthodontic tooth movement (Nakagawa *et al*., 1994; Karimbux and Nishimura, 1995; Bumann *et al*.,

1997; Redlich *et al*., 1998). Human gingival and periodontal fibroblasts have shown increased mRNA MMP-1 and MMP-2 production under continuous and cyclic stretch, whereas mechanical stress induced no mRNA MMP-9 or MT1-MMP expression in these cells (Carano and Siciliani, 1996; Bolcato-Bellemin *et al*., 2000). When the effect of orthodontic force on MMP-1 mRNA synthesis was analyzed in the gingival tissues of the dog during experimental tooth movement, orthodontic force significantly elevated MMP-1 mRNA production (Redlich *et al*., 2001). In addition, increased expression of MMP-8 and MMP-13 mRNA in the PDL of rats during active tooth movement has been demonstrated (Takahashi *et al*., 2003). Recently, orthodontic tooth movement in mice has been inhibited by the use of MMP inhibitors (Holliday *et al*., 2003b). Total collagenase activity in the human GCF of orthodontic patients treated with fixed appliances has been shown to be tenfold that of the control GCF (Sorsa *et al*., 1992a).

Bone resorption by osteoclasts involves demineralization of the bone inorganic matrix by acid and degradation of the bone organic matrix (primarily type I collagen) by cathepsin K and MMPs (Delaissé *et al*., 1993; Rice *et al*., 1997; Domon *et al*., 1999; Sahlberg *et al*., 1999; Ohba *et al*., 2000; Tsuji *et al*., 2001), although the precise role of MMPs in osteoclastic bone resorption is not yet understood. In addition, osteoclasts use different enzyme systems depending on the part of the skeleton where they exert their activity. Osteoclastic bone resorption is preceded by removal of the non-mineralized osteoid by the interstitial collagenase of osteoblasts (Everts *et al*., 1992). MMP-9 plays a major role in the osteoclast recruitment process, since mice deficient in MMP-9 exhibit a delay in osteoclast recruitment (Engsig *et al*., 2000). MMP-9 is produced by osteoclasts in the human bone tissues and degrades bone collagens in concert with MMP-1 and cysteine proteinases (Okada *et al*., 1995). MMP-1 has been found in osteoclasts (Deláisse *et al*., 1993), but controversy exists (Sakamoto and Sakamoto, 1984; Domon *et al*., 1999). It has been suggested that interstitial collagenase cleavage of type I collagen provides a signal that triggers osteoclasts to resorb bone (Holliday *et al*., 2003a).

Electron microscopic studies have revealed that orthodontically induced root resorption is mediated by odontoclasts (Rygh, 1977). The ultrastructural and histochemical resemblance of odontoclasts and osteoclasts suggests that odontoclastic resorption of the tooth root involves a mechanism similar to that of osteoclastic bone resorption (Domon *et al*., 1999; Tsuji *et al*., 2001). MMP-1 mRNA and MMP-9 are expressed by odontoclasts in bovine root-resorbing tissue (Okamura *et al*., 1993; Linsuwanont *et al*., 2002). PDL cells from the permanent dentition produce less MMP-1 than do human primary teeth undergoing physiological root resorption (Wu *et al*., 1999).

8. GINGIVAL CREVICULAR FLUID (GCF)

GCF is a complex mixture of substances derived from serum, host inflammatory cells, structural cells of the periodontium, and oral bacteria. GCF originates from the vessels of the gingival plexus of blood vessels and flows through the external basement membrane and the junctional epithelium to reach the gingival sulcus. GCF can be isolated from healthy sulcus, although only in small amounts. In the healthy periodontium, GCF represents the transudate of gingival tissue interstitial fluid produced by an osmotic gradient (Alfano, 1974). The volume of GCF increases during periodontal inflammation, primarily due to an increase in the permeability of the vessels underlying the junctional and sulcular epithelium, and also due to basement membrane changes. In addition, the enlarged intercellular spaces of the junctional epithelium act as a reservoir for GCF. At the same time, its composition starts to resemble that of an inflammatory exudate.

The collection and analysis of GCF have provided a non-invasive and site-specific means to assess the biochemical status of the marginal periodontium. GCF composition is well documented as reflecting also the metabolic state of the deeper-seated tissues of the periodontium, *e.g.*, alveolar bone turnover (Wilson *et al.*, 2003). A considerable number of bacteria and host-derived products found in the GCF have been associated with the initiation and progression of periodontal disease. Biochemical analysis of GCF shows promise as an effective means for early detection of periodontal disease (Sorsa *et al.*, 1999; Kinane *et al.*, 2003; Mäntylä *et al.*, 2003). In order to monitor non-invasively the expression of biologically active substances in humans, changes have been studied in the composition of GCF during orthodontic tooth movement (Sorsa *et al.*, 1992a; Grieve *et al.*, 1994; Uematsu *et al.*, 1996; Waddington and Embery, 2001; Ren *et al.*, 2002; Sugiyama *et al.*, 2003).

Several techniques have been employed for the collection of GCF, namely gingival washing (Skapski and Lehner, 1976), capillary tubing (Sueda *et al.*, 1969), and collection by absorbent filter-paper strips (Ren *et al.*, 1992a; Sugiyama *et al.*, 2003). Of these three, collection by filter-paper strips is most frequently used in GCF studies.

AIMS AND OUTLINES OF THE STUDY

1. AIMS OF THE STUDY

SRA has received little attention in the literature. The small number of reported cases may, at least in part, be due to lack of recognition of the condition. As some reports refer to familial occurrence, the hypothesis of this study was that the etiology of SRA is genetic. Population studies based on schoolchildren with developing dentitions have indicated varying prevalence figures for SRA, possibly due to differing ethnic backgrounds and ages in the populations studied. The purpose was to establish the prevalence of SRA in healthy young adult Finns.

Under normal conditions, the tooth-supporting tissues are constantly remodeled. Studies indicate that, in human periodontal ligament (PDL) cells *in vitro,* mechanical stress induces mRNA MMP production. The second part of this thesis tested whether the levels, molecular forms, and degree of activation of MMPs in gingival crevicular fluid (GCF) reflect an early stage of orthodontic tooth movement in humans *in vivo*. Another hypothesis was that the pattern of MMPs in GCF of SRA teeth differs from that of control teeth, thus reflecting characteristic periodontal remodeling in physiological conditions.

2. OUTLINES OF THE STUDY

Based on the background, the specific aims of the present study were to:

1. Analyze the phenotypic features of SRA and inheritance in a series of families with SRA, and any association with familial hypodontia and other dental anomalies.
2. Establish the prevalence of SRA in healthy Finnish young adults with fully developed dentitions.
3. Perform an observational biochemical characterization of gelatinases (MMP-2, -9) and collagenases (MMP-8, -13) present in the GCF of SRA patients.
4. Examine whether MMP-1 and MMP-8 activities can be determined *in vivo* in the GCF of human teeth exposed to orthodontic force.
5. Analyze the time-dependent levels, molecular forms, and degree of activation of MMP-1 and MMP-8 in the GCF of human teeth exposed to orthodontic force after fixed appliance activation during an eight-hour short-term period and one-month long-term follow-up.

PATIENTS AND METHODS

1. PATIENTS

1.1. Families with SRA (Study I)

The family probands (n = 8) were those in whom SRA was first diagnosed. These individuals were refered for consultation by their dentists or orthodontically treated at the Department of Pedodontics and Orthodontics, Institute of Dentistry, University of Helsinki or treated in a private dental practice. Four of the probands were referred by orthodontists who had noted short roots in post-treatment radiographs and sought a consultation because of what they felt was an unfavorable sequel of the treatment. The probands´ first-degree relatives and all other available relatives were contacted and invited to a dental and radiographic examination. In four families, it was possible to include second-degree relatives in the pedigrees. In total, the study group included 8 probands with SRA, two males, six females (age-range 14-46 years) and their first- and second-degree relatives, totaling 72 individuals in eight families. Of these, 28 belonged to one large family with nine sibships.

1.2. University students (II)

The Finnish Student Health Service (FSHS) archives in Helsinki contain approximately 30 000 panoramic radiographs. From these archives, 2 000 dental records with panoramic radiographs were randomly selected. Of these, 41 were rejected because of technically poor radiography, usually because the root apices, incisal edges, and intersections of crown and root contours were undefinable on the maxillary central incisors. The final sample comprised 1 959 panoramic radiographs (1289 females, 670 males; aged 19–30 years, mean age 20.6).

1.3. SRA patients (III)

SRA GCF samples were collected from five of the SRA patients included in Study I (3 females and 2 males, mean age 26) at the Institute of Dentistry, University of Helsinki.

1.4. Orthodontic patients (IV, V)

GCF samples in Study IV were obtained from 11 orthodontic patients (8 females, 3 males; aged 10–14 years except for three adults aged 37–38) undergoing fixed appliance treatment in a private dental practice. GCF samples in Study V came

from five orthodontic patients (3 females and 2 males, mean age 12 except for one patient age 36).

1.5. Controls (III-V)

Control patients in Studies III to V were the following: 4 females and 1 male, mean age 24 years (III); 4 females and 2 males aged 11 to 16 years, except for three adults aged 35 to 37 years (IV), and 3 females with a mean age of 36 years (V).

1.6. Exclusion criteria

All orthodontic, SRA, and control patients from whom the GCF samples were obtained were healthy, except for one with type I controlled diabetes. The patients had used no antibiotics within the preceding 6 months. All patients had clinically healthy periodontal tissues, as assessed by careful clinical and radiological periodontal examination.

1.7. Ethical permission

The studies were carried out with the informed consent of all the patients, and the protocols were approved by the Ethics Committee of the Institute of Dentistry, University of Helsinki, Helsinki, Finland.

2. METHODS

2.1. Diagnosis of SRA

Diagnosis was based on relative root length, defined as the ratio between root length (R) and crown length (C) (Lind, 1972). On maxillary central incisors, the crown and root lengths were compared with a ruler. In cases where the root length was of equal length or shorter than the crown ($R/C \leq 1.0$) in at least one pair of permanent teeth, the SRA diagnosis was made. Since this kind of measurement cannot be reliably performed on posterior teeth, these were visually evaluated. Only bilateral occurrence of short roots was recorded; any single teeth with short roots were ignored.

2.2. Clinical and radiographic examination (I)

Each of the probands´ first-degree relatives and, whenever possible, second-degree relatives underwent a radiographic and dental examination at the Institute of Dentistry, University of Helsinki. Panoramic radiographs, and in the majority of cases also full-mouth intraoral series were taken for the diagnosis of SRA. Dental

examination was performed by two of the authors (S. Apajalahti, S. Pirinen). Root development arrested by any environmental factor was excluded by the retrospective dental information collected from the dental files and anamnestic information given by the patient. Radiographs taken elsewhere were reviewed. In cases with orthodontic treatment included in their retrospective dental data, pre- and post-treatment radiographs were compared in order to evaluate root shortening due to orthodontic treatment. Other dental anomalies recorded included hypodontia (third molars excluded), peg-shaped maxillary lateral incisors, invaginations, supernumerary teeth, ectopic canines, and taurodontism. Invagination was recorded for any lateral incisors which showed any distinct lingual pit, or a filling at the location for invagination, or if radiographs showed distinct enamel notching. Individuals with missing maxillary laterals were excluded. Taurodontism was determined from panoramic radiographs according to Laatikainen and Ranta (1996). For available family members with SRA, mesiodistal dimensions of the maxillary incisors, and maxillary and mandibular premolars were measured from dental casts with a digimatic sliding caliper (Mitutoyo®, Mitutoyo, Mfg Co. Ltd, Minato-Ku, Tokyo, Japan). Measurements were performed twice. If the dimensions deviated by more than 0.1 mm, the tooth was remeasured until the difference did not exceed 0.1 mm, and the mean values for these two dimensions were used. All dimensions were compared to the Finnish standards (Alvesalo, 1971).

Cases of SRA become apparent only a year or two after tooth emergence, that is when root formation has been nearly completed. Of the 64 relatives, two were too young for reliable diagnosis of SRA in any of the tooth groups. In addition, root formation in canines, premolars, and second molars in four individuals was incomplete.

2.3. Radiographic examination (II)

The random sample of 1 959 existing panoramic radiographs were first visually evaluated by one author (S. Apajalahti). All intraoral radiographs available were also used. In 36 dentitions, the roots appeared as very short, and the crown and root lengths were compared with a ruler for diagnosing SRA. Of these 36 patients, seven were rejected because their crown and root lengths did not meet the SRA criteria described above. For the patients thus identified, the radiographs were re-examined together with an experienced orthodontist (S. Pirinen). Four more cases in which root shortening possibly due to orthodontic treatment could not be reliably excluded were rejected. Anamnestic information came from the forms the patients had filled in at the first appointment. Hypodontia was determined from panoramic radio-

graphs. Teeth were diagnosed as developmentally missing if the corresponding deciduous tooth persisted. Third molars were not included in the data collection.

3. GCF SAMPLE COLLECTION, WEIGHING, PROCESSING, AND STORAGE

GCF samples were collected from two SRA teeth, the upper central incisors or premolars, from each SRA patient, as well as from the control patients (III).

For each orthodontic patient in Study IV, the permanent upper incisor, upper canine or lower central incisor undergoing orthodontic tooth movement served as the experimental tooth. GCF samples were collected from each experimental tooth immediately before fixed appliance activation and every hour for 8 hrs following application of the orthodontic force. Control GCF samples were collected from the upper central incisors of six non-orthodontic patients every hour for 8 hrs (IV).

In study V, GCF samples were collected from five orthodontic patients undergoing fixed appliance treatment (Mini-mat brackets, 0.018 inch slot). Initial aligning archwire (Respond, Ormco corporation, Glendora, CA, USA) was placed during the same visit at which the GCF sample collection began. The samples were collected from the upper or lower central incisor or from the upper canine just before and every 24 hrs after fixed appliance activation for one month. Control GCF samples were collected from the upper central incisor of three non-orthodontic patients every 24 hours for one month (V).

The surfaces of the teeth were dried gently and kept dry with cotton rolls. Two filter-paper strips were placed at the gingival margin into the sulcus for 3 min. The GCF flow volume was measured by weighing the strips in polypropylene tubes according to the Mettler AJ 100/GWB scale (IV, V). Thereafter, the absorbed fluid was eluted from each strip into 25 µl buffer consisting of 0.2 M NaCl–1.0 mM $CaCl_2$ –50 mM Tris-HCl, pH 7.5, and stored at -20°C prior to analysis (III–V).

4. REAGENTS

Polyclonal rabbit anti-human MMP-8 was provided by Dr. J. Michaelis, Department of Pathology, Christchurch School of Medicine, Christchurch, New Zealand (Michaelis *et al.*, 1990). All other reagents were of the highest analytical reagent grade.

5. FUNCTIONAL ASSAYS

5.1. Western blot (III-V)

The molecular forms of MMP-1, -8, and -13 in the GCF samples were analyzed by the Western blot method with specific antibodies. Samples were treated with Laemmli buffer, pH 6.8, and heated for 5 min at 100°C. Low-range prestained sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) standards (Bio-Rad, Richmond, CA, USA) served as the molecular-weight markers. The samples were separated on 8 to 10% SDS-PAGE and then electrophoretically transferred onto nitrocellulose membranes. To block non-specific binding sites on the nitrocellulose membranes, the membranes were diluted in 3% gelatin in 10 mM Tris-HCl, pH 7.5 – 0.05% Triton X-100 – 22 mM NaCl (TTBS) for one hour at 37°C, after which the membranes were washed with TTBS four times, for 15 min each. The membranes were then incubated with monoclonal mouse anti-human MMP-1 antibody (1:250 dilution in TTBS; Oncogene, Cambridge, MA, USA) (IV, V), polyclonal rabbit anti-human MMP-8 antibody (1:500 dilution in TTBS) (Hanemaaijer *et al.*, 1997) (III-V) or monoclonal mouse anti-human MMP-13 antibody (1:150 dilution in TTBS; Oncogene) (III) at 22°C for 24 hrs. After four 15-min washes with TTBS, the membranes were incubated with goat anti-mouse IgG for MMP-1 (1:1000 dilution in TTBS) (IV, V) and MMP-13 (1:500 dilution in TTBS) (III), and goat anti-rabbit IgG for MMP-8 (1:500 dilution in TTBS; Sigma, St. Louis, MO, USA) (III-V) alkaline phosphatase conjugates for one hour. After four washings with TTBS, each for 15 min, one 15-minute wash with 10 mM Tris-HCl (pH 7.5, 22 mM NaCl), and one 15-minute wash with alkaline phosphatase buffer (100 mM NaCl – 5 mM $MgCl_2$ – 100 mM Tris, pH 9.5), the immunoblots were visualized by the addition of nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate diluted to N-N-dimethyl formamide (Sigma) in 100 mM Tris-HCl – 5 mM $MgCl_2$ – 100 mM NaCl, pH 9.5.

The intensities of different MMP isoforms were analyzed by means of an Imaging Densitometer (Model GS-700; Bio-Rad) equipped with the Molecular Analyst/PC program.

5.2. Zymography (III)

The SRA and control GCF samples in Study III were analyzed by zymography, with SDS-PAGE gels containing 1 mg/ml type-I gelatin as the substrate (Sigma). Before electrophoresis, the samples were incubated for 2 hrs at 22°C, after which the samples were loaded into 10% gels. Molecular-weight markers were low-range pre-stained standards (Bio-Rad). After electrophoresis, the gels were washed for 30

min in 50 mM Tris-HCl, 2.5% Tween 80 and 0.02% NaN_3, pH 7.5, and then for 30 min with the same buffer supplemented with 1 μM $ZnCl_2$ and 5 mM $CaCl_2$. The gels were then incubated overnight in 50 mM Tris-HCl, 5 mM $CaCl_2$, 1 μM $ZnCl_2$, 0.02% NaN_3, pH 7.5, at 37°C, and then stained with Coomassie Brilliant Blue R250 and destained as described by Ingman *et al*. (1994b).

5.3. Immunofluorometric assay (IFMA) (IV, V)

Concentrations of MMP-8 in the GCF samples from orthodontic and control patients were determined by a time-resolved immunofluoroscence assay (IFMA) (IV,V). The monoclonal MMP-8 specific antibodies 8708 and 8706 (Medix Biochemical, Kauniainen, Finland) served as the catching antibody and tracer antibody, respectively. The tracer antibody was labeled with europium chelate. The assay buffer contained 20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 5 mM $CaCl_2$, 50 μM $ZnCl_2$, 0.5% bovine serum albumin, 0.05% NaN_3, and 20 mg/l DTPA. Samples were diluted in assay buffer and incubated for one hour, followed by incubation for one hour with tracer antibody. Enhancement solution was added, and after 5 min, fluorescence was measured with the 1234 Delfia Research Fluorometer (Wallac, Turku, Finland). The specificity of the monoclonal antibodies against MMP-8 corresponds to that of polyclonal MMP-8 (Hanemaaijer *et al*., 1997; Sorsa *et al*., 1999; Mäntylä *et al*., 2003).

Mean MMP-8 concentrations (μg/l) assayed with IFMA in orthodontic GCF in the one-month follow-up were compared with the mean MMP-8 concentrations in gingivitis and periodontitis GCF published previously by Mäntylä *et al*. (2003).

6. STATISTICS

The standard chi square test was used to analyze differences in the prevalence of SRA between males and females. The same test was used to analyze the prevalence of hypodontia in SRA patients and non-SRA patients (II). The non-parametric Mann-Whitney *U*-test was used to define differences in MMP-8 levels between orthodontic and control GCF samples, and between orthodontic samples at 1 to 8 hours (IV). A *P* value of less than 0.05 was considered significant.

RESULTS

1. SRA IN FAMILIES (I)

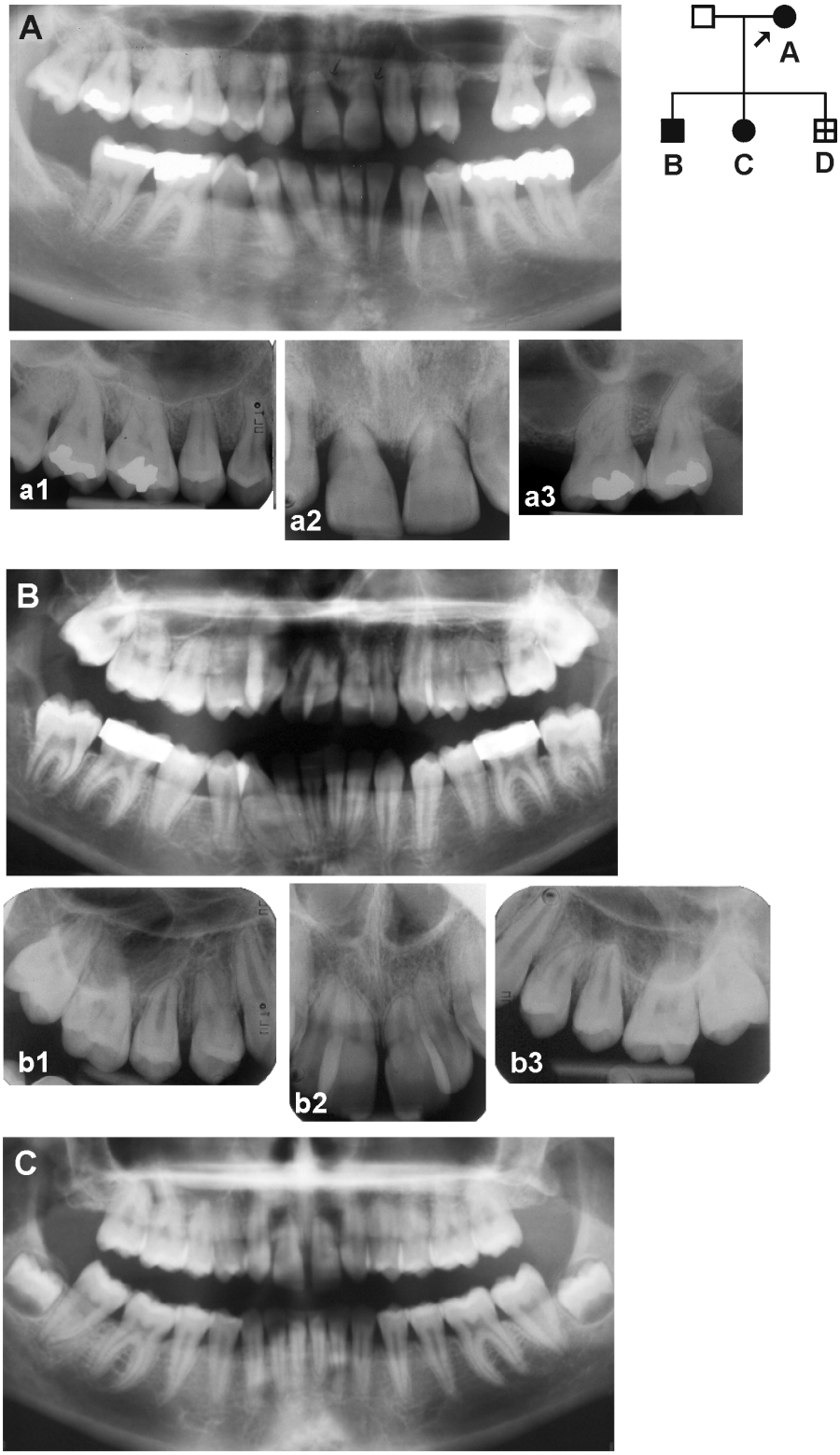
SRA was diagnosed only in first-degree relatives of the probands. Three of the pedigrees showed one or two children with SRA and an affected parent (Fig. 5). In addition, in two families, the anomaly existed in siblings but not in the parents. In three of the families, the proband was the only family member affected.

The tooth pairs most frequently affected (n = 48) were maxillary central incisors (27%), maxillary first premolars (18%), maxillary second premolars (14%), mandibular second premolars (8%), and maxillary canines (8%). Usually two to four tooth pairs were affected.

1.1. Occurrence of hypodontia in family members

Developmentally missing teeth occurred in seven of the eight pedigrees. Of the family members with SRA, 46% (7 of 15) presented with hypodontia. The missing teeth were mostly second premolars and upper lateral incisors. However, in one proband, a mandibular incisor was developmentally missing, and in one family both daughter and mother had one mandibular second molar missing. The maximum number of missing teeth was one or two, except for one family member with five missing teeth.

Figure 5 (right) (A) Panoramic radiograph of one proband with Short root anomaly (SRA) (also indicated by black arrow in the pedigree). Maxillary central incisors and all the maxillary teeth except the molars were short-rooted (for more detail, see **a1-a3**). Maxillary lateral incisors, left second premolar, and mandibular second premolars were developmentally missing. Maxillary central incisors were loose (**a2**), and they were later replaced by dental implants. (**B)** The eldest son (12 years) had short-rooted maxillary incisors and premolars (for more detail, see **b1-b3**). **C.** The daughter (16 years) had short roots in maxillary central incisors. Her palatal canines were orthodontically treated, and maxillary lateral incisors were developmentally missing. The youngest son (D in the pedigree) was too young for reliable diagnosis of SRA. Symbols in the pedigree: squares, males; circles, females; darkened, affected; arrow, proband; cross, too young for diagnosis.


A
A
B
C
D
a1
a2
a3
B
b1
b2
b3
C

1.2. Other dental features in family members

Both invagination(s) and ectopic maxillary permanent canine(s) were evident in 5 of 15 (33%) of the family members with SRA. Two affected family members had supernumerary teeth, and taurodontism in molars was recorded in two.

1.3. Mesiodistal dimensions of SRA teeth

The mesiodistal dimensions of the crowns of intact, fully erupted maxillary incisors and upper and lower premolars were evenly distributed around the population means (Alvesalo, 1972) (Table 1). No systemic increase or reduction in tooth sizes was noted. None of the SRA patients exhibited reduced mesiodistal crown size (≤ -2SD). One patient showed increased mesiodistal dimensions (> 2SD) in the maxillary central incisors, and in the maxillary and mandibular premolars.

2. PREVALENCE OF SRA IN HEALTHY YOUNG ADULTS (II)

Prevalence of SRA in this study group was 1.3% (25 of 1 959). Females were significantly more often affected than males ($P < 0.05$). Due to patient data-protection, no access was available to the pre-treatment records of the SRA patients. It was thus impossible to verify either pre-treatment root length and morphology or type and duration of orthodontic treatment performed.

2.1. Distribution of SRA teeth

Maxillary central incisors were the teeth most frequently involved in 72% (18 of 25) of the SRA patients. In addition, maxillary second premolars, maxillary first premolars, maxillary lateral incisors, mandibular second premolars, and maxillary canines (in that order) were involved. Overall, in 60% (15 of 25) of the SRA patients all maxillary central incisors and maxillary premolars were involved. Furthermore, short roots occurred in molars in six of the affected, in three of whom the molars were strongly taurodontic in shape.

2.2. Hypodontia

Of the 25 patients with SRA, three (12%) also showed hypodontia. The missing teeth were maxillary and mandibular second premolars and maxillary lateral incisors. Additionally, two subjects had peg-shaped lateral incisors.

Table 1. Mean tooth size in incisors and premolars of seven SRA patients.

Tooth	(n)	m-d* tooth size ± (SD) mm	Reference (Alvesalo, 1971)	SDS**
Girls				
Maxilla				
I1	(10)	8.69 (0.56)	8.62	+0.1
I2	(8)	6.89 (0.57)	6.67	+0.4
P1	(7)	7.06 (0.50)	7.03	+0.1
P2	(9)	7.07 (0.59)	6.75	+0.7
Mandible				
P1	(8)	7.21 (0.58)	7.03	+0.4
P2	(7)	7.33 (0.42)	7.04	+0.8
Boys				
Maxilla				
I1	(4)	8.51 (0.14)	8.82	-0.6
I2	(4)	6.66 (0.18)	6.87	-0.3
P1	(4)	7.24 (0.15)	7.10	+0.3
P2	(2)	6.86 (0.03)	6.84	0
Mandible				
P1	(2)	7.21 (0.03)	7.20	0
P2	(2)	7.24 (0.18)	7.28	-0.1
All	(67)			

* m-d = mesiodistal

** SDS = standard deviation score. SDS was calculated for each SRA tooth by use of means and standard deviations of the reference group (Alvesalo, 1971).

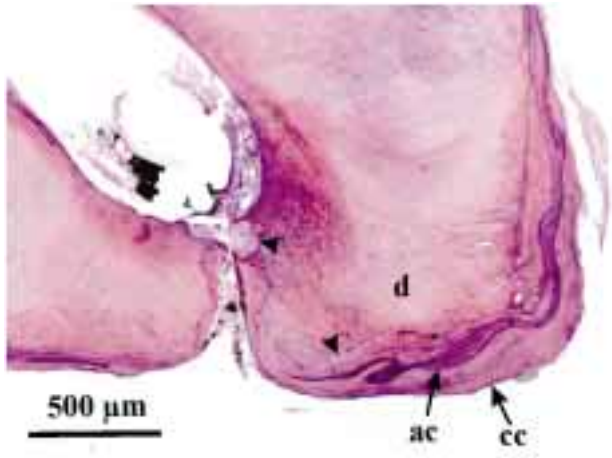


Figure 6. Photomicrograph showing the apical part of an maxillary second molar of a patient with short root anomaly (SRA). The apex is closed. The tip of the root appears blunt. The apical dentin (d) showing a regular tubular pattern is leyerwise covered by successive layers of cellular (cc) and acellular cementum (ac). The dentin-cementum interface is irregular (arrowheads). Minor resorption of the apical dentin, which has been replaced by cementum, may be inflammatory or represent response to endodontic treatment. Hematoxylin and eosin stain.

3. HISTOLOGICAL FINDINGS IN A TOOTH OF ONE PATIENT WITH SRA

The upper right second molar was removed from a 27-year-old woman with SRA because of poor response to endodontic treatment; it was submitted for histological examination. The tooth was fixed with 10% formalin, demineralized with ethylenediaminetetraacetic acid, bisected axially, dehydrated, and embedded in paraffin. A representative series of sections were cut and stained with hematoxylin and eosin. Consistent with the macroscopic appearance of this tooth, histological examination showed a short and cervically constricted root tapering in the apical direction. The apex was closed and the root tip appeared blunt. The pattern of the dentinal tubules was regular (Fig. 6). Two denticles with a discernable tubular pattern were embedded in the root dentin. The root canal contained filling material and more apically, a chronic inflammatory cell infiltrate was visible. The most apical part of the root canal was formed of dense-textured connective tissue. The apical dentin was covered by acellular and cellular cementum, which had replaced

the minor resorption of dentin. This resorption was likely to be inflammatory in nature and not the primary cause of the short root (Lukinmaa and Apajalahti, unpublished results).

4. GELATINASES (MMP-2, -9) AND COLLAGENASES (MMP-8, -13) IN GCF OF SRA PATIENTS (III)

Zymographic analysis of the GCF of SRA patients revealed both high- and low-molecular weight gelatinolytic proteinases. The major species detected in SRA GCF represented 92 kD gelatinase B (MMP-9). Of the 70–90 kD MMP-9 (30% of the total gelatinolytic activity), 18% was in the 90 kD proform and 12% was converted to the active enzyme with a molecular weight of 71–82 kD (Fig. 7A). High molecular-weight bands (120 kD), representing MMP-9 complex formation with other proteins, made up 37%, and low molecular-weight (< 50 kD) species, most likely representing the fragmented MMP-9 due to activation, made up 33% of the total gelatinolytic activity. Control GCF samples revealed MMP-9 in 90 kD proform (14% of the total gelatinolytic activity), but not in active 71–82 kD form. Other molecular species of gelatinases in the control group included high molecular-weight complexes (59%) and low molecular-weight fragments (27%). MMP-2 species were not detectable in SRA and control GCF samples. No MMP-8 and MMP-13 immunoreactivities were detectable by Western blot.

5. EXPRESSION OF MMP-1 AND MMP-8 IN GCF OF ORTHODONTIC PATIENTS (IV, V)

5.1. Eight-hour follow-up (IV)

Mean concentrations of MMP-8 obtained by IFMA assay in the GCF of orthodontically treated teeth were significantly higher at 4 to 8 hours after fixed appliance activation than before activation (0 hr), and when compared with the control teeth ($P < 0.05$) (Fig.7B).

In Western blot analysis, several immunoreactivities for the MMP-8 enzyme were detectable (Fig.7C). Bands in the range 60-80 kD corresponding to PMN-type pro- and active enzymes represented the majority (42–62%) of the total MMP-8 immunoreactivity, except at 0 and 5 hours. From baseline to one hour, the amount of PMN-active enzyme (60 kD) was elevated in relation to total PMN-type (60–80 kD) immunoreactivity from 31% to 79%, decreasing to 56% at 2 hours, and thereafter being less than or nearly equal to the amount of PMN pro-enzyme (75–80 kD). Of the total staining, the high molecular-weight band (>100 kD) was the next most frequent. Low molecular-weight staining at < 30 kD appeared in only one patient at

6 to 8 hours. Bands around 40–55 kD were presumed to represent fibroblast-type MMP-8, and were visible in four patients from 2 to 8 hrs with the maximum level at 2 hours (9% of the total MMP-8 immunoreactivity).

The majority of MMP-1 Western blots for experimental teeth failed to show any detectable immunostaining. Of the 11 orthodontic samples, three showed immunoreactivity at > 100 kD. In addition, one patient showed staining at 52 kD, corresponding to the MMP-1 proform, at 4 hours. No MMP-1 immunoreactivity was detectable in the control samples. GCF flow was not significantly affected by orthodontic treatment.

5.2. One-month follow-up (V)

The IFMA assay revealed on average 12-fold higher (56 µg/l ± 50 SD *versus* 4.6 µg/l ± 4 SD) levels of MMP-8 in orthodontic GCF compared with control GCF during the whole month of follow-up testing, but still lower levels of MMP-8 than in gingivitis and periodontitis (Fig.7D). IFMA analysis was confirmed by Western blot, also showing clearly more MMP-8 immunoreactivity in the GCF of orthodontic patients relative to control GCF during the whole month. Orthodontic samples revealed that 41% of the total MMP-8 was high molecular-weight complexes (>100 kD). The 75 kD proPMN-MMP-8 represented 32% of total MMP-8 immunoreactivity. Of the total MMP-8, 14% was in 60 kD active-PMN-MMP-8 form and 13% in 55 kD fibroblast-type pro-MMP-8 form. With Western blotting, MMP-8 levels in control samples were hardly detectable.

MMP-1 levels in orthodontic and control GCF samples were not detectable by Western blotting.

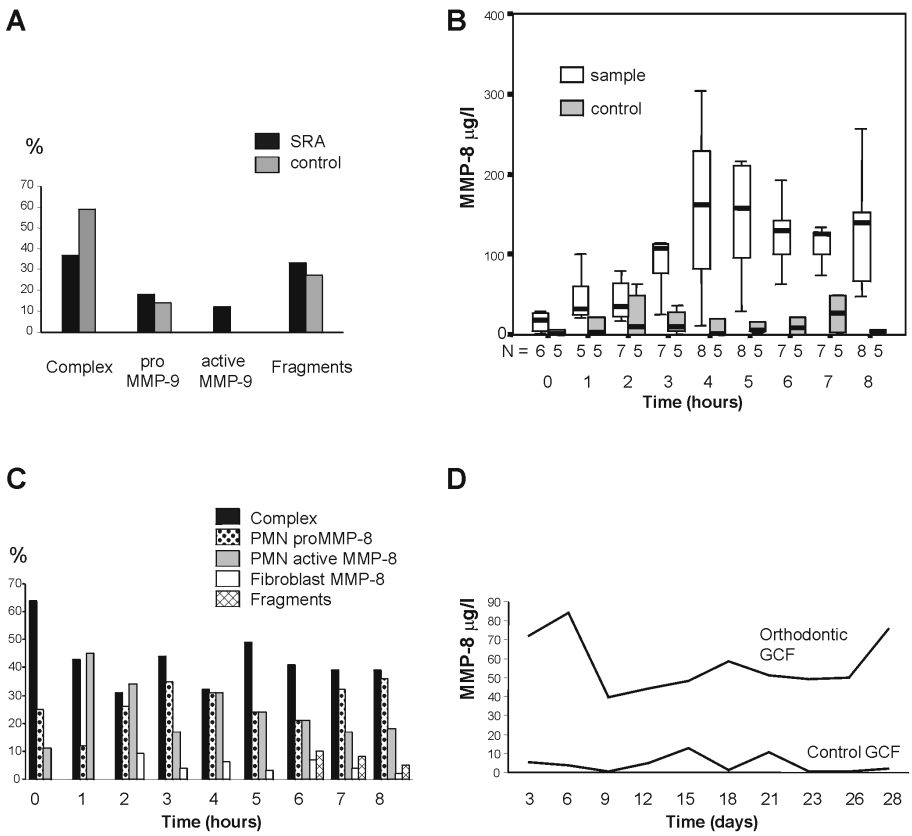


Figure 7. (A) Percentages of different molecular forms of MMP-9 for total MMP-9 gelatinolytic activity in gingival crevicular fluid (GCF) of short root anomaly (SRA) and control patients detected by zymography. **(B)** MMP-8 levels in the GCF of orthodontic and control patients detected with the IFMA analysis. The orthodontic samples were collected from the orthodontically moved teeth immediately before fixed appliance activation (0 hr), and at 1-8 hrs after force application. Represented as range, median and 25th and 75th percentiles. **(C)** Mean percentages of different molecular forms of MMP-8 in the GCF of orthodontic patients (n = 11) at 0-8 hrs using the densitometric quantitation of Western blots. **(D)** Mean MMP-8 levels in the GCF of orthodontic (n=5) and control (n=3) patients during the one-month follow-up detected with the IFMA analysis.

DISCUSSION

1. SRA - A RARE DEVELOPMENTAL ANOMALY

1.1. The genetic background of SRA

In the present study, a family series with SRA was collected in order to analyze the inheritance pattern of this rare anomaly. Of eight pedigrees, three showed one or two children with SRA and an affected parent, suggesting autosomal dominant transmission. Unlike the case of autosomal dominant disorders, however, SRA could not be traced in the second-degree relatives of the probands, although a large pedigree with 24 second-degree relatives was available for examination on the affected father's side. In three of the eight pedigrees, the proband was the only affected family member, suggesting either fresh mutation of an autosomal dominant trait, failure of penetrance of an autosomal dominant trait or autosomal recessive transmission, in which there is usually no family history.

Familial occurrence of SRA has been suggested (Lind, 1972; Newman, 1975; Edwards and Roberts, 1990), but the inheritance pattern of SRA has not thus far been studied by use of extensively collected family material as in the present study. The diagnosis of genetic SRA is not verified until similar short-rooted teeth appear in some family members, and when other causes for short-rooted teeth can be excluded. With these criteria the presence of true genetic SRA could be confirmed.

An autosomal dominant pattern of inheritance for SRA has been suggested (Lind, 1972; Newman, 1975; Edwards and Roberts, 1990). Based on our three sporadic cases in eight families with SRA, however, the anomaly may not be seen in first-degree relatives of the probands, unlike what is usually seen in autosomal dominant traits. In the present family data, the apparent genetic heterogeneity of the pedigrees allowed no conclusions as to the mode of inheritance.

Of the family members with SRA, 46% presented with hypodontia, nearly six times the Finnish population prevalence of 8% (Haavikko, 1971). The family data thus support earlier speculations as to any association between SRA and hypodontia (Brook and Holt, 1978; Edwards and Roberts, 1990). Isolated hypodontia is a genetic condition inherited as an autosomal dominant trait with incomplete penetrance and variable expression (Grahnen, 1956; Burzynski and Escobar, 1983; Arte *et al*., 2001). The missing teeth in our SRA patients were usually the same as shown in incisor-premolar hypodontia: second premolars and upper lateral incisors (Alvesalo and Portin, 1969; Seow and Lai, 1989; Schalk-van der Weide *et al*., 1993; Pirinen *et al*., 1996; Peck and Peck, 1997; Arte *et al*., 2001).

In the present study, ectopic canines were occurred in 33% of the family members with SRA, about 16 times that in Western countries of about 2% (Thilander and Jakobsson, 1968; Ericson and Kurol, 1986). A spectrum of dental anomalies such as ectopic canines, malformed upper lateral incisors, rotation of premolars, and taurodontism are all related to hypodontia (Arte, 2001). Our data thus allow the assumption that SRA may also belong to this spectrum.

1.2. The prevalence of SRA

The prevalence of 1.3% in healthy university students 18-30 years old affected females significantly more often than males. The prevalence of SRA in the present study is lower than the 2.4% or 2.7% reported for Western school children aged 11 to 14 years (Jakobsson and Lind, 1973; Brook and Holt, 1978), whereas our findings are somewhat higher than the 0.6% reported for Swedish school children aged 8 to 9 years (Bergström, 1977). Earlier population studies, based on school children with developing dentitions, and have focused on maxillary central incisors. The present study found that in most cases, other teeth, typically maxillary premolars, laterals, and mandibular second premolars, were also affected. Maxillary central incisors were defined as abnormally short if the roots were of equal length or shorter than the crowns bilaterally, whereas Jakobsson and Lind (1973) and Brook and Holt (1978) included maxillary central incisors with R/C ≤ 1.1 in SRA category. Obviously, because they have ignored the criteria of bilateral occurrence, the possibility of other reasons behind the shortening of the root of a single incisor (dental trauma being possibly the most important reason) cannot with absolute certainty be excluded. Moreover, the age range of the children studied (11 to 14 years) was considerably younger than in this study. Bergström (1972) did not define any criteria for short-rooted teeth. Thus, all these differences in sample size and population age, as well as in diagnostic criteria, may partly explain the differences between prevalences. Racial differences may, at least in part, explain the much higher prevalence of 10% found in Japan (Ando *et al*., 1967).

In the present study, diagnosis of SRA was based on evaluation of relative root length on panoramic radiographs. In cases with excessive labial inclination of the upper central incisors, some distortion may be expected in the radiographic image. However, this distortion was considered not to have influenced the results in the large sample of 1959 panoramic radiographs examined.

This study covered mesiodistal dimensions in incisors and premolars of the seven SRA patients. The measurements were compared to the means and SDs for Finns published by Alvesalo (1971). The material of his study was from Hailuoto island, where the prevalence of missing maxillary lateral incisor was 4.25%

(Alvesalo and Portin, 1969). Since reduction in the mesiodistal dimensions of tooth crowns has been reported in individuals with hypodontia and their relatives, it is possible that also the mesiodistal crown dimensions were smaller in the Hailuoto population. In the SRA patients, the mean mesiodistal dimensions were of the same order than those reported for the Hailuoto population. On the other hand, SRA patients also presented with hypodontia.

1.3. Possible developmental mechanisms of SRA

Tooth development is characterized by reciprocal interactions between the dental epithelium and mesenchyme. Several paracrine signaling molecules and transcription factors regulate these interactions during early tooth morphogenesis (reviewed by Thesleff and Mikkola, 2002a), whereas little is known about the signaling pathways regulating root development (Thomas, 1995; Yamashiro *et al*., 2003). Root formation is initated by the downgrowth of HERS, which directs root morphogenesis (Thomas, 1995). Recently, it was found that a homeobox transcription factor Msx2 is continuously expressed in HERS during mouse root development, indicating its involvement in root morphogenesis (Yamashiro *et al*., 2003). Since panoramic radiographs of the children with SRA in Study I showed that crown and root development appeared to have proceeded normally before tooth emergence, and since the fully developed roots had closed apices, it seems that the HERS has all the capacity needed to achieve a complete root. It thus appears that the pathogenesis of SRA results from a disturbance in signaling pathways in HERS, which results in premature closure of the apex.

Of the family members with SRA, 46% also presented with hypodontia, the missing teeth being mostly second premolars and upper lateral incisors. In humans, mutations in *MSX1* and *PAX9* genes have been demonstrated to cause oligodontia (Vastardis *et al*., 1996; Nieminen *et al*., 2001, 2003; Lammi *et al*., 2003), whereas the gene defects causing incisor-premolar hypodontia or other forms of hypodontia in man are unknown. It is evident that both hypodontia and oligodontia are genetically heterogenous traits (Arte, 2001).

Interestingly, according to our observations it appears that mandibular second premolars are more susceptible to SRA than are mandibular first premolars. The mandibular second premolar is the most frequently missing tooth in hypodontia (Haavikko, 1971), in which the last developing teeth in each tooth group seem to be most frequently affected, indicating the critical locations in the placods where agenesis occurs (Arte, 2001). Contrary to hypodontia, however, maxillary central incisors are almost always affected in SRA.

1.4. Clinical implications of SRA

Since SRA teeth are clinically normal, with the crowns being of normal size, SRA cannot be diagnosed based on clinical examination alone. Thus, in everyday clinical work, radiographic analysis of root length is important before orthodontic, endodontic or prosthetic treatment is instituted, because one person in a hundred may have short-rooted upper incisor teeth or premolars. This is particularly important in patients presenting with hypodontia and ectopic canines. Unlike tooth agenesis, which can be diagnosed after the age of six (Pirinen and Thesleff, 1995), SRA is difficult to diagnose in the mixed dentition. Cases of SRA become apparent only a year or two after tooth emergence, that is, when root formation is nearly completed. Large variation exists in root formation time by sex, as well as between individual children, and should always be taken into consideration when determining root lengths in developing SRA dentitions. In developing dentitions with bilateral occurrence of short-rooted maxillary central incisors, a careful screening of maxillary premolars, laterals, and mandibular second premolars is recommended, since these teeth are frequently involved in SRA. The present findings showed mandibular second premolars to be more frequently affected with SRA than mandibular first premolars. Thus, in patients presenting with crowding and when premolar extractions are needed, it is strongly advisable that root length be carefully analyzed in orthodontic treatment planning. Since SRA dentitions frequently show short roots in maxillary anterior region, alveolar bone height may be underdeveloped, and this may affect orthodontic treatment strategy.

During the course of this study, two of the SRA patients undergoing fixed appliance treatment presented with minor root resorption. An increased tendency towards root resorption during orthodontic treatment and due to pressure from embedded canines has been reported in SRA dentitions (Lind, 1972; Newman, 1975). It is most desirable that further root shortening of SRA teeth be avoided. Therefore, during orthodontic treatment, long treatment times and extensive tooth movement with heavy force should be avoided, with frequent radiographs recommended. However, it seems that short-rooted maxillary incisors function remarkably well unless they are under exceptionally heavy stress. Perhaps, this is due to their roots´ being blunt instead of tapered

2. A CHARACTERISTIC PROFILE OF GELATINASES (MMP-9) ACTIVITY IN GCF OF SRA PATIENTS

In the present study, zymographic analysis of the GCF of SRA patients revealed gelatinases of high- and low-molecular size. The major species detected were presumed to represent MMP-9 that had undergone activation associated with

fragmentation and complex formation. The high molecular-weight forms of MMP-9 may represent dimer-forms of MMP-9 or complex formation with other proteins. One cannot but speculate on the various cellular sources of the MMP-9 in the GCF of the SRA patients. Expression of MMP-9 in normal tissues is low, but it can be induced during inflammation, development, and bone resorption. MMP-9 has been shown to be abundantly expressed by osteoclasts in developing bone tissue in mice and in human bone tissues from normal subjects, implying that the enzyme plays a key role in physiological bone remodeling (Okada *et al*., 1995; Rice *et al*., 1997; Sahlberg *et al*., 1999). In the periodontal tissues, gelatinases are the primary matrix-degrading proteinases produced by the epithelial cells (reviewed by Kähäri and Saarialho-Kere, 1997), and MMP-9 expression by gingival epithelial cells has been detected *in vivo* (Salo *et al*., 1994). In periodontitis-affected gingival tissue, MMP-9 is the predominant gelatinase and is mainly derived from degranulating PMNs (Ingman *et al*., 1994b; Westerlund *et al*., 1996). In this study, the gingival tissues of SRA patients were clinically healthy with no signs of periodontal diseases evident in panoramic radiographs, indicating that PMNs were not the main cells involved in the production of MMP-9. In the present study, both epithelial cells and osteoclasts may thus be possible sources for MMP-9.

Previous studies have shown that degradation of PDL precedes root resorption and is followed by recruitment of resorptive cells that remove root structure (Brudvik and Rygh, 1993; Rygh, 1977). The collagen of the PDL is mainly of types I and III, and dentin organic matrix is mainly type I collagen. MMP-8 and MMP-13 both degrade types I and III collagen, MMP-8 being the most effective collagenase in initiating type I collagen degradation (Hasty *et al*., 1987). In addition, MMP-2 can cleave type I and II collagen to the characteristic N-terminal ¾ and C terminal ¼ fragments identical to those generated by collagenases (Aimes and Quigley, 1995). The present GCF analysis did not reveal any MMP-8 or -13 immunoreactivity, whereas zymographic analysis of the GCF of SRA patients revealed what seemed to be gelatinase (MMP-9) activity, but with no MMP-2 activity. Since MMP-2, MMP-8, and MMP-13, but not MMP-9, can split types I, II and III native fibrillar collagens, and thus can initiate collagenolysis (Weiss, 1989; Konttinen *et al*., 1998), the present findings from the GCF analysis allow the speculation that there existed no significant collagenolytic resorptive activity in the periodontal tissues of the SRA teeth.

The elastic fiber system in the gingival and periodontal tissues include oxytalan, elaunin and elastic fibers, the components of which include elastin and fibrillin (Chavrier *et al*., 1988). Elastin is highly resistant to proteolysis, and under physiological conditions it undergoes minimal turnover. MMP-9 has a capacity to

degrade elastin, whereas intersititial collagenases have virtually no elastolytic activity (Shipley *et al.*, 1996). Another *in vivo* substrate of MMP-9 is α_1-PI, which belongs to the serpin family and is a potent inhibitor of PMN elastase (Liu *et al.*, 2000). By proteolytically inactivating α_1-PI, MMP-9 may thus indirectly contribute to elastin turnover. The functional significance of MMP-9 in the periodontium of SRA teeth may be that the enzyme contributes to physiological remodeling of the elastic-fiber system in order to withstand increased pressure.

3. MMP-1 AND MMP- 8 IN ORTHODONTIC TOOTH MOVEMENT

3.1. Significance of MMP- 8 in periodontal remodeling induced by orthodontic force

The collagenous extracellular matrix of PDL is reorganized in orthodontic tooth movement (reviewed by Sandy *et al.*, 1993; Nakagawa *et al.*, 1994; Karimbux and Nishimura, 1995). Physiological remodeling involves phagocytosis by fibroblasts and collagen degradation by an extracellular MMP-mediated pathway. Since collagenases (MMP-1, MMP-8, and MMP-13) are able to initiate the degradation of native fibrillar collagen types I and II (Weiss, 1989; Konttinen et al., 1998), their involvement in PDL remodeling during periodontitis has been intensively studied (Ingman *et al.*, 1994a, b; Golub *et al.*, 1997; Mancini *et al.*, 1999; Romanelli *et al.*, 1999; Sorsa *et al.*, 1994, 1999; Tervahartiala *et al.*, 2000; Kiili *et al.*, 2002; Kinane *et al.*, 2003; Mäntylä *et al.*, 2003). Results from these studies indicate that the concerted action of these and other MMPs evidently plays a significant role in the initial destruction of PDL collagens.

Recently, increased expression of the MMP-8 gene was demonstrated in the PDL during experimental tooth movement in an animal model (Takahashi *et al.*, 2003). In the present study, the finding that fixed appliance activation significantly elevated MMP-8 levels in GCF of orthodontically treated teeth is in line with that obtained from the experiment in rats and indicates that MMP-8 also functions in human orthodontic tooth movement *in vivo*.

In the GCF samples, multiple forms of immunoreactive MMP-8 were detectable. Immunoreactivities in the range 60 to 80 kD represented the majority of the total MMP-8 immunoreactivity. According to molecular-weight, these immunoreactivities were assumed to represent pro- and active PMN-type enzyme. MMP-8 is synthesized by PMNs during their maturation in bone marrow, stored in specific granules and released by triggered degranulating PMNs at the site of inflammation (Sorsa *et al.*, 1992b; Ding *et al.*, 1995). MMP-8 is activated upon proteolytic cleavage by host-derived tissue and serine proteinases, resulting in a reduction of about 10-20 kD in molecular mass (Kerkelä and Saarialho-Kere, 2003). In the

orthodontic GCF from baseline to one hour, the activated form of MMP-8 was elevated in relation to total PMN-type immunoreactivity from 31% to 79%, indicating increased PMN degranulation and simultaneous activation of MMP-8. The enzyme remained at a high level during the one-month follow-up. During this time period, bands in the range 40 to 55 kD were also found, accounting for 13% of the total MMP-8 immunoreactivity. Bacterial proteinases present in plaque and dental caries lesions can activate and process PMN-type MMP-8 (60-80 kD) to low molecular-weight forms (Sorsa *et al*., 1995; Tjäderhane *et al*., 1998). However, since the SRA patients had no gingival inflammation, and plaque accumulation was minimal, it may be assumed that the 40 to 55 kD immunoreactive forms represented fibroblast-type MMP-8 produced by cells other than neutrophils within the PDL. MMP-8 has recently been shown to originate from gingival and PDL fibroblasts, osteoblasts, osteocytes, sulcular epithelial cells, and odontoblasts (Tervahartiala *et al*., 2000; Kiili *et al*., 2002; Sasano *et al*., 2002; Tsubota *et al*., 2002; Palosaari *et al*., 2003). Thus, MMP-8 may have multiple potential cellular sources during orthodontic tooth movement.

In this study, the GCF samples were collected from different teeth, *i.e*., upper central incisors, upper canines, and lower central incisors undergoing fixed appliance treatment. The orthodontic patients from whom these samples were collected included both children and adults. It has been suggested that there is a delay in the initial response to orthodontic force in adults (Melsen, 1999). This is probably due to biological changes with age such as decrease in bone density and vascularity, and proliferation of PDL cells, which can affect the responsiveness of periodontal tissues to orthodontic forces (reviewed by Sandy *et al*., 1993; Melsen, 1999). In addition, site-specific variations such as tooth structure and alveolar bone structure evidently affect the biomechanical responses within the PDL during orthodontic tooth movement (Melsen, 1999). Based on clinical observation, it seems that the maxillary central incisors are the first teeth to respond when subjected to fixed appliance activation. These interindividual and site-specific differences may thus have had a minor effect on the tissue reaction in the periodontium, and finally the enzyme levels in the GCF.

Animal studies have shown that application of force induces rapid vascular responses in the PDL, dental pulp, and alveolar bone (Davidovitch *et al*., 1988; Vandevska-Radunovic *et al*., 1994). According to one observation, experimental tooth movement leads to significant recruitment of cells belonging to the mononuclear phagocytic system (Vandevska-Radunovic *et al*., 1997). On the pressure side, local overcompression of the PDL results in vascular compression and leads to formation of a necrotic, hyalinized zone, which is a potent inflammatory stimulus.

Invading macrophages function as a part of a general defence mechanism and remove the peripheral areas of the necrotic tissue in the PDL (Rygh *et al*., 1986). Osteoclasts differentiate from the cells within the adjacent bone-marrow spaces to start undermining resorption. This indirect remodeling can be considered a sterile inflammation meant to remove ischemic bone under the hyalinized tissue (Melsen, 1999). Many investigations have shown raised levels of several inflammatory mediators and growth factors such as PGs, IL-1β, IL-6, TNF-α, and EGF in human GCF and in animal periodontal tissues within hours of the application of an orthodontic force (Grieve *et al*., 1994; Uematsu *et al*., 1996; Alhashimi *et al*., 2001; Ren *et al*., 2002). In addition, it has been suggested that susteined orthodontic force provokes neurogenic inflammatory blood flow changes in adjacent periodontal tissues (Davidovitch *et al*., 1988; Vandevska-Radunovic *et al.*, 1998). These studies indicate that the initial phase of orthodontic tooth movement involves many inflammatory-like reactions within the periodontal tissues.

In the present study, the apparent involvement of neutrophils as a major source of MMP-8 indicates that an inflammatory reaction takes place in the periodontium apparatus of the orthodontic patients. In periodontitis, MMP-8 is derived from PMNs when triggered by the periodontopathogenic bacteria or their virulence factors (Sorsa *et al*., 1992b; Ding *et al*., 1995). Since no signs of plaque accumulation or pocket formation were evident among the orthodontic patients in the study sample, bacteria and their virulence factors could not have been involved in triggering neutrophils to produce MMP-8. The evident participation of proinflammatory mediators may thus play a key role in the MMP-8 regulation during orthodontically induced PDL remodeling. The present findings suggest that orthodontic force *per se* causes an inflammatory response within the PDL which leads to secretion of MMP-8 from neutrophils and monocytes/macrophages and its expression from PDL resident cells.

The present study demonstrated that MMP-8 can be detected non-invasively in the GCF during orthodontic treatment. A chairside MMP-8 test has been recently developed to monitor the response to treatment of periodontitis (Sorsa *et al*., 1999; Kinane *et al*., 2003; Mäntylä *et al*., 2003). Future studies may show whether the same principal can be adopted to monitor site-specifically individual teeth during orthodontic tooth movement.

3.2. Possible role of MMP-1 during orthodontic tooth movement

In vitro studies on human gingival and PDL fibroblasts have shown elevated MMP-1 mRNA expression after a 12-hour mechanical stimulation (Bolcato-Bellemin *et al*., 2000), and elevated MMP-1 production after a 4-day cyclic stretch (Carano and

Siciliani, 1996). More recently, increased MMP-1 mRNA production in the gingival tissues of dogs after 3 days of force application was demonstrated during experimental tooth movement (Redlich *et al*., 2001), however, the study did not show evidence on the protein synthesis. In the present study, the majority of MMP-1 Western blots for orthodontically treated teeth failed to show any immunostaining during a period of 1 to 8 hours after fixed appliance activation or during the one-month follow-up, indicating that MMP-1 does not contribute in gingival or periodontal tissue remodeling during human orthodontic tooth movement.

Together with cathepsin K, MMP-1 is involved in degradation of collagenous bone matrix in bone resorption (Deláisse *et al*., 1993; Domon *et al*., 1999). Animal studies have reported that osteoclasts first appear on the pressure side during the initial stage of tooth movement 12 hours to 2 days after force application (Ohba *et al*., 2000). Some bone-specific markers in GCF for the measurement of alveolar bone remodeling such as osteocalcin, proteoglygans, and collagen telopeptide fragments have been evaluated (Giannobile *et al*., 1993; Golub *et al*., 1997; Waddington *et al*., 2001; Wilson *et al*., 2003). In bone resorption, osteoclastic collagenase is not released in the periodontal tissues, but is secreted in the subosteoclastic resorbing compartment (Deláisse *et al*., 1993). From this microenvironment, the diffusion of collagenases at levels possible to be detected in GCF may be unlikely. Unfortunately, the methods for studying biochemical responses in bone during human orthodontic tooth movement *in vivo* are very limited.

CONCLUSIONS

Conclusions of this work are as summarized below:

1. The present family data confirm the earlier suggestions of familial background for short-root anomaly (SRA). The SRA pedigrees showed strong genetic heterogeneity; both autosomal dominant and autosomal recessive modes of inheritance were possible. The sporadic cases may represent fresh mutations.

2. Genetic SRA is a condition with a prevalence close to 1.3% in healthy Finnish young adults, affecting females significantly more often than males, and with a predisposition for the maxillary incisors and premolars and mandibular second premolars to be affected. Therefore, in developing dentitions with bilateral occurrence of short-rooted maxillary central incisors, a careful screening of maxillary premolars, laterals, and mandibular second premolars is highly recommended in orthodontic treatment planning.

3. The results support the suggestion that hypodontia is related to SRA. The developmentally missing teeth are of the same frequency and quality as seen in incisor-premolar hypodontia. In addition, the ectopic maxillary canine appears to be related to SRA. Since SRA teeth are clinically normal, with the crowns being of normal size, in everyday clinical work it is strongly advisable that root length be carefully analyzed from the radiographs. This is particularly important in patients presenting with hypodontia.

4. A characteristic feature of short-rooted teeth was an ongoing gelatinase (MMP-9) activity in their periodontal apparatus. Apparently this reflects the physiological remodeling needed by short-rooted teeth in order to resist increased pressure due to short root structure.

5. The periodontium of SRA teeth, as judged from gingival crevicular fluid (GCF) analysis, had no collagenolytic resorptive or pathological activity. The normal histological appearance of a SRA tooth further supports a developmental etiology for this anomaly.

6. Significantly elevated levels of MMP-8 were found in the GCF of orthodontic patients after fixed appliance activation, indicating that the cells within the periodontium were up-regulated to produce MMP-8 induced by the orthodontic force. Application of orthodontic force quickly (in one hour) enhanced MMP-8 activation.

7. As judged from GCF analysis, MMP-8, possibly produced mainly by neutrophils, plays an key role in the remodeling of the PDL during initial orthodontic tooth movement.

8. According to molecular-weight, some MMP-8 immunoreactivity was presumed to correspond to the non-PMN-type enzyme, possibly produced by resident PDL cells such as fibroblasts. This immunoreactivity was more pronounced during the one-month follow-up.

9. Since MMP-1 was virtually absent, the participation of MMP-1 appears to be rather limited in the periodontal remodeling occurring during initial orthodontic tooth movement.

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