OROFACIAL AND DENTAL ANOMALIES IN HERITABLE COLLAGEN AND FIBRILLIN DISORDERS

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Many thanks to my beloved wife Trui, without whose encouragement, tolerance and unquestioning commitment the present work would never have been brought to a favourable conclusion.
Orofacial and Dental Anomalies in Heritable Collagen and Fibrillin Disorders

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Dankwoord
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- Letters to the Editor:


2/ As abstracts published in journals or abstract books


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Chapter I

Introduction

I. 1 CONNECTIVE TISSUE: COLLAGEN AND FIBRILLIN PROTEINS
I. 2 HERITABLE DISORDERS OF CONNECTIVE TISSUE
I. 3 OROFACIAL STRUCTURES AND EXTRACELLULAR MATRIX PROTEINS
I.1 Connective Tissue: Collagen and Fibrillin Proteins

I.1.1 Definition and Structural Aspects

The term ‘connective tissues’ embraces a large number of soft and mineralized tissues, such as skin, mucosa, joint ligaments, muscle, basement membranes (soft tissues), bone, cartilage, and dentin (mineralized tissues). All connective tissues are composed of cells imbedded in a highly organised extracellular matrix (ECM). The ECM is built up from a large number of different components. These include both structural proteins (e.g. collagens, fibrillin, fibronectin) and non-structural materials, such as proteoglycans. These molecules are synthesised by chondrocytes, fibroblasts, epithelial cells, and a variety of other specialised cell types, such as dentinoblasts and cementoblasts. The structure of the ECM varies with the specific function of the tissue: for example, basement membranes form a sheet-like network providing support, growth and differentiation of the cells (e.g. muscles and ligaments), whereas the matrix of bone becomes calcified and provides rigidity and strength, allowing bones to support weight [Nuytinck, 1999].

In the present study collagen and fibrillin will be studied, and more in particular the clinical and histological manifestations in the orofacial structures resulting from deficiency of these structural proteins.

I.1.2 Collagens

I.1.2.1 Definition and Biosynthesis

The collagens comprise a diverse family of structurally related ECM proteins, having a diverse set of functions involving structural, developmental (organogenesis, cell adhesion, cell movement) and physiological (wound healing, hemostasis) processes. The large number of collagen types with their different structures and defined tissue distribution reflects this variety of functions. The collagen proteins, although structurally related, are genetically and biochemically distinct with each collagen type having a characteristic tissue distribution [Chapman, 1989]. So far, 19 different types of collagen have been identified, encoded by at least 33 genes, dispersed over the human genome. As the structure and especially the biological role of some uncommon collagen types are being elucidated, the classification is continuously being adapted [Kuivaniemi et al., 1997].

All collagens share similar biosynthetic pathways (Figure I.1). All collagen types consist of three polypeptide chains (known as α-chains or procollagen chains) twisted into a triple helix, which go on to form various supramolecular structures in the ECM. Collagen molecules containing three identical polypeptide chains are called homo-trimers (e.g. type III collagen), whereas collagen molecules, composed
of two or three different α-chains, are known as hetero-trimers (e.g. types I and V collagen). To distinguish one collagen type from another, vertebrate collagens were assigned Roman numerals in order of discovery (I, II, III, etc.). Individual polypeptide chains are called α1, α2, α3 etc. followed by, in parentheses, the collagen type in which they occur. For example, Type I collagen (collagen I), which consists of two identical α1-chains and one α2-chain, is designated as [α1(I)]2[α2(I)]. Each type of α-chain is encoded by a different gene. In collagen I, for example, COL1A1* codes for the α1-chains [OMIM Entry 120150], and COL1A2* for the α2-chain [OMIM Entry 120160] [Online Inheritance in Man; http://www3.ncbi.nlm.nih.gov/Omim]. As for collagen I, COL1 indicates the type of collagen, whereas A1/A2 stand for the α1/α2-chains being encoded.

* By international convention, human gene symbols are written in italicized capital letters and Arabic numerals [HUGO Gene Nomenclature Committee; http://www.gene.ucl.ac.uk/nomenclature].

After glycosylation of some of the amino acids, the triple helix starts to fold. Once the protein has folded, it is secreted from the cell as a procollagen molecule. After secretion, specific enzymes in the extracellular matrix remove both the N- and COOH terminal propeptide ends and give rise to the collagen molecule. These molecules associate into thick fibrils, which, in turn, assemble to form a meshwork. Both the thickness of the fibrils and the meshwork organization are dependent on the function and the mechanical properties of the tissue.

Figure I.1  Biosynthesis of Type I collagen in fibroblasts (or odontoblasts). Collagen I molecules (heterotrimer) are built up from two identical α1- and one α2-procollagen-chains, which are encoded by two different genes, i.e. COL1A1 (α1) and COL1A2 (α2). Collagen I molecules associate into fibrils in order to form a structural framework.
I.1.2.2 Function, Distribution and Genetics

The group of fibrillar collagens represent the most abundant and important proteins in the human body, and comprises collagen types I, II, III, V and XI. Table I.1 displays the function, distribution and genetics of the most prevalent fibrillar collagen types (I, III and V). Each type has been shown to form fibrils on its own, and some types appear to associate with another type of fibrillar collagen to form a heterotypic fibril [Birk et al., 1988]. Collagen V is associated with type I and III collagens in non-cartilagenous tissues, and is believed to control fibrillogenesis of its ‘associated’ collagen types [Niybizi & Eyre, 1994].

Table I.1 Distribution, function, genes and clinical manifestations of deficiency of the major fibrillar collagens (types I, III and V)

<table>
<thead>
<tr>
<th>Type</th>
<th>Distribution</th>
<th>Function</th>
<th>Gene*</th>
<th>Major manifestations of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>Bone, muscle, ligaments, skin</td>
<td>Provides structural framework, strength and resilience</td>
<td>COL1A1</td>
<td>Brittle bones, joint hypermobility, hearing loss, cardiac pathology</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>COL1A2</td>
<td></td>
</tr>
<tr>
<td>Collagen III</td>
<td>Bone, ligaments, skin, blood vessel walls</td>
<td>Scar tissue formation, transition to mineralization, elasticity</td>
<td>COL3A1</td>
<td>Vessel wall rupture, organ ruptures</td>
</tr>
<tr>
<td>Collagen V</td>
<td>Bone, muscle, ligaments, skin</td>
<td>Regulation of fibril formation of collagen I</td>
<td>COL5A1</td>
<td>Hyperextensible skin, increased tissue fragility</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>COL5A2</td>
<td></td>
</tr>
</tbody>
</table>

*Collagen I and V are heterotrimers; collagen III is a homotrimer

I.1.2.3 Collagen Deficiency

Abnormal collagen biosynthesis results in deficient production of collagen molecules. This may be caused either by mutation in the genes encoding the different procollagen parts (e.g. COL1A1 or COL1A2 for collagen I), or in the genes coding for enzymes that are essential to extracellular synthetic pathways (e.g. proteases, removing the N- or -COOH terminal propeptide ends). As a result, abnormal collagen molecules are secreted into the extracellular matrix. These abnormal molecules fail to associate into fibrils of normal shape and thickness and, hence, to organize into (structural) frameworks. The pathological expression, however, is entirely dependent on both the collagen type and the tissue (Table I.1).

I.1.3 Fibrillin

I.1.3.1 Definition

Fibrillin is a cysteine-rich glycoprotein that exists in three homologous forms [Keene et al., 1991], of which fibrillin-1 [OMIM Entry 134797] and fibrillin-2 [OMIM Entry 121050], respectively encoded by the FBN1 gene on 15q21 and the
FBN2 gene on 5q23 [Lee et al., 1995] are the best characterized. Fibrillin-1 provides the major structural (i.e. load-bearing and limiting expansive tissue growth) function of microfibrils [Dietz & Pyeritz, 1995; Dietz & Pyeritz, 2001], whereas expression of fibrillin-2 directs the assembly of elastic fibers during early embryogenesis [Zhang et al., 1995].

1.1.3.2 Function, Distribution and Genetics

Table I.2 displays the distribution of fibrillin in the human body. Although fibrillin is known to mainly provide soft tissues with elasticity, these properties may vary along with the tissue.

**Table I.2** Distribution, function and clinical manifestations of deficiency of fibrillin (miscellaneous for types I and II)

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Function</th>
<th>Clinical manifestations of mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periosteum</td>
<td>Restraint of skeletal growth</td>
<td>Skeletal overgrowth, scoliosis</td>
</tr>
<tr>
<td>Eye lens</td>
<td>Elastic lens suspension</td>
<td>Ectopia lentis</td>
</tr>
<tr>
<td>Cartilagenous tissue</td>
<td>Growth modelling</td>
<td>Crumbled ears</td>
</tr>
<tr>
<td>Heart valves</td>
<td>Tissue elasticity</td>
<td>Mitral valve prolapse</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Tissue elasticity</td>
<td>Arterial dilatation / dissection</td>
</tr>
<tr>
<td>Lung tissue</td>
<td>Tissue elasticity</td>
<td>Spontaneous pneumothorax</td>
</tr>
<tr>
<td>Muscle</td>
<td>Tissue elasticity</td>
<td>Hypotonia</td>
</tr>
<tr>
<td>Joint ligaments</td>
<td>Joint stability during function</td>
<td>Hypermobility, dislocations</td>
</tr>
</tbody>
</table>

1.1.3.3 Fibrillin Deficiency

Mutations in FBN1 or FBN2 result in fibrillin deficiency, i.e. abnormal fibrillin molecules are secreted in the extracellular matrix. Clinical manifestations are widespread and may involve the skeletal, ocular, cardiovascular and pulmonary systems, muscle, skin and integumentum, hence reflecting the distribution of fibrillin in periosteum, eye lens, vessel walls, lamina propria of the skin, joint ligaments etc. (Table I.2).

1.2 Heritable Disorders of Connective Tissue

1.2.1 Definition

The term 'heritable disorders of connective tissue' (HDCT) was first introduced by McKusick [1950] to encompass a group of conditions involving connective tissue. These were reported to be inherited as a mendelian trait (single gene defect),
in contrast with the entirely different group of ‘acquired disorders of connective tissue’. The latter group, including rheumatoid arthritis, systemic lupus erythematosus, scleroderma, dermatomyositis and vasculitides, were known to occur as a familial, though non-mendelian trait, and to have a varying etiology. McKusick’s first compilation included the Marfan syndrome, osteogenesis imperfecta, Ehlers-Danlos syndrome, pseudoxanthoma elasticum, and gargoylism. Subsequently, other disorders, such as achondro- and hypochondroplasia, Alport syndrome, epidermolysis bullosa etc., have been added, and much has been learned about their clinical variability and genetic heterogeneity. Most of them are now understood at the resolution of nucleotides in their gene mutation, and have been studied extensively by use of transgenic mice.

As mutations can occur in different parts of the gene and in different ways (base pair substitution, deletion, insertion, duplication etc.), it is clear that combinations may result in a variety of protein expression. Moreover, allelic variants (i.e. different DNA sequences on the same chromosome locus as a result of natural mutation, such as e.g. the color of the eyes) may add to the complexity of the expression [Jorde et al., 2000]. For instance, 62 allelic variants have been reported for mutation in COL1A1 (a1-chain) on 17q21.31-q22 [OMIM Entry 120150], and 51 variants for mutation in COL1A2 (a2-chain) on 7q22.1 [OMIM Entry 120160]. Although resulting from mutation in the same genes, the expression and the severity of the clinical manifestations may vary considerably among these variants [Dalgleish, 1997; Dalgleish, 1998; www.le.ac.uk/ genetics/ collagens].

I.2.2. Heritable Disorders of Connective Tissue (HDCT) Presenting with Oral Manifestations

Few HDCT have yet been reported in association with oral manifestations. A number of dental and craniofacial features have been related to Osteogenesis Imperfecta (OI) [OMIM Entry 166220], a heterogeneous group of disorders caused by mutation in COL1A1 or COL1A2. Four OI types have been designated, sharing brittle bones with major skeletal deformity, blue eye sclerae and joint laxity as most important clinical manifestations (Table I.3) [Sillence, 1988].

Dental manifestations in association with OI have been termed dentinogenesis imperfecta (DI), and are characterized by an opalescent tooth discoloration, short roots, progressive pulp obliteration and extensive wear of the enamel, all of which present in both the deciduous and the permanent dentition (see also Chapter VII.3.1) [Rao & Witkop, 1971; Levin, 1980]. Dentin ultrastructure in OI has been shown to be abnormal, even in teeth that appear clinically unaffected (see also Chapter VII.5) [Witkop & Rao, 1971; Sillence et al., 1979a; Sillence et al., 1979b; Waltimo et al., 1996]. Deformity of the craniofacial bones has been reported to result in Class III malocclusion (70-80%) with a high incidence of cross bites and/or open bites [Schwartz & Tsipouras, 1984; O’Connell & Marin, 1999]. Aberrant dental development [Malmgren & Norgren, 2002] and multiple
mandibular cysts have been documented in various cases [Jones et al., 1993; Vorast et al., 2000]. The oral manifestations in patients with OI can be considered either as the direct consequence of the monogenic defect in one of the collagen I genes, or as the result of a complex interaction between genes (collagen genes and genetic modifiers) and environmental factors.

Table 1.3 Classification and clinical characteristics of Osteogenesis Imperfecta (OI) [after Sillence, 1988]

<table>
<thead>
<tr>
<th>OI Type</th>
<th>Clinical features</th>
<th>Inheritance*</th>
<th>DI**</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal or mild short stature, blue sclerae, hearing loss</td>
<td>AD yes</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Severe osseous fragility, stillbirth or neonatal death</td>
<td>AD no</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Very short stature, progressive bone deformity, blue sclerae, hearing loss</td>
<td>AD/AR yes</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Variable short stature, mild bone deformity, normal sclerae, hearing loss</td>
<td>AD yes</td>
<td></td>
</tr>
</tbody>
</table>

* AD autosomal dominant; AR autosomal recessive
** DI dentinogenesis imperfecta

Apart from OI, few other associations of ECM protein deficiency and oral abnormalities have been reported. However, since both collagens and fibrillin make up an important part of ECM proteins of the orofacial structures (i.e. craniofacial skeleton, alveolar bone and periosteum, masticatory muscles, pulpdentin complex, temporomandibular joint components, periodontium and mucosae), it is most likely that deficiency of one of these proteins may produce oral manifestations with high diagnostic specificity. The Ehlers-Danlos syndromes (EDS) and Marfan syndrome (MFS) are the most obvious diseases to be studied as models of disease with maximum expression of deficiency of respectively collagen (EDS) and fibrillin (MFS).

1.2.3 Ehlers-Danlos Syndromes: State-of-the-Art Review

1.2.3.1 Definition

The Ehlers-Danlos syndromes (EDS) comprise a heterogeneous group of heritable disorders of connective tissue, commonly characterized by articular hypermobility, skin hyperextensibility, and increased tissue fragility (Figure 1.2). The clinical features, modes of inheritance, and molecular causes differ according
to the type. Most of EDS types are caused by mutations in genes coding for different types of collagen (types I, III or V), or for enzymes essential to collagen biosynthesis. The presence of one or more major criteria is necessary for clinical type diagnosis and warrants laboratory confirmation whenever possible (Table I.4).

![Figure I.2](image)

Figure I.2  
Cardinal skin and joint features of Ehlers-Danlos syndromes: hyperextensible skin (A), joint hypermobility (B) and increased tissue fragility with extensive scarring (C)

I.2.3.2 Classification Systems and Prevalence

At present, two classifications of EDS are commonly used, with the more recent taxonomy being based on recent developments in the elucidation of the biochemical and molecular bases of EDS. Classification of EDS began in the late 1960s [Beighton, 1970; McKusick VA, 1972], and in 1986 the so-called Berlin-nosology was proposed to formalize the nomenclature of the various types known at that time [Beighton et al., 1988]. As displayed in Table I.5, more than ten subconditions were delineated according to differences in clinical features, modes of inheritance, and biochemical defects.

![Table I.4](image)

Table I.4  
Diagnostic specifications of most prevalent Ehlers-Danlos syndromes

<table>
<thead>
<tr>
<th>Type</th>
<th>Major criteria</th>
<th>Minor criteria</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>Skin hyperextensibility</td>
<td>Smooth, velvety skin</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Tissue fragility</td>
<td>Molluscoid pseudotumors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Joint hypermobility</td>
<td>Muscle hypotonia</td>
<td></td>
</tr>
<tr>
<td>Hypermobility</td>
<td>Skin hyperextensibility</td>
<td>Recurrent joint dislocations</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Severe joint hypermobility</td>
<td>Chronic joint/ limb pain</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>Thin, translucent skin</td>
<td>Small joint hypermobility</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Arterial/ intestine rupture</td>
<td>Tendon/ muscle rupture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extensive bruising</td>
<td>Pneumothorax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Facial dysmorphism</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In 1997, a simplified classification of EDS into six major types was proposed [Beighton et al., 1998]. The guiding principle in formulating the classification was its usefulness to the ‘generalist’. For each type major and minor diagnostic criteria, respectively with high and low diagnostic specificity, were defined (Table I.5). As a rule of thumb, six clinical features are commonly shared (with varying expression and, thus, varying diagnostic weight) by the different types: skin hyperextensibility, generalized joint hypermobility, easy bruising, tissue fragility, mitral valve prolapse and proximal aorta dilatation, and chronic joint and limb pain. The molecular basis of EDS types is displayed in Table I.6.

Table I.5  Current classifications of Ehlers-Danlos syndromes (EDS).
(New classification : Villefranche nosology - Beighton et al., 1998; Former classification : Berlin nosology - Beighton et al., 1988)

<table>
<thead>
<tr>
<th>New Type</th>
<th>Former Type</th>
<th>OMIM*</th>
<th>Inheritance**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical type Gravis (EDS type I)</td>
<td>Mitis (EDS type II)</td>
<td>130000</td>
<td>AD</td>
</tr>
<tr>
<td>Hypermobility type Hypermobile (EDS type III)</td>
<td>Arterial-echymotic (EDS type IV)</td>
<td>130020</td>
<td>AD</td>
</tr>
<tr>
<td>Vascular type Arterial-echymotic (EDS type IV)</td>
<td>Ocular-scoliotic (EDS type VI)</td>
<td>225400</td>
<td>AR</td>
</tr>
<tr>
<td>Kyphoscoliosis type Arthrodysplasia multiplex (EDS types VIIA and VIIB)</td>
<td>Human dermatosparaxis (EDS type VIIC)</td>
<td>225410</td>
<td>AR</td>
</tr>
<tr>
<td>Dermatosparaxis type Human dermatosparaxis (EDS type VIIC)</td>
<td>Other forms X-linked EDS (EDS type V)</td>
<td>305200</td>
<td>XL</td>
</tr>
<tr>
<td>Other forms Periodontitis type (EDS type VIII)</td>
<td>Fibronectin-deficient type (EDS type X)</td>
<td>130080</td>
<td>AD</td>
</tr>
<tr>
<td>Other forms Familial hypermobility syndrome (EDS type XI)</td>
<td>Progeroid EDS</td>
<td>130070</td>
<td>?</td>
</tr>
</tbody>
</table>

* OMIM (Online Mendelian Inheritance in Man) Entry : www.ncbi.nlm.nih.gov/OMIM; ** AD autosomal dominant; AR autosomal recessive; XL sex linked

The most recent classification (Villefranche Nosology) [Beighton et al., 1998] discriminates classical EDS [OMIM Entry 130000-130010], hypermobility EDS [OMIM Entry 130020], vascular EDS [OMIM Entry 130050], kyphoscoliosis EDS [OMIM Entry 225400], arthrodysplasia EDS [OMIM Entry 130060], and dermatosparaxis EDS [OMIM Entry 225410].

In terms of prevalence, hypermobility EDS is assumed to occur in 80%, classical EDS in 10%, whereas the other types are assigned a lower frequency [Beighton et al., 1998]. Although there is conflicting evidence in the literature concerning the prevalence of the distinct EDS types, estimates of approximately 1:10,000-20,000 for classical EDS type (former EDS types I and II), and 1:100,000 for vascular EDS type (former EDS type IV) have been reported in various studies [McKusick VA, 1972; Gorlin et al., 1990a]. The actual prevalence, however, may be
greater as diagnostic skills at the molecular/biochemical level are steadily improving.

**Table I.6  Molecular basis of Ehlers-Danlos syndromes** [after Beighton et al., 1998]

<table>
<thead>
<tr>
<th>Type</th>
<th>Cause</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>Structural defects in pro?1(V) or pro?2(V) chains of collagen type V</td>
</tr>
<tr>
<td></td>
<td>(COL5A1 and COL5A2 genes)</td>
</tr>
<tr>
<td>Hypermobility</td>
<td>Currently unknown collagen type</td>
</tr>
<tr>
<td>Vascular</td>
<td>Structural defects in pro?1(III) chain of collagen type III</td>
</tr>
<tr>
<td></td>
<td>(COL3A1 gene)</td>
</tr>
<tr>
<td>Kyphoscoliosis</td>
<td>Deficiency of lysyl hydroxylase (PLOD) (homozygosity or compound</td>
</tr>
<tr>
<td></td>
<td>heterozygosity for mutant PLOD alleles)</td>
</tr>
<tr>
<td>Arthrochalasia</td>
<td>Mutations leading to deficient processing of the amino-terminal</td>
</tr>
<tr>
<td></td>
<td>end of pro?1(I) (type A) or proA2(I) (type B) chains of collagen type</td>
</tr>
<tr>
<td>Dermatosparaxis</td>
<td>Mutations in ADAMTS2-gene, leading to deficient processing of</td>
</tr>
<tr>
<td></td>
<td>collagen type I</td>
</tr>
</tbody>
</table>

**I.2.4  Marfan Syndrome: State-of-the-Art Review**

**I.2.4.1  Definition and Prevalence**

The Marfan syndrome (MFS) [OMIM Entry 154700] is a heritable multisystem disorder with a variable phenotype. Table I.7 summarizes the MFS characteristics. Until the late 1990s, the diagnosis of the Marfan syndrome has relied completely on clinical criteria [McKusick, 1972], codified in 1986 in the so-called Berlin Nosology [Beighton et al., 1988]. Since molecular [Dietz et al., 1993] and therapeutic [Shores et al., 1994] developments have further enhanced the need for reliable, uniform diagnostic criteria, De Paepe and co-workers [1996] proposed revised diagnostic criteria based largely on a combination of major and minor clinical manifestations in the various organ systems, and on the nature of the family history. Table I.8 summarizes the pleiotropic features of the Marfan syndrome. Multiple organ systems are affected, with most features being age-related. There is an important distinction between a ‘major’ criterion, that carries high diagnostic specificity, being present in a system, and the system being ‘involved’. ‘Minor’ clinical manifestations, that occur occasionally in the disorder, are given a more specific nuance in scoring the ‘affected’ systems [De Paepe et al., 1996]. Kainulainen and co-workers [1990] localized the gene causing MFS to 15q21, providing assurance that the gene encoding the microfibrillar protein fibrillin-1 (FBN1) was causal. This finding enabled scientists to make a distinction between a number of clinically identical ‘marfanoid’ conditions, such as e.g. familial mitral
valve prolapse syndrome, congenital contractural arachnodactyly, and Stickler syndrome. Transmission of MFS is in autosomal dominant fashion [Dietz et al., 1993; De Paepe et al., 1996].

Table I.7 Characteristics of Marfan syndrome

<table>
<thead>
<tr>
<th>Multisystem disorder affecting tissues with elastic properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal overgrowth with elongated limbs</td>
</tr>
<tr>
<td>Structural defects of major artery walls</td>
</tr>
<tr>
<td>Generalized joint hypermobility</td>
</tr>
</tbody>
</table>

Autosomal dominant mode of inheritance

Caused by mutation in \( FBN1 \) gene

Deficient biosynthesis of fibrillin-1

Prevalence estimated at about 3-4:10,000

New mutation in about 1:4 cases

Fibrillin is a major component of these elastin-associated fibrils, which are widely distributed in the extracellular space. Because the distribution of microfibrils corresponds closely to the tissues affected in MFS, their deficiency is believed to account for the striae of the skin, pulmonary bullae, dural ectasia [Hollister et al., 1990; Tsipouras et al., 1994], and even skeletal overgrowth [Dietz & Pyeritz, 1995; Westling & Mohlin, 1996].

Table I.8 « Ghent » diagnostic criteria for the Marfan Syndrome (MFS)
[after De Paepe et al., 1996]

<table>
<thead>
<tr>
<th>Somatic system / Family history</th>
<th>Major criteria *</th>
<th>Minor criteria **</th>
<th>System involvement ***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeleton</td>
<td>Pectus deformity, long limbs, scoliosis</td>
<td>Joint hypermobility, high palate, retrognathia, dolichocephaly</td>
<td>At least two major criteria, or one major plus two minor criteria</td>
</tr>
<tr>
<td>Eye</td>
<td>Eye lens subluxation</td>
<td>Flat cornea, hypoplastic iris</td>
<td>At least two minor criteria</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Dilatation and/or dissection of the ascending aorta</td>
<td>Mitral valve prolapse, dilatation and/or dissection of thoracic aorta</td>
<td>At least one minor criterion</td>
</tr>
<tr>
<td>Lungs</td>
<td>None</td>
<td>Spontaneous pneumothorax</td>
<td>Minor criterion</td>
</tr>
<tr>
<td>Skin and integumentum</td>
<td>None</td>
<td>Striae atrophicae, recurrent herniae</td>
<td>At least one minor criterion</td>
</tr>
<tr>
<td>Dura</td>
<td>Lumbosacral dural ectasia</td>
<td>None</td>
<td>Major criterion</td>
</tr>
<tr>
<td>Family history</td>
<td>Mutation in, or haplotype around ( FBN 1 )</td>
<td>None</td>
<td>At least one of the major criteria</td>
</tr>
</tbody>
</table>

* Major criterion: symptom with high diagnostic specificity; ** Minor criterion: symptom with low diagnostic specificity

*** A somatic system can be considered involved on a combination of major and/ or minor criteria
Prevalence estimates of MFS vary from 5-8:100,000 [Gorlin et al., 1990b] to 3-4:10,000 in the general population [Dietz & Pyeritz, 2001]. Individuals with Marfan syndrome have been identified in many ethnic, racial and geographic groups [De Paepe et al., 1986].

I.2.4.2 Clinical Aspects and Diagnosis

Although involvement of the skeletal, ocular, and cardiovascular system is often responsible for the most prominent clinical manifestations, other organ systems may also display abnormalities, including the skin, fascia, lungs, dura, skeletal muscle, and adipose tissue [Pyeritz, 2000b].

The symptoms are numerous and frequently are age-related. The ocular features of MFS include severe myopia and eye lens subluxation (ectopia lentis), found in over 60% of MFS patients. Skeletal abnormalities comprise skeletal overgrowth (tall stature) with elongation of the extremities (dolichostenomelia), fingers and toes (arachnodactyly), a long and narrow skull (dolichocephaly), scoliosis, and pectus deformities [De Paepe et al., 1996] (Figure I.3). Generalized joint hypermobility and muscle hypotonia may be present in a majority of MFS patients. Dilatation of the ascending aorta, with or without aortic regurgitation, involving the sinuses of Valsalva, mitral valve prolapse, dilatation of the main pulmonary artery in the absence of valvular or peripheral pulmonary stenosis below the age of 40, and calcification of the mitral annulus below the age of 40, may be present in MFS [Pyeritz & McKusick, 1979]. Neuropsychologic impairment, including learning disability and attention deficit disorder, still need broader evaluation before inclusion into the minor criteria.

Figure I.3 Skeletal and joint features of Marfan syndrome: tall stature with elongated limbs (A), arachnodactyly (B), and joint hypermobility (C: knee hyperextension).

MFS is serious largely because of its cardiovascular complications, which cause 95% of deaths in patients with the condition and reduce life expectancy by up to 40% [Shores et al., 1994; Putnam et al., 1995]. The leading cause of premature
death in MFS is progressive dilatation of the aortic root and ascending aorta with resultant aortic incompetence and dissection [Gray & Davies, 1996]. Mitral valve disease may be the earliest cardiovascular manifestation of MFS, and may progress to cause significant mitral insufficiency in a majority of patients. The average life expectancy of individuals with MFS, however, has risen significantly throughout the past decades [Silverman et al., 1995], due mainly to improved management of the cardiovascular complications, including beta-adrenergic blockade [Shores et al., 1994], routine imaging of the aorta, and prophylactic surgical replacement of the aortic root [Gott et al., 1999].

The diagnosis of MFS relies primarily on clinical criteria as defined in the so-called Ghent Nosology [De Paepe et al., 1996]. The phenotypes of affected individuals form a continuum of severity and include many features such as scoliosis and mitral valve prolapse that are relatively common in the general population. Other connective tissue disorders share features with MFS and may give rise to diagnostic dilemmas. The Ghent Nosology attempts to address these difficulties by defining major criteria with high diagnostic specificity and minor criteria with less specificity. To make the clinical diagnosis, a constellation of findings including major criteria in two organ systems and the involvement of a third organ system are required. Table I.8 displays the “Ghent” diagnostic criteria for involvement of the different somatic systems [De Paepe et al., 1996]. Laboratory confirmation consequently may be given by means of mutational analysis of \textit{FBN1}. From literature, it is known that mutations in \textit{FBN1} can be established in about 60% of MFS using standard screening tests (conformation sensitive gel electrophoresis and single stranded conformation polymorphism). Recently it was shown that this can be extended by up to 90% by using combinations of the latter tests and denaturing high performance liquid chromatography, direct DNA sequencing and Southern blot analysis [De Paepe et al., 2004].

Reported subtypes of MFS comprise severe classical form, mild variable form, atypical and neonatal form, with numerous types of mutations being mapped for each subtype [OMIM Entry 154700]. Other type-1 fibrillinopathies [OMIM Entry 134797] include familial ectopia lentis, familial aortic aneurysm, and marfanoid skeletal syndrome, all of which conditions share skeletal overgrowth, joint laxity, and structural alterations of the aortic root wall as common features.

### I.3 Orofacial Structures and Extracellular Matrix Proteins

#### I.3.1 Mineralized Orofacial Connective Tissues

Table I.9 displays the distribution of the most prevalent extracellular matrix (ECM) proteins in orofacial structures. Collagen I makes up the bulk of the ECM proteins of the mineralized orofacial connective tissues, i.e. bone [Nanci et al., 2003a], dentin [Nanci, 2003] and cementum [Nanci et al., 2003b]. Although collagen
V is known to be associated with collagen I in non-cartilagenous tissue [Niyibizi & Eyre, 1994], it is unclear whether its presence is of any decisive structural importance in bone. Synthesis and secretion of collagens in these tissues is expressed respectively at osteoblast, odontoblast or cementoblast level. Deposition of minerals is initiated at specific telodomain locations on the surface of collagen I molecules, resulting in calcification of the respective matrices [Ten Cate et al., 2003a].

From a histopathological point of view, collagen deficiency may be expressed at either or both the level of tissue growth and development, or of (maintainance of) tissue capacities, including regeneration processes [Ten Cate et al., 2003]. This may be reflected clinically as morphological aberrations or as alterations of the mechanical tissue properties.

Table I.9 Distribution of major organic components in orofacial structures

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>Craniofacial skeleton, TMJ ligaments and disc, mucosa, periodontium, dentin, pulp</td>
</tr>
<tr>
<td>Collagen III</td>
<td>Pulp, distal ligaments of TMJ, predentine matrix</td>
</tr>
<tr>
<td>Collagen V</td>
<td>Mucosa, periodontium, TMJ ligaments, dentin, pulp</td>
</tr>
<tr>
<td>Fibrillin</td>
<td>Craniofacial periosteum and perichondrium, distal ligaments of TMJ, intramaxillary stroma</td>
</tr>
</tbody>
</table>

I.3.2 Soft Orofacial Connective Tissues

As for mineralized tissues, collagen I is the most prevalent ECM protein in soft orofacial connective tissues, i.e. periosteum [Nanci et al., 2003a], mucosa [Squier et al., 2003], gingiva and periodontal ligament [Nanci et al., 2003b], masticatory muscles, temporomandibular joint (TMJ) ligaments and disk [Ten Cate, 2003], and the dental pulp [Nanci, 2003]. Collagen III is found in dental pulp, predentine matrix [Chiego, 2001], distal TMJ ligaments [Gage et al., 1990], and in mucosal/gingival scar tissue [Ten Cate et al., 2003]. Collagen V co-distributes with both collagen types I and III in the former tissues [Niybizi & Eyre, 1994]. As for mineralized connective tissues, collagen deficiency may be expressed clinically at either or both the level of tissue growth and development, or of (maintainance of) tissue capacities, including regeneration processes. In soft connective tissue, however, this may present as increased tissue fragility, or as alterations of the mechanical and regenerative [Ten Cate et al., 2003] tissue properties.
In the orofacial region, fibrillin is found in maxillary bone stroma, periosteum [Giganti et al. 1996, Giganti et al., 1999a], dental pulp [Berkovitz et al., 2002a], and distal TMJ ligaments [Berkovitz et al., 2002b]. Within the framework of this study, the expression of fibrillin deficiency is being investigated in the (growth of the) craniofacial skeleton, the periodontium, dentin-pulp complex, and the TMJs. Since some of these soft tissues are closely associated with growth and development of mineralized tissues, e.g. periosteum with bone [Giganti et al., 1999a], structural aberrations in those soft tissues may produce morphological abnormalities in the associated mineralized tissues as well.

1.3.3 Is Oral Health at Risk in Heritable Disorders of Connective Tissue?

From the formerly described associations between ECM proteins and the form and function of connective tissues, it is clear that deficiency of these proteins may produce morphological/functional abnormalities in the orofacial region. This may be expressed as clinical manifestations with a high diagnostic specificity in a number of orofacial structures.

As a consequence, some of these structural/functional tissue alterations may predispose to either or both an early development or increased liability to infectious (decay, inflammation and breakdown of periodontal tissues) and/or functional (muscle or disk disorders of the TMJs) oral disease. Previous studies reported that in OI with DI, there may be clinical problems in affected teeth (fractures, abscesses) due to structural alterations of dentin. At present, however, no such data are available on other heritable disorders of collagen and fibrillin.
Chapter II

Aims of the Study

II.1 INTRODUCTION
II.2 OROFACIAL STRUCTURES AND ORAL HEALTH
II.3 AIMS
II.4 APPROVAL
Patients affected with inherited disorders of the connective tissues constitute a large and heterogeneous group, sharing multisystem expression of the molecular anomaly as a common characteristic. The clinical symptoms result from an altered metabolism of specific extracellular matrix (ECM) proteins, such as collagen and fibrillin, often leading to major alterations of tissue architecture and of tissue responses to mechanical and/or infectious influences. Deficiency of these proteins also affects the structural properties of connective tissues, such as strength and elasticity, causing malformation and/or increased liability for dysfunction of a number of organ systems. In general, the spreading of clinical symptoms is accepted to reflect the distribution of the deficient ECM proteins throughout the body and organ systems.

The clinical expression of the causal deficiency, however, appears to be strictly determined by (1) the type of the affected ECM protein, with regard to the distribution and the nature of the symptoms, and (2) by the type of mutation. The latter association may be illustrated by the great variety in distribution and gravity of symptoms and in prognosis that presents with Osteogenesis Imperfecta (OI), a heterogeneous group of inherited disorders of the connective tissues comprising four major types with distinct clinical and molecular features. Although all OI types result from mutation in the genes encoding the a1- (COL1A1) or a2-chain (COL1A2) of procollagen I, both the number, nature and management of clinical symptoms, as well as life expectancy, may vary considerably along with the disease type. In each of four OI types, different mutation types can be assessed that are believed to account for the phenotypic heterogeneity [Dalgleish, 1997].

Since ECM proteins make up the bulk of orofacial structures (craniofacial skeleton and alveolar bone, temporomandibular joints, teeth, and oral soft tissues), deficiency of these proteins may result in a variety of clinical expressions in the orofacial region. By analogy with general medical aspects of inherited disorders of the connective tissues, orofacial symptoms may be highly specific as to the molecular cause of the disease, and may probably compromise oral health. However, literature on these topics is yet sparse. Hence, it may be of interest to analyse the clinical effects of deficiency of the major ECM proteins on oral health and on the structure and function of orofacial structures in conditions that result from deficiency of these proteins.

The null hypothesis of this study stated that patients affected with inherited deficiency of collagens or fibrillin do not present orofacial symptoms with (high) diagnostic specificity, and that oral health in these patients is not compromised.

To test the null hypothesis, two populations affected with EDS (caused by deficiency of either collagen I, III or V according to EDS subtype) on the one hand, or MFS (caused by fibrillin deficiency) on the other, were selected for study.

II.2 Orofacial Structures and Oral Health
II.2.1 Clinical Symptoms in Orofacial Structures

Analyzing dental defects and/or clinical symptoms in the orofacial region can be extremely helpful in making differential diagnosis in diseases with similar phenotypes, or in diagnosing carriers of autosomal dominant disorders. With respect to inherited disorders of the connective tissues, this has extensively been studied for Osteogenesis Imperfecta (OI) [Levin, 1978; Levin, 1980; Schwartz et al., 1984; Dean et al., 1984; Lukinmaa et al., 1987a; Lukinmaa et al., 1987b; Levin et al., 1988; Lygidakis et al., 1996]. Classification of OI into a subgroup with (A-subtype) and another without oral manifestations (B-subtype) has been based on the presence of dentinogenesis imperfecta (DI) [Sillence et al., 1979; Sillence, 1981].

At present, a great need is felt to integrate oral findings in clinical diagnostics of EDS and MFS. Literature on oral manifestations of these syndromes, however, is sparse. In addition, conflicting evidence exists as to the diagnostic specificity of a number of oral symptoms presenting in patients affected with EDS or MFS. Some of these symptoms may be directly related to the molecular cause of the disease, whereas others may be secondary to the condition, i.e. resulting from interaction with environmental factors (predisposing to development of disease, dysfunction, or influencing growth processes). In view of refining differential diagnostics, it is of great importance to assess the diagnostic specificity of these clinical symptoms.

In conclusion, a first objective of the present study was to analyse the orofacial manifestations of patients affected with EDS or MFS.

II.2.2 Oral Health

As reported in populations with OI, some aspects of oral health may be compromised as a result of the underlying collagen I deficiency. Although in OI periodontal disease and caries experience have been reported to be comparable to controls, extensive enamel wear, abnormal tooth development (delayed or accelerated) and subsequent dental malocclusion, and mandibular cysts in some patients may lead to functional oral problems and/or oral disease [O’Connell & Marini, 1999; Malmgren & Norgren, 2002].

At present, no data are available on oral health in patients affected with EDS or MFS. Since a large number of these patients are at risk for cardiovascular complications, and restitution of oral connective tissue after infectious disease may be seriously hampered, it is important to assess the different aspects of oral health in these populations. As a result, preventive measures should be adjusted to their medical condition, and tissue responses should be anticipated (see also Chapter II.2.3).
From clinical experience, it is clear that oral health may be at risk in patients with EDS or MFS due to a probably increased liability to the development of infectious oral disease and of dysfunction of the masticatory system. Recent advancements in oral genetics yielded a multifactorial disease model (diagram B), that, compared to the former model (diagram A), permitted the functional interrelations of gene products with each other and with environmental factors to be better understood. At present, however, no epidemiological data are available as to the prevalence and the development of oral infection in EDS and MFS. An analysis of these data would be a first step towards a better understanding of the functional interrelation of etiologic factors in infectious disease affecting these patients.

Scheme: Evolution of insights into the functional interrelation of etiological factors contributing to development of oral infectious disease [adapted after: Hart, 2004]. Disease expression is indicated by shaded overlap area.

In conclusion, a second objective of the present study was to assess different aspects of oral health in patients affected with EDS or MFS, as compared to unaffected controls.

II.2.3 Therapeutic Guidelines

Since it was hypothesised that a number of epidemiological-clinical and morphometrical findings might be highly specific to the syndromes in focus, some practical considerations compelled to formulate guidelines for dental examination and treatment of patients with deficiency of collagen or fibrillin.

First, assessment of oral symptoms with high diagnostic specificity in EDS or MFS may allow for an early diagnosis and timely treatment, both of which medical acts can be of vital importance to the affected subject. Hence, it may prove useful to formulate clinical guidelines for the outlook for affected persons.
Second, it should be assessed whether a number of tissue responses and precautions should be anticipated when considering dental treatment of a patient with any known deficiency of collagen or fibrillin. This not only may considerably enhance dental treatment outcome, but also should enable the clinician to avoid often life-threatening complications.

In conclusion, a third objective of the present study was to formulate therapeutical guidelines to be integrated in dental treatment strategies of patients affected with deficiency of collagen (EDS) or fibrillin (MFS).

II.2.4 **Ultrastructural Analysis of Dental Hard Tissues**

Histological analysis of dentin matrix has been proposed as a useful adjunctive tool in diagnosing DI as a symptom of OI [Lukinmaa et al., 1987a; Waltimo, 1994; Lindau et al., 1999]. It was shown that ultrastructural morphological aberrations might be found in any teeth from patients affected with OI, even in those that appear clinically unaffected [Waltimo et al., 1996].

Consequently, and since collagen I makes up the bulk of dentin, it can be assumed that also in types of collagen I deficiency different from OI, structural abnormalities may be found in dentin. Recently, some ‘new’ types of collagen I deficiency, resulting in phenotypes different from OI, have been described [Nusgens et al., 1992; Nuytinck et al., 2000]. Clinical and/or histological data on dentinal involvement in these types, however, are still lacking. From a comparative histological analysis of dentin from patients with the presently known types of collagen I deficiency, one could expect to learn how these different gene defects, which each in different ways account for production of abnormal collagen I molecules, are expressed during dentinogenesis. It would be of great interest to see if any diagnostic specificity could be attributed to morphological characteristics of dentin in these types of collagen I deficiency.

In conclusion, a fourth objective of the present study was to assess the value of ultrastructural analysis of dentin as a diagnostic tool for the determination of collagen I deficiency.

II.3 **Aims of the Study**

According to the null hypothesis, which states that patients affected with deficiency of collagen or fibrillin do not present orofacial manifestations with (high) diagnostic specificity, and that oral health in these patients is not compromised, the following aims of the study can be postulated:

1. **Analysis of the orofacial (oral soft tissues, dental hard tissues, craniofacial skeleton, and temporomandibular joints) manifestations of patients affected with EDS or MFS.**
2. Assessment of oral health (caries experience, periodontal health, condition of the oral soft tissues, and temporomandibular disorders) in patients affected with EDS or MFS.

3. Formulation of therapeutical guidelines for dental treatment of patients affected with EDS or MFS.

4. Assessment of the value of ultrastructural analysis of dentin as a diagnostic tool for the determination of collagen I deficiency.

II.4 Approval

This doctoral research project was presented to the Faculty Board of the Faculty of Medicine, Ghent University, and approved on May 7, 2002.

The study design, protocols and consenting forms had previously been approved by the Ethics Committee of the Ghent University Hospital on January 22, 2001 as projects nos. 2000/308 and 2000/309.

The study in part was sustained by grant nr. B/05088 from the Special Research Fund of Ghent University, Section IV.1.
Chapter III

Oral Manifestations and Oral Health In Ehlers-Danlos Syndromes

Expression of Collagen Deficiency in Oral Structures of Patients Affected with Ehlers-Danlos Syndromes

ABSTRACT

III.1 INTRODUCTION

III.2 AIMS

III.3 MATERIAL AND METHODS

III.4 RESULTS

III.5 DISCUSSION

III.6 GUIDELINES FOR DENTAL TREATMENT

III.7 CONCLUSION

Part of this chapter has been accepted as:


1 Prof. dr. Anne de Paepe (MD, PhD) is affiliated to the Centre for Medical Genetics, Ghent University Hospital
Abstract

Alterations in the molecular structure of collagen, caused by mutation in the encoding genes, result in altered mechanical properties of the connective tissues. This may interfere with processes of growth and development as well as with the function and regenerative capacities of the orofacial tissues. To analyze the impact of collagen deficiency on oral health, Ehlers-Danlos syndromes (EDS) were selected as a model of disease. The EDS comprise a heterogenous group of heritable disorders of connective tissue, characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. Most EDS types are caused by mutations in genes encoding different types of collagen or enzymes, essential for normal processing of collagen.

Oral health was assessed in 31 subjects with EDS (16 with hypermobility EDS, 9 with classical EDS and 6 with vascular EDS), including caries experience, alterations of the dental hard tissues, oral mucosa and periodontium, and was compared to matched controls (n=49).

The overall mean dmf/DMF-s score (P=0.001), mean Plaque Index (P<0.001), and mean pocket depth (P=0.002) were significantly higher in EDS than controls. Caries experience was not dependent on EDS type. There was an increased liability for soft tissue pathology and a rapid progression of periodontal disease in a majority of EDS subjects. Periodontal treatment needs were high (CPITN = III in at least one sextant) in 62% of patients, especially in hypermobility EDS. This could be related to a high Plaque Index and high Gingival Index. In these cases, restraint joint mobility of the wrists was found to influence brushing habits and brushing frequency. Among subjects reporting of increased mucosal fragility (74% of EDS population), a low brushing frequency, high Plaque Index, and high dmf/DMF-s-score proved significantly interrelated.

Pathological manifestations of the dental hard tissues were found to be significantly dependent on EDS type. Abnormal pulp shape presented exclusively in classical EDS (P<0.001), and pulp calcification was found in both hypermobility and classical EDS (P=0.004). Further investigation of larger samples, however, is needed to validate the diagnostic specificity of these findings.

On the basis of these findings, it was concluded that oral health may be compromised in patients affected with EDS. When considering dental treatment in EDS, a number of tissue responses (mucosa, periodontium, pulp) and precautions (TMJ dislocation) should be anticipated.
III.1 Introduction

Collagens make up the bulk of extracellular matrix components in the orofacial structures (see also Chapter 1.2.4). Alterations in the molecular structure of the different collagen types, caused by mutation in the responsible genes, result in altered mechanical properties of the connective tissues. This may interfere with processes of growth and development as well as with the function and regenerative capacities of the tissues. As a result, oral health may be considerably compromised.

Previously, oral health in subjects affected with collagen I deficiency was assessed in a population with Osteogenesis Imperfecta (OI) [O’Connell & Marini, 1999; Malmgren & Norgren, 2002]. A large number of studies on oral manifestations of OI mainly documented on dentin defects (dentinogenesis imperfecta) in the deciduous and permanent dentition as a symptom of OI (Schwartz & Tsipouras, 1984; Lukinmaa et al., 1987; Levin et al., 1988; Aldred, 1992; O’Connell & Marini, 1999; Stephen & Beighton, 2002; Malmgren & Norgren, 2002). In order to assess the impact on oral health of mutations in other ‘major’ fibrillar collagen types (III and V) and in molecules that are important for posttranslational processing of these collagens, Ehlers-Danlos syndromes (EDS) were selected as model of disease. The EDS comprise a heterogenous group of heritable disorders of connective tissue, characterized by joint hypermobility, skin hyperextensibility, and tissue fragility. The clinical features, modes of inheritance, and molecular bases differ according to the type. EDS are caused by a genetic defect causing an error in the synthesis or processing of collagen types I, III or V. The distribution and function of these collagen types are displayed in Table III.1.

Table III.1 Distribution and function of the major fibrillar collagens

<table>
<thead>
<tr>
<th>Collagen type</th>
<th>Function</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen I</td>
<td>Providing functional tissues with a structural framework</td>
<td>Bone, muscle, ligaments, skin, mucosae, organs</td>
</tr>
<tr>
<td></td>
<td>Initialization of mineralization in calcified tissues</td>
<td></td>
</tr>
<tr>
<td>Collagen III</td>
<td>Scar formation</td>
<td>Bone, ligaments, skin, organs, vessels</td>
</tr>
<tr>
<td></td>
<td>Transition to late mineralization stage in calcified tissues</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Providing elastic properties to vessel walls</td>
<td></td>
</tr>
<tr>
<td>Collagen V</td>
<td>Regulation of fibril formation of collagen I</td>
<td>Bone, muscle, ligaments, skin</td>
</tr>
</tbody>
</table>

At present, two classifications of EDS are commonly used of which the most recent taxonomy is largely based on recent developments in the elucidation of the biochemical and molecular bases of the disorder [Beighton et al., 1988; Beighton et al., 1998]. As displayed in Table III.2, more than ten subconditions were delineated according to differences in clinical features, modes of inheritance,
and biochemical defects. Six clinical features are commonly shared with varying expression and varying diagnostic weight by the different types: skin hyperextensibility, generalized joint hypermobility, easy bruising, tissue fragility, mitral valve prolapse, proximal aorta dilatation, and chronic joint and limb pain. The most recent classification discriminates the classical type [OMIM Entry 130000-130010] [Online Mendelian Inheritance in Man, http://www3.ncbi.nlm.nih.gov/OMIM], hypermobility type [OMIM Entry 130020], vascular type [OMIM Entry 130050], kyphoscoliosis type [OMIM Entry 225400], arthrochalasia type [OMIM Entry 130060], and dermatosparaxis type [OMIM Entry 225410] as chief conditions of EDS (Table IV.2). In terms of prevalence, hypermobility EDS type is thought to occur in 80% of EDS; classical type in 10%; whereas the other types are assigned a lower frequency. Although there is conflicting evidence in the literature concerning the prevalence of the distinct EDS types, estimates of approximately 1:15,000-25,000 for classical EDS type (former EDS types I and II), and 1:100,000 for vascular EDS type (former EDS type IV) have been reported in various studies [Gorlin et al., 1990; Pyeritz, 2000]. The actual prevalence, however, may be greater as diagnostic skills at the molecular/ biochemical level are steadily improving.

Table III.2  
Current classifications of Ehlers-Danlos syndromes (EDS)

<table>
<thead>
<tr>
<th>New 'Villefranche' classification</th>
<th>Former 'Berlin' classification</th>
<th>OMIM°</th>
<th>Inheritance°°</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical type</td>
<td>Gravis (EDS type I)</td>
<td>130000</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Mitis (EDS type II)</td>
<td>130010</td>
<td>AD</td>
</tr>
<tr>
<td>Hypermobility type</td>
<td>Hypermobile (EDS type III)</td>
<td>130020</td>
<td>AD</td>
</tr>
<tr>
<td>Vascular type</td>
<td>Arterial-ecchymotic (EDS type IV)</td>
<td>130050</td>
<td>AD</td>
</tr>
<tr>
<td>Kyphoscoliosis type</td>
<td>Ocular-scoliotic (EDS type VI)</td>
<td>225400</td>
<td>AR</td>
</tr>
<tr>
<td>Arthrochalasia type</td>
<td>Arthrochalasis multiplex (EDS types VIIA and VIIB)</td>
<td>130060</td>
<td>AD</td>
</tr>
<tr>
<td>Dermatosparaxis type</td>
<td>Human dermatosparaxis (EDS type VIIIC)</td>
<td>225410</td>
<td>AR</td>
</tr>
<tr>
<td>Other forms</td>
<td>X-linked EDS (EDS type V)</td>
<td>305200</td>
<td>XL</td>
</tr>
<tr>
<td></td>
<td>Periodontitis type (EDS type VIII)</td>
<td>130080</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Fibronectin-deficient type (EDS type X)</td>
<td>225310</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>Familial hypermobility syndrome (EDS type XI)</td>
<td>147900</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Progeroid EDS</td>
<td>130070</td>
<td>?</td>
</tr>
</tbody>
</table>

* 'Villefranche' classification (2)
** 'Berlin' classification (1)
° OMIM Entry (Online Mendelian Inheritance in Man) : www3.ncbi.nlm.nih.gov/OMIM (3)
°° AD autosomal dominant, AR autosomal recessive, XL sex linked

At present, a small number of reports on oral health and oral features in EDS is available in literature. During the 1960s, the first clinical and histological observations on the oral manifestations of EDS were published. Oral manifestations of nine unclassified EDS patients included fragility of the oral mucosa with delayed healing, microdontia, short or malformed roots, large pulp stones and calcification of the pulp, bleeding after tooth brushing, and periodontal disease [Barabas & Barabas, 1967]. Under light microscopy, absence of scaloping at
the dentinoenamel junction, vascular inclusions in dentin, and fibrous degeneration of the pulp were scattered findings in a sample of teeth from six unspecified EDS patients. It was concluded that these features were caused by abnormal collagen formation [Barabas, 1969].

Table III.3 Characteristics of most prevalent EDS types

<table>
<thead>
<tr>
<th>Type</th>
<th>Major criteria</th>
<th>Minor criteria</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical</td>
<td>Skin hyperextensibility</td>
<td>Smooth, velvety skin</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Tissue fragility</td>
<td>Molluscoid pseudotumors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Joint hypermobility</td>
<td>Muscle hypotonia</td>
<td></td>
</tr>
<tr>
<td>Hypermobility</td>
<td>Skin hyperextensibility</td>
<td>Recurrent joint dislocations</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Severe joint hypermobility</td>
<td>Chronic joint/limb pain</td>
<td></td>
</tr>
<tr>
<td>Vascular</td>
<td>Thin, translucent skin</td>
<td>Small joint hypermobility</td>
<td>AD</td>
</tr>
<tr>
<td></td>
<td>Arterial/intestine rupture</td>
<td>Tendon/muscle rupture</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extensive bruising</td>
<td>Pneumothorax</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Facial dysmorphism</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

During the last decades, a number of case reports have documented oral [Gosney, 1987; Hoff, 1977; Slootweg & Beemer, 1987; Sadeghi et al., 1989; Welbury, 1989; Leung et al., 1989; Fridrich et al., 1990; Pope et al., 1992; Bond et al., 1993; Melamed et al., 1994], temporomandibular [Goodman & Allison, 1969; Sacks et al., 1990; McDonald & Pogrel, 1996; Miller et al., 1997; Norton & Assael, 1997], and histological [Ooshima et al., 1990; Pope et al., 1992] findings in patients with often unclear (clinical) EDS type diagnosis. The diverse oral features comprised microdontia, supernumerary teeth, partial anodontia, yellowish tooth discoloration, easy gingival bruising, root deformity, and often generalized pulp calcification (Table III.4). Subluxation of the temporomandibular joints was reported as a frequent finding. Aggressive periodontal disease (early-onset periodontitis) was reported in hypermobility EDS (former EDS type III) [Reichert et al., 1999] and in periodontitis EDS (former EDS type VIII) [Perez et al., 2002; Rahman et al., 2003]. In some of these patients, however, a genotype overlap between vascular EDS (former EDS type IV) and periodontitis EDS can not be excluded. Defective dentinogenesis, pulp and root deformities have been reported in classical EDS type (former type I) [Pope et al., 1992]. Ultrastructural and immunohistochemical examination demonstrated an abnormal pattern of collagen fiber network in pulp and dentin in a primary tooth of a patient with (former) EDS type VII [Ooshima et al., 1990], and absence of collagen type III in dentin in EDS type I [Pope et al., 1992].

The number of investigated subjects, however, is very small and most reports lack a clear EDS type diagnosis (Table III.4). Furthermore, there is no clear information on whether the reported features are age-related or may be confounded by the conciding presence of other inherited oral traits. As a
consequence, the diagnostic weight of a number of these reported oral findings can be questioned.

Table III.4  Oral manifestations in EDS - review of literature

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>EDS type</th>
<th>Cases</th>
<th>Age</th>
<th>Oral observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barabas GM et al.</td>
<td>1967</td>
<td>?</td>
<td>9</td>
<td>1.5m - 49yrs</td>
<td>Microdontia, short roots, pulp stones</td>
</tr>
<tr>
<td>Hoff M</td>
<td>1977</td>
<td>?</td>
<td>1</td>
<td>18 yrs</td>
<td>pulp calcification</td>
</tr>
<tr>
<td>Gosney MB</td>
<td>1987</td>
<td>II (classical)</td>
<td>1</td>
<td>14 yrs</td>
<td>pulp calcification, stunted roots, peg teeth</td>
</tr>
<tr>
<td>Slootweg PJ et al.</td>
<td>1987</td>
<td>VIII (periodontitis)</td>
<td>1</td>
<td>7yrs</td>
<td>early onset periodontitis</td>
</tr>
<tr>
<td>Leung AKD et al.</td>
<td>1989</td>
<td>?</td>
<td>1</td>
<td>4m</td>
<td>&quot;abnormal&quot; gingiva</td>
</tr>
<tr>
<td>Welbury RR</td>
<td>1989</td>
<td>?</td>
<td>1</td>
<td>9yrs</td>
<td>pulp calcification, short roots</td>
</tr>
<tr>
<td>Sadeghi EM et al.</td>
<td>1989</td>
<td>?</td>
<td>1</td>
<td>11yrs</td>
<td>pulp calcification, root deformity</td>
</tr>
<tr>
<td>Fridrich KL et al.</td>
<td>1990</td>
<td>III (hypermobility)</td>
<td>1</td>
<td>30yrs</td>
<td>gingival bruising, TMJ dislocation</td>
</tr>
<tr>
<td>Pope FM et al.</td>
<td>1992</td>
<td>I (classical)</td>
<td>2</td>
<td>12yrs</td>
<td>pulpcalcification, root deformity</td>
</tr>
<tr>
<td>Bond PJ et al.</td>
<td>1993</td>
<td>VIII (periodontitis)</td>
<td>1</td>
<td>8yrs</td>
<td>early onset periodontitis</td>
</tr>
<tr>
<td>Melamed Y et al.</td>
<td>1994</td>
<td>III (hypermobility)</td>
<td>2</td>
<td>21yrs / ?</td>
<td>supernumerary teeth</td>
</tr>
</tbody>
</table>

At present, there are no clear epidemiological data concerning oral manifestations in the ‘general’ EDS population. Moreover, the recent insights into the molecular basis of the distinct EDS types compel one to question the validity of the previous reports, since patient selection may have been considerably biased in the absence of clear diagnostic criteria. This means, inclusion of patients affected with phenotypically EDS-related conditions (e.g. benign joint hypermobility syndrome) may have confounded the research outcome, as it may become apparent from the conflicting evidence in literature.

III.2  Aim of the Study

The aim of this study was to analyze the different manifestations in the orofacial region and to assess oral health in a population consisting of the three most prevalent EDS types (i.e. classical, hypermobility and vascular EDS). The null hypothesis stated that oral health is not compromised in patients with congenital deficiency of collagen. In addition, therapeutical guidelines for dental treatment were formulated.

III.3  Material & Methods

III.3.1  Patient Selection

An invitation for participation to the study was communicated to the Belgian Association of Patients Affected with Ehlers-Danlos Syndromes. Forty-eight subjects volunteered and consented to be examined at the Ghent University Hospital, Belgium. The study group comprised thirty-one patients (n=31) who were diagnosed according to the above mentioned criteria [Beighton et al., 1998] at the Centre for Medical Genetics, Ghent University Hospital, Belgium. The EDS
subtype distribution was as follows: sixteen subjects had hypermobile EDS (former EDS type III), nine had classical EDS (former types I and II) and six had vascular EDS (former type IV). Mean age of the study group was 28 ± 15.3 yrs (age range: 4 to 61 yrs), and gender distribution was 35% males to 65% females. Forty-nine healthy subjects (n=49), presenting for general dentistry purposes and without a history of cardiovascular, endocrine, haematological or infectious diseases, were enrolled in a control group. The control subjects were matched for age, sex and dental attendance (on ordinal scale: attending regular recalls once or twice a year; attending periodontal check-ups twice or more year; only in case of emergency). Mean age of the control population was 29.2 ± 15.9 yrs (age range: 5 to 62 yrs), and sex distribution was 39% males to 61% females.

III.3.2 Methods

All subjects were examined and interviewed by the same trained investigator (PDC), using standardized criteria as recommended by the WHO’s report on oral health surveys [WHO, 1997]. The oral assessment included the following sections: general medical information, extra-oral information, temporomandibular joint (TMJ) assessment, oral mucosa, enamel opacities/hypoplasia, caries experience and oral cleanliness, periodontal status, loss of gingival attachment, frequency of visiting the dental office, and prosthetic status [WHO, 1997]. In view of analyzing previously reported phenomena in the syndrome, the WHO oral assessment was extended with structured queries on TMJ dislocation and the condition of the oral mucosa (including occurrence, initiating factors and management of increased mucosal fragility). Panoramic radiographs and bite-wings (taken with standardized Rinn-device) were examined for anomalies of tooth number, shape and structures.

Assessment of general medical status included registration of EDS type and acute and chronically administered medication. Extra-oral information comprised clinical assessment of extra-oral ulcerations, swellings, sores, erosions, scars or fissures.

Signs and symptoms of temporomandibular disorders (TMD) were recorded during clinical examination and patient interview, based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) [LeResche, 1992; Widmer, 1992]. Results are discussed in Chapter VII - Generalized joint hypermobility and temporomandibular disorders.

The condition of the oral mucosa was assessed by recording evidence of increased mucosal fragility (bruises, grazes, regenerative patches, or ulcerations) at different locations of the lining (floor of the mouth, underside of the tongue, inside of the lips, soft palate, cheeks, and alveolar processes) and masticatory mucosa (hard palate and gingiva). Bruises were defined as dark coloured (red to blueish-grey) mucosal swellings, not related to dental/periodontal pathology or neoplasmata, scar tissue, or hyperpigmentation. Grazes were defined as
circumscribed, dark red or purple coloured areas, usually displaying exposure of capillary vessels, indicated by the presence of small red points. Regenerative patches were defined as circumscribed spots, either of white, grey or brown colour, usually with a paler outline and reflecting recent bruising and/or erosion with deficient wound healing. These patches should be distinguished from abscesses and ulcerations (aphthous, herpetic or traumatic), and mucosal conditions with a more durable character such as leukoplakia, lichen planus and candidiasis. A positive history of recurrent mucosal trauma was required to confirm the clinical diagnosis of increased mucosal fragility. In these cases, the occurrence (daily; once or more a week; less), initiating factors (mastication; brushing teeth; consumption of hot, spicy or crusty foods or candy; spontaneously), and individual management (nihil; mouth rinses; topical gels; professional help) of the lesions were scored on an ordinal scale.

Enamel abnormalities were classified into three types on the basis of their appearance: demarcated opacities, diffuse opacities, and hypoplasia [FDI, 1992]. Caries experience was assessed according to the World Health Organisation (WHO) [WHO, 1997; http://www.whocolab.od.mah.se] and the British Association for the Study of Community Dentistry (BASCD) guidelines, using the dmft/DMF Index, recording decay at the D₃ diagnostic threshold [Pitts & Fyffe, 1988; Fyffe et al., 2000]. The dmft/DMF Index [Klein et al., 1938] is the most commonly used criterion to validate an individual's experience with dental decay, and can be expressed either at tooth (t) or at tooth surface (s) level. It is a sum score index composed by the numbers of decayed (d/D), missing (m/M) and filled (f/F) teeth or tooth surface per individual. In deciduous teeth, the dmft Index (indicated in small font) is used, whereas permanent teeth are scored by means of the DMF Index (in capitals). The D₃ threshold is the conventional diagnostic threshold, scoring caries at the level of cavitation [Mitropoulos & Pitts, 1993] and/or at level 2 or higher of approximal radiolucency (radiolucency in enamel up to enamel-dentine junction or deeper) [Kidd et al., 1996]. Data were obtained under artificial light by using a mouth mirror, a WHO/CPITN type E probe, cotton rolls, and bite-wings after professional mechanical tooth cleaning [Mitropoulos & Pitts, 1993; WHO, 1997]. White spots and pit-and-fissure sealants were not scored. Exclusion was made of third molars and teeth extracted for orthodontic purposes. The dental care level was expressed as F/ (D+F) (Restorative Index) [Jackson, 1973]. Visual presence of plaque was scored on tooth surface level using Silness & Löe's Plaque Index system (PII) [Lang, 1998]. The periodontal status was assessed by use of two indicators: Löe & Silness' Gingival Index system (GI) [Lang, 1998] and probing depth of periodontal pockets. A blunt WHO/CPITN type E probe with an end diameter of 0.5mm was used for examination of the tooth surfaces and gingival attachment [Mitropoulos et al., 1992; WHO, 1997; Lang, 1998]. Information on loss of gingival attachment was collected by probing pockets depths of all teeth except third molars, permitting comparison between population groups [WHO, 1997]. Probing pocket depth was not recorded for children under the age of 14 years. Periodontal treatment needs were indicated on a scale of 0 to III on the basis of the CPITN score [Ainamo et al., 1982; Lang, 1998]. The impact of dental attendance,
brushing frequency, the patient’s physical condition, and the condition of the oral mucosa on oral care and oral hygiene habits was evaluated by recording qualitative data from the patients.

The examinator (PDC) was calibrated at baseline and inter-examiner agreement was tested for dmf/ DMF-s score using Kappa statistics (Cohen’s Kappa or ?) (? = 0.78 ; 95% CI 0.69 - 0.87). Inter-examiner agreement was tested for probing pocket depth (? = 0.96; 95% CI 0.92 - 1.01), and scoring gingival inflammation (Silness & Loë’s Gingival Index) (? = 0.83; 95% CI 0.74 - 0.92), and dental plaque (Silness & Loë’s Plaque Index) (? = 0.88; 95% CI 0.80 - 0.96). These Kappa values stand for an acceptable interreliability with an experienced examiner as a benchmark [Clayton & Hill, 1993; Petrie & Sabin, 2000].

Statistics were performed with Fischer’s exact test and chi-square test for comparison of proportions, Mann-Whitney U-test (unpaired Wilcoxon test) to compare means between two groups, one-way analysis of variance (ANOVA) to analyze the effect of qualitative factors on continuous variables, and rank correlation analysis (Spearmann’s correlation coefficient) and regression analysis to describe the relationship between two continuous variables. Differences at the P<0.05 level were considered statistically significant (Clayton & Hill, 1993; Petrie & Sabin, 2000).

III.4 Results

III.4.1 Medical Status

Limb and/or joint pain was reported in 67.7% of EDS population, manifesting in an acute or chronic (circadian) pattern. Chronic TMJ pain was a frequent finding in over 50% of EDS. All subjects with limb or joint pain were >18 years of age, and no gender difference was found with regard to pain report (P=1.0). Chronical administration of non-steroidal anti-inflammatory drugs was recorded in 29% of EDS population to relieve chronic limb and joint pain. In 48.4% of EDS population, chronic pain was recorded in the presence of mild to severe restraint of physical activities. There was no difference in reporting of restricted mobility between males and females (P=0.456). No differences were found between EDS types in frequency of reports of pain (P=0.285) or restraint joint mobility (P=0.441).

III.4.2 Caries Experience

The mean d/ D-s, m/ M-s, f/ F-s, and dmf/ DMF-s scores in EDS (n=31) were significantly higher than controls (n=49) (P=0.001) (Table III.5). A more detailed analysis of caries data among EDS and controls as a function of age category (Table III.5 and Figure III.1) revealed that EDS subjects aged 0-17 yrs (n=9) showed significantly higher dmf/ DMF-t(-s) scores than contemporaneous
controls (respectively $P=0.019$ and $P=0.006$). D-scores in this age group were significantly higher both on tooth ($P<0.001$) and surface ($P=0.011$) level. Among subjects aged 18 years and older ($n=22$), there were no significant differences in DMF-t/-s scores as compared to controls. D-scores, however, showed significantly different, both on tooth ($P=0.008$) and surface ($P=0.022$) level. There was a similar significant increase in caries experience, both on tooth and surface level, with increasing age among both EDS ($n=31$) and controls ($n=49$). Individual DMF-t/-s scores were not different among EDS types ($P=0.085$).

The mean dental care index value $[(F/D+F)-t/-s]$ in EDS ($n=31$) was situated around 0.80, yielding no statistically significant difference compared to controls ($n=49$). In all EDS age categories, care levels were acceptable to good and varied from 0.74 to 0.80 (surface level). With respect to dental attendance in EDS, attending regular dental recalls (once or twice a year) was recorded in 58%, attending periodontal check-ups (twice or more a year) in 6%, whereas 36% reported to visit the dentist only in cases of emergency (pain, loss of filling).

There was no association between individual dmf/DMF score and dental attendance ($P=0.632$), brushing habits ($P=0.424$), and reports of recurrent mucosal erosions ($P=0.247$) in EDS.

**Figure III.1** Caries experience (dmf/DMF) in EDS ($n=31$) and controls (Ctrl, $n=49$) as a function of age category

**III.4.6 Oral Cleanliness**

The mean plaque index in EDS was $2.3 \pm 0.6$, which was significantly different from controls (i.e. $1.7 \pm 0.6$) ($P<0.001$). No difference was found between EDS types. There was no correlation between the individual plaque index and...
frequency of tooth brushing (P=0.524), or reports of oral hygiene being influenced by the general condition (P=0.160) in EDS population. The dmf/DMF-scores were significantly higher in EDS subjects with a high plaque index (rank correlation analysis; \( r_s = 0.3; P=0.042 \)), which was in contrast with controls (\( r_s = 0.467; P=0.067 \)). This finding could be related to the presence of complaints of chronic (limb) pain (P<0.001) and/or restraint joint mobility of the wrists (P<0.001), resulting in poor oral hygiene.

IV.4.7 Periodontal Status

The mean Gingival Index (GI) in EDS population was 1.2 ± 0.4. No significant difference was found between EDS and controls (i.e. 1.2 ± 0.7) (P=1.0) or among EDS types (P=0.486). Both in EDS and controls, GI was found to increase with increasing age.

The mean pocket depth in EDS was 3.1 ± 0.9, which was significantly different from controls (i.e. 2.5 ± 0.8) (P=0.002). No difference could be established between EDS types (P=0.093). A CPITN = III in at least one sextant, indicating a high periodontal treatment need (improvement of oral hygiene and professional scaling needed), was recorded in 62% of EDS subjects. CPITN was significantly different among EDS types (P=0.047), i.e. a high CPITN was most frequent in hypermobility EDS, and less frequent in vascular EDS.

Rank correlation analysis (Spearman’s correlation coefficient) yielded significantly different interrelations between the presence of dental plaque and gingival inflammation (respectively Plaque Index and Gingival Index), gingival inflammation and mean pocket depth, and dental plaque and mean pocket depth between EDS and controls. Regression analysis showed the mean individual pocket depth to be correlated with age in both EDS and controls, but yielded a significantly different interrelation between gingival inflammation (Gingival Index) and age in both groups (Table III.8).

IV.4.3 Oral Mucosa

Increased mucosal fragility was recorded in 74% of EDS population, without any significant difference between EDS types (P=0.752). Local evidence of
mucosal fragility (mostly bruises and regenerative patches) was present in 96% at two or more locations at the alveolar processes, in 78% at the hard palate, in 4% at the soft palate, and in 35% at the cheeks. In 52% a combination of at least four locations was recorded, without any significant difference between locations at the lining or the masticatory mucosa (P=0.238). The occurrence of bruising/grazing events was recorded in 61% as once or more a day, in 26% once or more a week, and in 13% less than once a week. The most prevalent initiating factors were mastication of food in 4%, brushing teeth in 9%, and a combination of both in 87%. In 52% of EDS population, regenerative patches appeared as pale red, circumscribed spots at locations with a high incidence of bruises and/or grazes, and hence might be interpreted as clinical manifestations of prolonged and deficient tissue healing (Figure III.2).

Subjects with increased mucosal fragility were found to have adopted special brushing techniques (P=0.024), although brushing less frequently (P=0.041). Both their mean Plaque Index (P=0.024) and dmf/DMF-score were significantly higher (P=0.041). There was no difference in dental attendance between subjects with and without increased mucosal fragility in EDS (P=0.465).

### III.4.4 Abnormalities of the Dental Hard Tissues

No abnormalities in tooth number were recorded in EDS population. Demarcated enamel opacities were found in 9.7% of EDS population (3:31). These defects exclusively presented in permanent premolars and could be related to carious processes of the deciduous dentition (local infectious etiology). No

<table>
<thead>
<tr>
<th>Variable X</th>
<th>Variable Y</th>
<th>Significancea</th>
<th>EDS (n=31)</th>
<th>controls (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Plaque Index</td>
<td>Mean Gingival Index</td>
<td>0.847°</td>
<td>&lt;0.001°</td>
<td></td>
</tr>
<tr>
<td>Mean Gingival Index</td>
<td>Mean probing depth</td>
<td>0.107°</td>
<td>&lt;0.001°</td>
<td></td>
</tr>
<tr>
<td>Mean Plaque Index</td>
<td>Mean probing depth</td>
<td>0.424°</td>
<td>0.015°</td>
<td></td>
</tr>
<tr>
<td>Mean Gingival Index</td>
<td>Age</td>
<td>0.868°°</td>
<td>&lt;0.001°°</td>
<td></td>
</tr>
<tr>
<td>Mean probing depth</td>
<td>Age</td>
<td>0.002°°</td>
<td>&lt;0.001°°</td>
<td></td>
</tr>
</tbody>
</table>

* P ≤ 0.05 were considered statistically significant
° Rank correlation analysis (Spearman’s correlation coefficient)
°° Single regression analysis
abnormal tooth crown morphology was recorded. Root deformity was recorded in 6.4% of overall EDS population (not significant when compared to controls). The presence of abnormal pulp shape (29% of EDS group) was found to be dependent on EDS type (P<0.001), i.e. exclusively presenting in classical EDS, and showed significantly different from controls (P<0.001). Demarcated calcification of the pulp (pulp stones) was recorded in 19% of hypermobile EDS, in 78% of classical EDS, and was absent in vascular EDS (Figure III.3A-B and Table III.7). This was found to be significantly different from controls (P=0.002) and showed dependent on EDS type (P=0.004).

**Figure III.3**

Root deformity (A) and abnormal pulp shape with progressive obliteration and pulp stones (B) are frequent findings in classical EDS, and may present in both dentitions.

![Figure III.3](image)

**Table III.7  Prevalence of dentin defects in EDS population (n=31)**

<table>
<thead>
<tr>
<th>RX Manifestations</th>
<th>Classical EDS (n=9)</th>
<th>Hypermobility EDS (n=16)</th>
<th>Vascular EDS (n=6)</th>
<th>Significance P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root deformity</td>
<td>2 (22%)</td>
<td>0</td>
<td>0</td>
<td>0.119</td>
</tr>
<tr>
<td>Abnormal pulp shape</td>
<td>9 (100%)</td>
<td>0</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pulp calcification</td>
<td>7 (78%)</td>
<td>3 (19%)</td>
<td>0</td>
<td>0.002</td>
</tr>
</tbody>
</table>

* Considered significant at P<0.05 (Chi-square test)

**III.4.5  Power Analysis**

Techniques measuring the statistical power of a study estimate the probability that a study could establish a statistically significant difference if a
specified true-size difference actually exists in the larger population. The consequence of using small sample sizes like in the present study, however, is that the sample sizes may vary widely from one sample to another and from the true numerical value in the large population. As a result, in small sample sizes the probability is quite great that one will fail to show a statistically significant difference when a true difference actually exists (Type II error or β). In these cases, a 20% (β=0.20), or even greater risk (up to 25%) may be accepted that one will fail to demonstrate a statistically significant difference even when a true difference exists in the larger populations. The value 1−β is called the power of the test. A high power means a low chance of a type II error, or, alternatively, a large chance of detecting significantly a particular result. In general terms, the more discrepant a reality is from the null hypothesis (i.e. the greater the effect is in the population), the greater is the power of the significance test [Daly & Bourke, 2000].

A post-hoc power analysis was performed on selected outcome variables with assumed high clinical and/or diagnostic relevance for the different items being investigated [Power Calculator, http://calculators.stat.ucla.edu/powercalc]. With regard to oral manifestations carrying assumed high diagnostic specificity in EDS (binomial variables), excellent power levels were found for the presence of symptoms of circumpulpal dentin malformation (0.99) and for pulp calcifications, especially those associated with classical EDS (0.94). As for oral health determinants (continuous variables), acceptable to excellent levels of power were computed for DMF-s (0.87), Plaque Index (1.0) and mean individual pocket depth (0.90).

III.5 Discussion

Ehlers-Danlos syndromes (EDS) are inherited disorders of collagen biosynthesis that may present with specific manifestations in the oral cavity. In mild and undiagnosed cases, assessment of these manifestations may lead to EDS diagnosis. Since metabolism of the most prevalent fibrillar collagens (type I, III and V) is altered in EDS [Beighton et al., 1998], structural abnormalities of connective tissue may produce clinical symptoms in the different orofacial systems.

The clinical expression of increased mucosal fragility appears to be closely related to deficiency of extracellular matrix (ECM) proteins of the lamina propria underlying the oral epithelium. The fibroblasts of both the superficial (papillary) and the deep (reticular) layer of the lamina propria are typical of those found in loose connective tissues. Collagen fibers (Types I, III and small amounts of non-fibrous forms of collagen) form the bulk of ECM proteins of both the lamina propria and the basal lamina, i.e. the epithelial-connective tissue interface [Berkovitz et al., 2002]. Deficiency in one or more of these proteins may alter the structural and mechanical properties of these layers, and consequently may elicit ‘tearing’ of the interface during e.g. mastication of food or brushing teeth.
In contrast with previous reports [Barabas et al., 1967; Fridrich et al., 1990; Letourneau et al., 2001; Melamed et al., 1994], no abnormalities were recorded in tooth number and tooth crown morphology in classical and hypermobility EDS. During recent years an increasing number of genes have been identified that are involved in the regulation of tooth initiation and tooth morphogenesis. The majority of these genes are associated with the signalling pathways transmitting interactions between cells and epithelial and mesenchymal tissue layers. Mutations in several of these genes have been identified as causes of dental defects, mainly hypodontia and arrested tooth crown formation [Thesleff, 2000]. Since genetic and biochemical research did not yet elucidate a possible interrelation between mutation in collagen genes and early tooth development, the former dental defects most likely originate from those regulatory genes. Hence, previous reports on abnormal tooth number and/or abnormal tooth crown morphology in EDS might concern accidental findings rather than pathognomonic signs related to collagen deficiency.

Increased mucosal fragility was a dominant finding in all examined EDS types. An interrelation between the location of the lesions and the type of the oral mucosa (lining or masticatory) could not be demonstrated, hence suggesting that neither the thickness, keratinization, or flexibility of the mucosal surface might influence the occurrence of tissue damage. As has previously been reported for skin fragility in EDS [Beighton et al., 1986; Beighton et al., 1996], structural alterations of the lamina propria, i.e. the connective tissue supporting the (oral) epithelium, might account for an increased fragility of the oral mucosa. The collagen in the lamina propria is primarily type I and type III, with type V occurring in inflamed or regenerative tissue [Squier et al., 2003]. Deficiency of collagen in the subepithelial connective tissue accounts for a decreased stretching and shear resistance of the epithelium, hence resulting in a high liability to ‘tearing’ of the mucosal tissues. Hence, extravasation of blood may occur in the lamina propria or between the lamina propria and oral epithelium, clinically presenting as a bruise, and the oral epithelium may be prone to easy grazing during mastication or brushing teeth. In addition, tissue regeneration may be hampered by a defective assembly of collagen fibrils.

Abnormal pulp shape and moderate generalized pulp calcification (pulp stones) were frequent findings in (classical) EDS. Pulp calcification localized in one or a few teeth most commonly follows after traumatic injury, whereas generalized pulp obliteration is much more rare and can be related to age changes or to pulpal pathology, caused by long-standing chronic irritation, such as abrasion, erosion, periodontal disease, extensive dental restorations, or carious lesions [Schindler & Gullickson, 1988; Cohen & Burns, 2002]. Generalized pulp calcification is, moreover, present in dental anomalies such as dentinogenesis imperfecta or dentin dysplasia [Witkop, 1988], or may be associated with systemic or genetic disease [Cohen & Burns, 2002]. The number and the size of the calcified bodies in classical EDS could not be proportionally related to the age of the individuals, neither to the formerly mentioned long-standing irritating factors. Although the histogenesis of
pulp calcifications is unknown, a relationship between pathologic alterations in collagen molecules within the pulp and pulpal calcification has been suggested [Cohen & Burns, 2002]. Calcified spheroids with comparable histologic appearance may be found subcutaneously in about 30% of classical EDS, caused by calcification in hyalinized scar tissue [Beighton & Thomas, 1969; Beighton et al., 1998]. Increased cross linkage between (reparative) collagen molecules is thought to enhance the tendency for collagen fibers to calcify [Cohen & Burns, 2002; Beighton & Thomas, 1969], a process which may also account for the presence of pulpal calcification in EDS subjects, irrespective of age and/or tooth eruption stage.

The mechanical properties of dentin are largely determined by the intertubular dentin matrix, which is a complex composite of collagen I fibers and a carbonate-rich mineral phase [Herold, 1972; Piesco, 2001]. Previous studies on this fiber/mineral composite architecture demonstrated that nucleation and growth of the apatite phase occur within periodic gaps in the collagen fibers [Kinney et al., 2001a; Kinney et al., 2003]. In normal dentin, these mineralized fibers were perpendicular to the dentinal tubules and parallel with the mineralization growth front [Dai et al., 1991; Kinney et al., 2001a]. Evidence was provided that intrafibrillar mineralization may be absent in dentinogenesis imperfecta, leading to decreased mechanical strength [Kinney et al., 2001b]. The similarity of ultrastructural findings in dentin defects, irrespective of the molecular cause (i.e. caused by mutation in either COL1 or DSPP genes), was related to the complicated interactions between the extracellular matrix macromolecules [Waltimo et al., 1995]. With regard to the present findings and on the basis of the present knowledge, it can be postulated that abnormalities in both the molecular structure (i.e. inconsistency in the sequence and the ‘accessibility’ of the periodic gaps in the collagen fibers) and the network-organizing properties (i.e. irregular pattern of fiber organization) of collagen I may interfere with intrafibrillar mineralization and may result in abnormal dentin structure. Consequently, the variety in the molecular structure of collagen I, caused by a variety of mutations, may account for the wide phenotypical spectrum of dentin defects.

The present study clearly demonstrates that oral health may be at risk in EDS. Caries experience (dmf/DMF score) was significantly higher in EDS population compared to controls. A high dmf/DMF-s score could individually be related to poor oral hygiene in EDS, which in turn was influenced by an increased mucosal fragility and chronic limb pain in the presence of restricted joint mobility (mostly wrists, interfering with proper brushing habits). The latter features appeared to hamper proper oral hygiene techniques. However, since oral health in patients with chronic medical problems very often is influenced by their general condition, one can assume that the above findings may not be specific to EDS, but may rather result from an interaction between physical, psychosocial, and environmental factors that are secondary to their disease.

There is no obvious explanation for gingival health in EDS being not clinically different from controls (P=1.0), although both mean Plaque Index and
mean pocket probing depth are significantly greater in EDS (respectively $P<0.001$ and $P=0.002$). Loss of gingival attachment was significantly greater in EDS population, and was found to be unrelated to gingival bleeding tendency and oral hygiene. These findings were contradictory to controls, and hence might result from specific structural (decreased resistance to mechanical and bacterial assaults) and/or biochemical (deficient healing or restitutional capacities) alterations of collagens in EDS, leading to rapid loss of attachment in the presence of pathogenic bacteriae. Given the above inconsistency in clinical features of periodontal health in EDS, a recommendation can be offered to examine on a routine basis the periodontal soft tissues in any patients with EDS, in order to disclose periodontal disease which might be present at the subclinical level.

Table III.9 Theoretical oral manifestations of deficiency of collagen I, III and V in orofacial structures of EDS

<table>
<thead>
<tr>
<th>Structure</th>
<th>Collagen types</th>
<th>Clinical expression of collagen deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>I - III - V</td>
<td>Slow socket healing after tooth extraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deficient restitution after periodontal treatment</td>
</tr>
<tr>
<td>TMJ</td>
<td>I - III - V</td>
<td>TMJ hypermobility with recurrent dislocations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High susceptibility to TMD development</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High susceptibility to TMJ hemarthrosis with consequent restraint TMJ mobility</td>
</tr>
<tr>
<td>Tooth / dentin</td>
<td>I</td>
<td>Root deformity</td>
</tr>
<tr>
<td>Tooth / pulp</td>
<td>I - III</td>
<td>Abnormal pulp chamber</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulp calcification</td>
</tr>
<tr>
<td>Mucosa</td>
<td>I - III - V</td>
<td>Increased bruisability with prolonged healing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increased bleeding tendency</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tearing of sutures</td>
</tr>
<tr>
<td>Periodontal ligament</td>
<td>I - III - V</td>
<td>Rapid loss of attachment (also juvenile variants)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Deficient restitution after professional root scaling</td>
</tr>
</tbody>
</table>

In previous studies, types I, III and V collagen have been identified in gingival connective tissue and the periodontal ligament. Type I collagen is the principal extracellular matrix protein in periodontal soft connective tissues [Narayanan et al., 1985], and is found in association with type III collagen throughout the tissue. Type III collagen is a major constituent of vascular walls [Romanos et al., 1991], whereas type V collagen has a more filamentous distribution, and has been observed in association with type I collagen fibril formation [Narayanan et al., 1983]. Type I collagen also accounts for 95% of total cementum collagens, especially presenting in Sharpey's fibers, and was found to be coated by type III collagen [Bartold et al., 2000]. In periodontitis, the collagens become more soluble, and the ratios of collagen types are altered. Furthermore, the
amount of type V collagen increases and a new, unstable collagen, type I trimer, may appear [Ruoslashti & Yamagichi, 1991]. There does not appear to be a change in localization and distribution of constituent collagen types in periodontitis [Salonen et al., 1990]. Restitution of the periodontal tissues after root scaling may be seriously compromised in EDS by deficient processing of new collagen proteins and fibrils, leading to a decreased regenerative capacity of connective tissues. The major challenge in contemporary periodontal therapy, i.e. re-establishing soft tissue attachment to newly formed cementum on the root surface, may be hampered by defective interdigitation of newly assembled (abnormal) collagen fibers to old fibers on the root surface [Barthold, 1995]. This may lead to failure of restitution of the periodontal ligament in disorders with deficient collagen biosynthesis.

The present study demonstrates an interrelation between deficiency of specific collagen types and clinical manifestations in the different orofacial structures (Table III.9). Previous reports on oral manifestations of collagen I deficiency in subjects affected with Osteogenesis Imperfecta (OI), mainly documented on dentin defects (dentinogenesis imperfecta) in the deciduous and permanent dentition [Schwartz & Tsipouras, 1984; Lukinmaa et al., 1987a; Levin et al., 1988; Aldred, 1992; O’Connell & Marini, 1999; Stephen & Beighton, 2002; Malmgren & Norgren, 2002]. A high prevalence of Class III dental malocclusion, anterior and posterior cross bites, and ectopic tooth eruption have also been reported in OI, but these characteristics most likely seem related to abnormal growth and development of the craniofacial bones as a result of abnormal collagen I biosynthesis [O’Connell & Marini, 1999]. In general, the clinical and molecular characteristics of collagen I disorders (e.g. OI and dermatosparaxis EDS type) are strictly determined by the causal gene mutation, which interferes in specific pathways in collagen synthesis. These distinct interferences may result in a wide range of clinical features in the connective tissues. At their best, the present findings are suggestive for some genotype-phenotype correlations, such as the presence of pulp calcification in classical EDS. However, in the absence of epidemiological evidence obtained from studies using larger sample sizes, no such correlations can yet be made on the basis of the present findings. Future investigation should address this issue.

III.6 Guidelines for Dental Treatment

When dental treatment is considered for any patient with EDS, a number of tissue responses and precautions should be anticipated. Oral mucosae may be fragile and easily bruised. Gingival tissues may be more liable to injury and to rapid progression of periodontal disease. Because of inborn problems with tissue repair, slow and deficient healing after tooth extraction and invasive periodontal treatment may occur. Dislocation of the TMJs during dental treatment have to be anticipated. Oral hygiene instructions have to take account of the individual’s restraint joint mobility and/or increased mucosal fragility. Pulp chamber
deformity and progressive pulp obliteration may considerably compromise endodontic treatment.

Whenever cardiovascular risks are present, patients must be properly premedicated in order to prevent bacteriaemia during or after dental treatment. In patients affected with hypermobility EDS (former Type III EDS), the depth of analgesia after local lidocaine infiltration has been reported to be significantly less than in controls [Arendt-Nielsen et al., 1990]. In these cases, both the type and dosage of local anesthetics must be chosen in close consultation with the physician and/or cardiologist.

III.7 Conclusion

Oral health was assessed in a population with EDS (n=31) and compared to matched controls. The overall mean DMF-s score (P=0.001), mean Plaque Index (P<0.001), and mean pocket depth (P=0.002) were significantly higher than controls. Caries experience was not dependent on EDS type. The overall dental care level was 0.80. Although the overall gingival condition (mean GI) was comparable to controls, there was an increased liability for soft tissue pathology and a rapid progression of periodontal disease in a majority of EDS subjects. Periodontal treatment needs were high (CPITN = III in at least one sextant) in 62% of patients, especially in hypermobility EDS. This could be related to a high Plaque Index and high Gingival Index. In these cases, restricted joint mobility of the wrists was found to influence the presence of dental plaque. Among subjects reporting increased mucosal fragility (74% of EDS population), a low brushing frequency, high Plaque Index, and high DMF-score proved significantly interrelated. On the basis of these findings, it was concluded that oral health may be compromised in patients affected with EDS.

Pathological manifestations of the dental hard tissues were found to be significantly dependent on EDS type. Abnormal pulp shape presented exclusively in classical EDS (P<0.001), and pulp calcification was found in both hypermobility and classical EDS (P=0.004). Further investigation of larger samples, however, is needed to validate the diagnostic specificity of these findings.
Chapter IV

Oral Manifestations and Oral Health in Marfan Syndrome

Expression of Fibrillin Deficiency in Oral Structures of Patients Affected With Marfan Syndrome

ABSTRACT

IV.1 INTRODUCTION
IV.2 MATERIAL AND METHODS
IV.3 AIMS
IV.4 RESULTS
IV.5 DISCUSSION
IV.6 GUIDELINES FOR DENTAL TREATMENT
IV.7 CONCLUSIONS

Parts of this chapter have been published or accepted as:


1Prof. dr. Anne De Paepe (M D, PhD) is affiliated to the Centre for Medical Genetics, Ghent University Hospital
ABSTRACT

Mutations in the gene encoding fibrillin, a widespread matrix protein involved in providing elastic properties to the connective tissues, may affect several organ systems. A number of craniofacial manifestations with minor diagnostic specificity (retrognathia, dolichocephaly, high palate) have previously been assigned to conditions caused by deficiency of fibrillin (Marfan syndrome, congenital contractural arachnodactyly). To analyze the impact of fibrillin deficiency on oral health, Marfan syndrome (MFS) was selected as a model of disease. MFS is a heritable multisystem disorder with a variable phenotype, caused by mutation in FBN1, the gene encoding fibrillin-1.

Oral health was assessed in 23 subjects with MFS (n=23), including caries experience, gingival health, and alterations of the oral soft and hard tissues, and was compared to matched controls (n=69). Caries experience (D-t/s scores) was significantly higher than controls (resp. P=0.004 and P=0.002), and dental care level on average was low (P=0.043). There was an increased mucosal fragility in 61% of MFS. The overall gingival condition was poor as compared to controls (P<0.001) with 66% of MFS showing severe gingivitis. Spindly, tapered roots, abnormal pulp shape, and pulp calcification were frequent findings in over one-third of MFS patients.

From these findings, it was concluded that oral health may be compromised in subjects affected with MFS, and that a number of dysmorphic oral features may be specific to the syndrome. However, since the pathologic pathways of expression of fibrillin deficiency in orofacial structures is still poorly understood, further investigation is needed to validate the diagnostic specificity of these findings. When considering dental treatment in MFS, a number of tissue responses (periodontium, pulp) and precautions (anesthetics, bacteriaemia, endodontics) should be anticipated. An early diagnosis and timely treatment of dental problems in MFS may help preventing life-threatening situations.
IV.1 Introduction

Marfan syndrome (MFS) (OMIM Entry 154700) [Online Mendelian Inheritance in Man, http://www3.ncbi.nlm.nih.gov/OMIM] is a heritable multisystem disorder with a variable phenotype, caused by mutation in FBN1, the gene encoding fibrillin-1 [De Paepe et al., 1996]. Table IV.1 summarizes the pleiotropic features of MFS. Multiple organ systems are affected, with most features being age-related. There is an important distinction between a ‘major’ criterion, that carries high diagnostic specificity, being present in a system, and the system being ‘involved’. ‘Minor’ clinical manifestations, that occur occasionally in the disorder, are given a more specific nuance in scoring the ‘affected’ systems (see also Chapter I.2.4).

Table IV.1 Pleiotropic features of Marfan Syndrome (MFS) [after De Paepe et al., 1996]

<table>
<thead>
<tr>
<th>Somatic system</th>
<th>Major criteria</th>
<th>Minor criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeleton</td>
<td>Pectus deformity, long limbs, scoliosis</td>
<td>Joint hypermobility, high palate,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>retrognathia, dolichocephaly</td>
</tr>
<tr>
<td>Eye</td>
<td>Eye lens subluxation</td>
<td>Flat cornea, hypoplastic iris</td>
</tr>
<tr>
<td>Cardiovascular system</td>
<td>Dilatation and/or dissection of the ascending aorta</td>
<td>Mitral valve prolapse, dilatation and/or dissection of thoracic aorta</td>
</tr>
<tr>
<td>Lungs</td>
<td>None</td>
<td>Spontaneous pneumothorax</td>
</tr>
<tr>
<td>Skin and integumentum</td>
<td>None</td>
<td>Striae atrophicae, recurrent herniae</td>
</tr>
<tr>
<td>Dura</td>
<td>Lumbosacral dural ectasia</td>
<td>None</td>
</tr>
<tr>
<td>Family history</td>
<td>Mutation in, or haplotype around FBN1</td>
<td>None</td>
</tr>
</tbody>
</table>

Fibrillin is a major component of these elastin-associated fibrils, which are widely distributed in the extracellular space. Because the distribution of microfibrils corresponds closely to the tissues affected in MFS, their deficiency is believed to account for the striae of the skin, the pulmonary bullae, the dural ectasia [Hollister et al., 1990; Tsipouras et al., 1994], and even the skeletal overgrowth [Dietz & Pyeritz, 1995; Westling & Mohlin, 1996].

Prevalence estimates of the ‘classic’ MFS phenotype vary from 5-8:100,000 [Gorlin et al., 1990b] to 3-4:10,000 in the general population [Dietz & Pyeritz, 2001]. Individuals with Marfan syndrome have been indentified in many ethnic, racial and geographic groups. Transmission of the syndrome is in autosomal dominant fashion [Dietz et al., 1993; De Paepe et al., 1996].

To date, the rare orofacial/dental literature on MFS has largely been confined to reporting on the presence of a high palatal vault as a clinical
manifestation with minor diagnostic specificity [Pyeritz & McKusick, 1979; Gazit & Lieberman, 1981; Gorlin et al., 1990b; Westling & Mohlin, 1996; Pirinen, 1998]. Recent cephalometric surveys indicate a prevalence of a high and deep palate of 50% [Westling & Mohlin, 1996; Westling et al., 1998]. Cleft palate or bifid uvula has been reported in several instances [Wilson, 1957; Lynas, 1958; McKusick, 1972]. The teeth have been noted to be long and narrow and frequently maloccluded, commonly associated with mandibular retrognathia [Gorlin et al., 1990b; Westling et al., 1998]. Oligodontia has been reported in association with bilateral aniridia [Sachdev et al., 1986]. Temporomandibular joint disorders are found more frequently than expected [Gorlin et al., 1990b]. Pulpal structures and gingival texture have been reported to be normal, however conventional light transmission microscopy has revealed moderate separation of the tunica media of arteries in both pulp and gingiva. A considerable increase in abnormal elastin fibers and dilatation of blood vessels was shown in these tissues by electron microscopy [Temtamy et al., 1989]. Table IV.2 summarizes the distribution and function of fibrillin in the orofacial structures.

### Table IV.2 Distribution and function of fibrillin in orofacial structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Part</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial skeleton</td>
<td>Perichondrium, periosteum</td>
<td>Restraint of growth, transmission of modelling forces on bone growth</td>
</tr>
<tr>
<td>TMJ</td>
<td>Upper lamella of posterior ligament</td>
<td>Joint stability during function</td>
</tr>
<tr>
<td>Tooth</td>
<td>Pulp (vessel walls)</td>
<td>Blood supply (dentinogenesis etc.)</td>
</tr>
<tr>
<td>Mucosa</td>
<td>Lamina propria</td>
<td>Restoring tissue form after stretching (lining mucosa)</td>
</tr>
<tr>
<td>Periodontal ligament</td>
<td>Oxytalan fibers (cervical region of ligament)</td>
<td>Regulation of vascular flow in relation to tooth function</td>
</tr>
</tbody>
</table>

At present no data are available with respect to oral health of patients affected with MFS. As these patients are cared for by dental professionals, it is essential to report on different aspects of oral health, in particular because these patients intrinsically are at high risk for cardiovascular complications. Prevention of bacteremia caused by advanced tooth decay, pulpal infection or periodontitis should be given high priority in dental treatment strategies.

### IV.2 Aims of the Study

The aim of this study was to analyze the different manifestations in the orofacial region and to assess oral health in a population affected with MFS.
The null hypothesis stated that oral health is not compromised in patients with congenital deficiency of fibrillin-1. In addition, therapeutical guidelines for dental treatment of these patients were formulated.

IV.3 Material and Methods

IV.3.1 Patient Selection

An invitation for participation to the study was communicated to the Belgian Association of Patients Affected With Marfan Syndrome. Forty-one unrelated patients volunteered and consented to be examined at the Ghent University Hospital, Belgium. A clinical and laboratory diagnosis, according to the Ghent nosology [De Paepe et al., 1996], was established at the Centre for Medical Genetics, Ghent University Hospital, and 23 patients participated (n=23). Mean age was 26.2 ± 13.6 years (range 9–53 yrs), and gender distribution was 39.1 % females to 60.8 % males. The control group comprised sixty-nine subjects (n=69), presenting for general dentistry purposes, and without a history of cardiovascular, endocrine, haematological or infectious diseases. Controls were individually matched to the study subjects for age, gender and dental attendance.

IV.3.2 Methods

Oral examinations were performed by the same investigator (PDC), assessing oral health using standardized indices as recommended by the WHO [WHO, 1997]. Caries experience was assessed according to the WHO [WHO, 1997; http://www.whocollab.od.mah.se] and the British Association for the Study of Community Dentistry (BASCD) guidelines, using the dmf/DMF Index, recording decay at the D₃ diagnostic threshold [Pitts & Fyffe, 1988; Fyffe et al., 2000]. The dmf/DMF Index [Klein et al., 1938] is the most commonly used criterion to validate an individual’s experience with dental decay, and can be expressed either at tooth (-t) or at tooth surface (-s) level. It is a sum score index composed by the numbers of decayed (d/D), missing (m/M) and filled (f/F) teeth or tooth surface per individual. In deciduous teeth, the dmf Index (indicated in small fonts) is used, whereas permanent teeth are scored by means of the DMF Index (in capitals). The D₃ threshold is the conventional diagnostic threshold, scoring caries at the level of cavitation [Mitropoulos & Pitts, 1993] and/or at level 2 or higher of approximal radiolucency (radiolucency in enamel up to enamel-dentine junction or deeper) [Kidd et al., 1996]. Data were obtained under artificial light by using a mouth mirror, a WHO/CPITN type E probe, cotton rolls, and bite-wings after professional mechanical tooth cleaning [Mitropoulos & Pitts, 1993; WHO, 1997]. White spots and pit-and-fissure sealants were not scored. Exclusion was made of third molars and teeth extracted for orthodontic purposes. The dental care level was expressed as F/(D+F) (Restorative Index) [Jackson, 1973]. Clinical presence of calculus was assessed by using Volpe & Manhold’s Calculus Index system. Loë & Silness’ Gingival Index (GI) system was used to assess gingival health [Lang, 1998]
by calculating the mean GI for each subject. Frequency of brushing and attendance of dental recalls were scored on an ordinal scale.

The examiner (PDC) was calibrated at baseline and intra-examiner agreement was tested for dmf/DMF Index (\(\gamma = 0.78\); 95% CI 0.69 - 0.87) using Kappa statistics (Cohen’s Kappa or \(\gamma\)). Inter-examiner agreement was tested for scoring oral calculus (Volpe & Manhold’s Calculus Index) (\(\gamma = 0.79\); 95% CI 0.64 - 0.89), and gingival inflammation (Loë & Silness’ Gingival Index) (\(\gamma = 0.83\); 95% CI 0.74 – 0.92). These Kappa values stand for an acceptable interreliability with an experienced examiner as a benchmark [Clayton & Hills, 1993; Petrie & Sabin, 2000].

Since epidermal tissues and integumentum may be involved in MFS, the oral mucosa was examined for evidence of structural abnormalities, i.e. striae, healing defects and pigmentation not related to racial characteristics or iatrogenic tattoo (post-restorative or -surgical mucosal staining). Local evidence of increased mucosal fragility (bruises, grazes, regenerative patches, or ulcerations) at different locations of the lining (floor of the mouth, underside of the tongue, inside of the lips, soft palate, cheeks, and alveolar processes) and masticatory mucosa (hard palate and gingiva) were recorded. Bruises were defined as dark coloured (red to blueish-grey) mucosal swellings, not related to dental/periodontal pathology or neoplasma, scar tissue, or hyperpigmentation. Grazes were defined as circumscribed, dark red or purple coloured areas, usually displaying exposure of capillar vessels, indicated by the presence of small red points. Regenerative patches were defined as circumscribed spots, either of white, grey or brown colour, usually with a paler outline and reflecting recent bruising and/or erosion with deficient wound healing. These patches should be distinguished from abscesses and ulcerations (aphthous, herpetic or traumatic), and mucosal conditions with a more durable character such as leukoplakia, lichen planus and candidiasis. A positive history of recurrent mucosal trauma was required to confirm the clinical diagnosis of increased mucosal fragility. In these cases, the occurrence (daily; once or more a week; less), initiating factors (mastication; brushing teeth; consumption of hot, spicy or crusty foods or candy; spontaneously), and individual management (nihil; mouth rinses; topical gels; professional help) of the lesions were scored on an ordinal scale.

Marked orofacial characteristics, i.e. both anthropometric (facial characteristics) and intra-oral features (high and narrow palatal vault), of MFS individuals were recorded.

Structural defects of the dental hard tissues were assessed by visual and tactile observation of the tooth surfaces, and evaluating tooth color and tooth dimensions. Enamel abnormalities were classified into three types on the basis of their appearance: demarcated opacities, diffuse opacities, and hypoplasia [FDI, 1992]. Panoramic and intra-oral radiographs were examined for abnormalities of root anatomy and pulp canal shape, and for evidence of pulp obliteration (calcification). Teeth with deep caries and/or fillings, or with a history of trauma
were excluded for assessment of pulp calcification. A generalized or symmetrical spreading of pulp calcification was assigned high diagnostic specificity, except when long-standing chronic irritation factors (abrasion, erosion, periodontal disease, extensive dental restorations, or carious lesions) were present [Schindler et al., 1988; Cohen & Burns, 2002].

Statistics were performed using MedCalc® Statistics for Biomedical Research, version 6.0 (MedCalc Software, Mariakerke, Belgium) [Schoonjans et al., 1995] and SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago, Illinois, USA). Chi-square test or Kolmogorov-Smirnov test were used to evaluate the distribution of variables. Student’s t-test or unpaired Wilcoxon test (Whitney-Mann U-test) were used to compare means, and Fischer’s exact test and chi-square test to compare proportions between groups. Associations between continuous variables were studied by means of rank correlation analysis (Spearman’s correlation coefficient) and regression analysis. Differences at the 5 % level of probability were considered statistically significant (P=0.05) [Clayton & Hill, 1993; Petrie & Sabin, 2000].

IV.4 Results

IV.4.1 Caries Experience

Except for significantly higher mean D-t/-s scores in MFS (n=23) as compared to controls (n=69), there were no significant differences in the mean M-, F- and DMF-scores, both on tooth (-t) and surface (-s) level (Table IV.3). A more detailed analysis of caries data among MFS and controls as a function of age category (Table IV.3 and Figure IV.1) revealed that MFS subjects aged 0-17 yrs (n=8) had significantly higher dmf/DMF scores both on tooth and surface level (respectively P=0.001 and P=0.007) than contemporary controls. M-scores in this age group were significantly higher both on tooth (P=0.008) and surface (P=0.003)

Figure IV.1 Caries experience (dmf/DMF) in MFS (n=23) and controls (Ctrl, n=69) as a function of age category
level, whereas D-scores showed significantly different on surface \((P=0.002)\) level compared to contemporary controls \((n=24)\). Caries data of the age group 18-26 yrs \((n=6)\) were largely comparable to controls. MFS subjects aged 26 yrs and older \((n=9)\) had significant higher Dt/\(-s\) scores \((P=0.027\) and \(P=0.031)\) than controls \((n=27)\).

In the control population \((n=69)\), caries experience \((D\)-score\), both on tooth \((P=0.036)\) and surface \((P<0.001)\) level, increased significantly with increasing age (Figure IV.1). This association was also found for m/ M-, f/ F- and dmf/ DMF-scores. In MFS population \((n=23)\), however, an interrelation between age and respectively d/ D-, m/ M-, f/ F-, and dmf/ DMF-scores, both on tooth and surface level, was absent.

IV.4.2 Dental Care Level

On tooth level, the mean dental care index \([(F/ D+F)-t]\) of MFS population \((n=23)\) was 0.55, i.e. 45% of the average dentition was decayed and in need of professional dental treatment. This was not significantly different from controls \((n=69)\). On surface level, however, dental care index differed significantly between MFS \((0.62)\) and controls \((0.74)\) \((P=0.043)\). On tooth level, a significant difference was also found between dental care index of MFS subjects aged > 26 yrs \((0.50)\) compared to contemporaneous controls \((0.71)\) \((P=0.038)\) (Table IV.3). The highest care level in MFS subjects, both on tooth \((0.64)\) and surface \((0.78)\) level, was found in patients aged 18-25 yrs.

On average, dental attendance in the MFS population was low, i.e. only 4% regularly attended dental recalls (i.e. once or more a year). The overall brushing frequency was low (13% brushed twice or more a day, 35% once a day, and 52% irregularly but less than once a day) and was significantly related to complaints of gingival bleeding \((P=0.042)\) and/ or increased mucosal fragility \((P=0.032)\), i.e. both factors negatively influenced the brushing frequency. There was no significant interrelation between brushing frequency and dmf/ DMF-score.

IV.4.2 Oral Mucosa

In contrast with previous reports, no oral clefts were recorded in MFS population. Occasional findings in the oral soft tissues in MFS were striae, exhibited by 17.4% of the examined subjects. Among these patients, no reports of healing defects after tooth extraction, difficult tooth exfoliation, or eruption problems of the temporary and/ or permanent dentition were recorded. The control subjects displayed these findings in 4.4%, hence yielding no statistically significant difference when compared to MFS \((P=0.114)\). Increased mucosal fragility was reported by 61% of MFS subjects, i.e. mucosal bruises or grazes occurred frequently during mastication of food (75%) and/ or brushing teeth (100%). Subjects reporting increased mucosal fragility had a low brushing frequency \((P=0.025)\). There was no interrelation between increased mucosal
fragility and DMF score. In five patients partial prostheses were present, which were well integrated without any signs of mucosal irritation or ulceration.

Figure IV.2

Circumscribed hypoplastic defects of enamel in premolars of a young adult affected with MFS

IV.4.3 Facial and intra-oral characteristics

The majority of MFS patients had typical facial characteristics, such as dolichocephaly (long, narrow face), deep-set eyes with slight ptosis, and a small and long nose. These features were found to be independent on age and gender. A high and narrow palate was clinically present in 52% of MFS.

IV.4.4 Dental hard tissues

With respect to tooth number (agenesia), no significant difference was found between MFS and controls. Enamel defects, not related to caries processes, in a majority of cases clinically presented as circumscribed opacities with local hypoplasia, and were recorded in 34.8% of MFS subjects (n=23). In both MFS and controls, these structural defects mostly presented at vestibular/occlusal surfaces of permanent maxillary incisors and premolars in both jaws (Figure IV.2). In most cases an association between the local anomaly and trauma or infection of the decayed preceding deciduous tooth could be determined. The high occurrence of these clinical findings in MFS proved significantly different compared to controls (P=0.045), and concurred with the high caries experience in children (0-17yrs).

Figure IV.3 Typical morphological features of MFS dentition: spindly tapered roots (A), abnormal pulp shape (A), and pulp calcification (B-C).
For structural dentin defects being observed, no abnormal discolorations were recorded in the temporary or permanent dentition. Root deformity, not associated with any reported trauma or premature orthodontic tooth displacement, was recorded significantly more frequently in MFS ($P=0.003$), in most cases presenting as long and spindly tapered roots (Figure IV.3). Pulp inclusions or evidence of generalized pulp calcification were present in 21.7% of MFS subjects, proving significantly different compared to controls ($P=0.013$). No association was found between the occurrence of pulp calcification and the dentition type, gender or eruption stage. When focusing on morphological abnormalities of the pulp chamber in MFS, some distinct findings were noted as to the topologic distribution of anomalies of the pulp canal shape: 47.8% exhibited one or more unilateral abnormality, whereas in 30.4% there was a symmetrical spreading (Figure IV.3). The occurrence of the former morphological findings proved statistically different from controls ($P<0.001$) (Table IV.4).

**Table IV.4 Abnormal features of enamel and dentin among MFS (n=23) and controls (n=69)**

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>MFS n=23</th>
<th>Controls n=69</th>
<th>P-value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enamel defects</td>
<td>34.8</td>
<td>13.0</td>
<td>0.043</td>
</tr>
<tr>
<td>Abnormal pulp chamber</td>
<td>30.4</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Root deformity</td>
<td>30.4</td>
<td>4.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Pulp calcification</td>
<td>21.7</td>
<td>2.9</td>
<td>0.013</td>
</tr>
</tbody>
</table>

* Differences considered significant at $P<0.05$ (Chi-square test)

When studying interdependent associations of these morphological abnormalities, a simultaneous presence of at least three of the four distinct records of dentinal involvement (root deformity, pulp calcification, abnormal pulp chamber shape, and bilateral occurrence of abnormal pulp chamber shape) was found in 13 per cent of MFS subjects. In 8.7 per cent all four findings were simultaneously present, which proved significantly different compared to controls ($P=0.002$). Since no reports of this nature have yet been made in literature, a possible overlap with familial inherited dentin defects, however, may not be excluded.

**IV.4.5 Gingival Health and Oral Calculus**

The mean individual Gingival Index value in MFS population (n=23) ($GI=1.94 \pm 0.70$) significantly differed from controls ($GI=1.06 \pm 0.69$) ($P<0.001$), indicating that gingival health in MFS on average was poor. However MFS subjects aged 18-25 yrs exhibited the best clinical gingival condition (i.e. lowest GI), no significant
difference in GI was detected among age groups (P=0.518) or gender (P=0.635) in MFS. Figure IV.4 displays the distribution of GI values in MFS and controls, revealing a remarkable difference in (the clinical manifestations of) gingival health between both groups. Sixty-six % of MFS subjects presented severe gingivitis (2.1<GI<3.0) compared to 12% in controls (Figure III.5). However in controls GI increased with increasing age, this correlation was absent in MFS. There was a significant interrelation between GI and brushing frequency (P=0.012).

**Figure IV.4** Distribution (Box-and-Whisker plot: mean values, standard deviation and extreme values) of Gingival Index values among MFS (n=23) and controls (n=69) as a function of age category [Loë & Silness’ Gingival Index System]

The mean individual amount of oral calculus in MFS (n=23) (VM =9.8 ± 3.6) was significantly greater (P<0.001) than in controls (VM =2.9 ± 3.1), which was a constant finding in all age categories (Table IV.5). In contrast with controls, no interrelations were found between the amount of oral calculus and age or brushing frequency in MFS. In both MFS and controls groups, the calculus amount was significantly correlated to dmf/ DMF score (P<0.001).

**Figure IV.5**

Distribution of individual mean Gingival Index (GI) values in MFS (n=23) and controls (Ctrls, n=69). Clinical diagnoses of gingival inflammation correspond to GI values: 0.0-1.0 mild gingivitis, 1.1-2.0 moderate gingivitis, 2.1-3.0 severe gingivitis [Loë & Silness]
Table IV.5  Comparison of Volpe-Manhold Index (mean values and standard deviations) for oral calculus between MFS (n=23) and controls (n=69) as a function of age category

<table>
<thead>
<tr>
<th>Status</th>
<th>0-17 yrs Mean</th>
<th>SD</th>
<th>18-25 yrs Mean</th>
<th>SD</th>
<th>26 yrs &amp; older Mean</th>
<th>SD</th>
<th>Overall Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFS</td>
<td>10.37*</td>
<td>5.6</td>
<td>6.33*</td>
<td>4.63</td>
<td>11.55*</td>
<td>3.38</td>
<td>9.78*</td>
<td>5.66</td>
</tr>
<tr>
<td>Controls</td>
<td>0.59</td>
<td>0.81</td>
<td>2.65</td>
<td>2.53</td>
<td>5.07</td>
<td>3.32</td>
<td>2.88</td>
<td>3.13</td>
</tr>
</tbody>
</table>

* Differences considered significant at P<0.05 (Mann-Whitney U-test)

IV.4.6 Correlation analysis

With regard to periodontal health, there were some additional significant differences between MFS and controls as to the interrelation between age and gingival index (i.e. absence of correlation in MFS: r=0.085 P=0.690; strong correlation in controls: r=0.471 P<0.001) and the number of missing teeth and gingival index (i.e. absence of correlation in MFS: r=0.279 P=0.191; strong correlation in controls: r=0.485 P<0.001). This may suggest that, compared to unaffected individuals, periodontal pathology may develop and evolve differently in subjects with MFS than in unaffected persons.

IV.4.7 Power of the Study

A post-hoc power analysis was performed on selected outcome variables with assumed high clinical and/or diagnostic relevance for the different items being investigated (see Chapter III.4.5) [Power Calculator, http://calculators.stat.ucla.edu/powercalc]. With respect to oral manifestations carrying high diagnostic specificity in MFS (binomial variables), acceptable power levels were found for the presence of symptoms of root malformation (0.88) and for pulp calcifications (0.75) in MFS compared to controls. As for oral health determinants (continuous variables), an excellent level of power was computed for Gingival Index (0.99). Although the overall D-t/-s and Care Level(-s) values were significantly different compared to controls (P<0.05), the tests of both variables were found to be underpowered (respectively 0.56 and 0.54).

IV.5 Discussion

The present study demonstrates that oral health may be at risk in subjects affected with MFS. Although dmf/DMF scores in MFS were comparable to controls, it was demonstrated that MFS subjects had significantly higher D-t/-s
scores (respectively P=0.004 and P=0.002) than controls. Caries experience in MFS was also found to vary as a function of age category, i.e. youngsters aged 0-17 yrs had the highest D-t/-s score. This was significantly different from controls, where caries experience, as well as M(issing)- and Filled-scores, were found to increase with increasing age (Figure IV.1). Dental care level in MFS (n=23) on average was low (0.55) as compared to controls (0.65) (P=0.043). The selection of age categories was based, on the one hand, on the epidemiological relevance of DMF scores [Schaub & Eijkman, 1981], and, on the other hand, on the unfavourable age distribution among the enrolled MFS subjects younger than 18 yrs, permitting no subdivision into e.g. 0-12 yrs and 13-18 yrs. The relatively high D-t/-s scores in the present study may be related either, or both, to the use of interproximal radiographs for detection of decay, the low dental attendance of MFS population, and poor oral hygiene (low brushing frequency). Because of the small sample size and the low level of power, however, restraint has to be called on assigning any ‘absolute’ epidemiological significance to the respective counts and scores among the different age groups in MFS and controls. Moreover, since oral health in patients with chronic medical problems very often is influenced by their general condition, one can assume that the above findings may not be specific to MFS, but may rather result from an interaction between physical, psychosocial, and environmental factors that are secondary to their disease.

The mean gingival condition in MFS was poor compared to controls: nearly 66 per cent of MFS exhibited severe gingivitis (i.e. GI > 2.1) versus 11 per cent in controls (Figure IV.5). This finding may be explained by the previously reported high occurrence of vascular defects in the periodontium [Temptamy et al., 1989], although this idea still needs further investigation. The absence of any relationship between GI and respectively age, brushing frequency, and the number of missing teeth in MFS, as presenting in unaffected controls, possibly indicates that periodontal pathology may develop and evolve differently in MFS. Structural alterations of the vascular walls may result in an altered tissue response to inflammatory activity of pathogens, and, hence, may lead to an increased liability for periodontal pathology. However, other factors than fibrillin deficiency, such as restricted physical capacities for maintaining good oral hygiene or poor dental mindedness, may very well influence the periodontal condition in patients with MFS. Further epidemiological and ultrastructural investigation is needed to explore the periodontal tissue characteristics of patients with MFS.

Structural alterations of the basal lamina of oral mucosae and gingiva may account for a number of clinical oral manifestations, such as recurrent bruising/grazing and the occasional presence of regenerative patches, found in 17.4 per cent of MFS. Deficiency of fibrillin/elastic fibers in the lamina propria of the skin and in the lining mucosa results in defective restoring of tissue form after stretching [De Paepe et al., 1996], and may lead to localized subdermal disrupture of mucosae, e.g. during mastication or brushing. Hence, an increased bruisability of the oral soft tissues may be present in a number of subjects affected with MFS.
Alterations of dentin structure, i.e. (bilateral) abnormal pulp shape, root deformity and pulp calcification, were frequent findings in over one-third of MFS population (Table IV.4). However, further investigation is required in order to assess the diagnostic specificity of these morphological features in fibrillinopathies, and to elucidate the role of the microfibrillar system in the development of these dentin defects. On the basis of the present knowledge, a possible link with vascular wall defects in MFS cannot be excluded. Endothelial ruptures of pulp arterioles might induce specific repair processes in the pulpal organ, that are comparable with the induction of reactive dentin formation as seen in teeth with advanced caries or a history of trauma. A possible explanation for the presence of abnormal pulp shape and root deformity (long and spindly tapered roots) might be provided by altered modeling forces that might be generated by microfibrillar deficiency in the stroma and periosteum, surrounding the developing tooth germ.

When presenting in any MFS subject, these features should be integrated in dental treatment strategies. Pulp obliteration, sometimes in combination with abnormal pulp canal shape, may considerably complicate endodontic treatment and should be anticipated by proper preoperative radiographic documentation and an appropriate technical approach. It remains uncertain whether or not these abnormal tooth structures are normally resistant to the application of orthodontic forces. Furthermore, tooth extraction can be complicated by fracturing of the root. Whenever possible, atraumatic or surgical extraction should be considered when any of these dentin features are present.

A majority of MFS patients in this study presented with typical facial characteristics, such as dolichocephaly (long, narrow face), deep-set eyes with slight ptosis, and a small and long nose. These findings were consistent with previous case reports [Ayers & Drummond, 2003]. Although a number of studies have documented an association between palatal clefts and MFS [Wilson, 1957; Lynas, 1958; McKusick, 1972], no clefts were found in the present MFS population. However, caution is called on the assignment of palatal clefting to the syndrome, as put forward by other authors [Ayers & Drummond, 2003], since an enlargement of the palatal shelves (so-called byzantine arch palate), featuring in a number of marfanoid syndromes such as Shprintzen-Goldberg syndrome (OMIM Entry 182212) and Stickler syndrome (OMIM Entry 108300), may easily be confused with a central palatal cleft [Gorlin et al., 1990b]. A high and narrow palate was clinically present in over 50% of MFS, which was consistent with previous reports [Westling & Mohlin, 1996; Westling et al., 1998]. Other craniofacial manifestations of MFS, such as long face and mandibular retrognathia, are studied in Chapter V.

Table IV.6 summarizes the oral manifestations that may be considered as the clinical expression of fibrillin deficiency on the basis of the literature and the present findings. The high caries experience in MFS population, however, cannot be explained by tissue alterations intrinsic to deficiency of the microfibrillar system, but certainly is of importance in view of preventing bacteriaemia. As previously reported in populations with restrictions and/or chronic illness [Nunn
et al., 1993; Glassman & Miller, 2003] individual physical, psychological and socio-
economical [Hobdell et al., 2003; Swedberg & Noren, 2003; Johnson, 2004] aspects
may impair oral hygiene, and hence may considerably contribute to compromizing
oral health.

Table IV.6  Clinical expression of fibrillin deficiency in the orofacial structures

<table>
<thead>
<tr>
<th>Structure</th>
<th>Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Craniofacial skeleton</td>
<td>Long face, mandibular retrognathia, deep-set eyes</td>
</tr>
<tr>
<td>TMJ</td>
<td>Recurrent TMJ dislocation</td>
</tr>
<tr>
<td>Tooth</td>
<td>Spindly tapered roots, abnormal pulp shape, pulp calcification</td>
</tr>
<tr>
<td>Mucosa</td>
<td>Increased bruisability</td>
</tr>
<tr>
<td>Periodontium</td>
<td>Increased bleeding tendency, increased susceptibility for pathology</td>
</tr>
</tbody>
</table>

IV.6  Guidelines for Dental Treatment

When considering dental treatment in any patient with MFS, a number of
tissue responses and precautions should be anticipated and integrated in treatment
strategies. Caries experience, liability for oral soft tissue pathology, and occurrence
of pulp calcification may be considerably increased in MFS. Dental treatment
should focus on prevention in view of anticipating often life-threatening
bacteraemia. Altered structural properties of the aortic walls compel to paying
attention to general systemic management during dental treatment. The use of
local anesthetics containing epinephrine needs to be carefully managed in MFS,
since this drug may enhance the cardiac output. The administration of articaine, or
prilocaine with felypressin, causes the least of an increase in cardiac function.
Monitoring of blood pressure, systolic time intervals and aortic pulse wave
velocity is strongly recommended during dental treatment [Hirota et al., 1986;
Hirota & Sugiyama, 1993]. In addition, patients must be premedicated in order to
prevent bacteraemia during and after invasive dental surgery. A high risk
protocol is indicated whenever any cardiovascular risks are present.

IV.7  Conclusions

Oral health was assessed in a population with MFS (n=23) and compared to matched controls. Caries experience (D-t/-s scores) (P=0.004 and
P=0.002) and mean Gingival Index (P<0.001) were signifiantly higher than controls, and Dental Care Level on average was low (P=0.043). Mucosal fragility was
increased in 61% of MFS subjects. There was an overall increased liability for
gingival bleeding and soft tissue pathology, suggestive of an unusual development
and evolution of periodontal disease. On the basis of these findings, it was concluded that oral health may be compromised in MFS.

Characteristic facial features, morphological aberrations of the dentin-pulp complex, and an increased bleeding tendency and susceptibility to periodontal pathology, presenting in a majority of the MFS study population, may be considered as clinical manifestations of fibrillin-1 deficiency. However, further investigation is needed to validate the diagnostic specificity of these findings.
Chapter V

The Craniofacial Complex in Marfan Syndrome

Expression of Fibrillin Deficiency in the Craniofacial Skeleton in Marfan Syndrome

ABSTRACT

V.1 INTRODUCTION
V.2 AIMS
V.3 MATERIAL AND METHODS
V.4 RESULTS
V.5 DISCUSSION
V.6 CONCLUSION

Part of this chapter has been accepted as:

De Coster P, De Pauw G\textsuperscript{1}, Martens L, De Paepe A\textsuperscript{2}.

Craniofacial structure in Marfan syndrome - a cephalometric comparative study.

\textit{Am J Med Genet}; in press.

\textsuperscript{1}Dr. Guy De Pauw (DDS, PhD), Dept. of Orthodontics, Dental School, and
\textsuperscript{2}Prof. dr. Anne De Paepe (MD, PhD), Centre for Medical Genetics, Ghent University Hospital
Abstract

Marfan syndrome (MFS) is a multisystem disorder with autosomal dominant inheritance. Mutations in FBN1 gene cause deficient processing of fibrillin-1, the main constituent of extracellular microfibrils, affecting tissues displaying elastic properties. Clinical manifestations are widespread and involve the skeletal, ocular, cardiovascular and pulmonary systems, skin and integumentum, and dura. A highly arched palate and retrognathia have been assigned to the symptoms with minor diagnostic specificity, although epidemiological data on prevalence are lacking yet.

Twenty-six patients with MFS (n=26) were studied for craniofacial characteristics using cephalometric measurements on lateral cranial radiographs. The purposes of this study were (1) to compare cephalometric variables of MFS group with age- and sex-matched population norms, and (2) to assess differences in sagittal palatal vault dimensions among adult MFS (n=17) and matched controls (n=32) by means of cephalometric measurements.

Significant differences with population norms were found in the structures of the cranial base, the maxillary complex, the mandibular body, and the relations of the jaws with respect to the cranial base and to each other. Palatal height and palatal length were significantly greater in MFS, and were significantly correlated to each other and to the height of the maxillo-alveolar processes.

The present data disprove in part previously reported findings, possibly due to biased patient selection in these studies or demographic differences. However, a strong correlation was found between maxillary/mandibular retrognathia, long face, highly arched palate, and MFS. A combination of both intrinsic genetic factors and environmental factors is suggested as a possible explanation for specific morphogenetic aspects of the craniofacial complex in MFS.
V.1 Introduction

The recent identification of the genetic basis of hereditary skeletal disorders has provided important insights into the intricate processes of skeletal formation, growth, and homeostasis [Thesleff, 1998; Rice et al., 2003]. A wide range of heritable diseases of the skeleton is caused by mutations in components of the extracellular matrix in cartilage and bone and in molecules that are important for posttranslational processing of such components. Mutations of the genes encoding the two polypeptide subunits of collagen I cause defects of the structure of bone matrix, while mutations in genes coding for cartilage-specific collagens (e.g. collagen II) are responsible for several chondrodysplasias. Abnormalities in the structure and function of bone and cartilage can also be due to mutations in structural noncollagenous components (aggrecan) or to abnormalities in sulfate transport and regulation of bone matrix homeostasis [Mundlos & Olsen, 1997b]. Defects in any of the transcription factors (e.g. transforming growth factor-ß) or signaling pathways, which are responsible for patterning events and differentiation of mesenchymal cells to form bone or cartilage, may also give rise to skeletal abnormalities [Mundlos & Olsen, 1997a; Thesleff, 1998; Rice et al., 2003].

In conclusion, skeletal abnormalities may result either from deficient biosynthesis of the structural bone matrix components (e.g. osteogenesis imperfecta and achondroplasias), resulting in a ‘direct’ interference with normal bone/cartilage formation, or from deficiency of extracellular matrix components of the surrounding tissues, influencing skeletal morphogenesis. The latter process may be held responsible for specific morphogenetic aspects of the skeleton in disorders caused by fibrillin deficiency, such as Marfan syndrome.

Marfan syndrome (MFS) is an autosomal dominant multisystem disorder with variable clinical manifestations [De Paepe et al., 1996]. Prevalence is estimated at 34:10,000 individuals and is not dependent on race or gender [De Paepe et al., 1996; Dietz & Pyeritz, 2001]. Mutations in the gene coding for fibrillin-1 (FBN1) cause MFS [Kainulainen et al. 1990; Dietz et al., 1991] and other related disorders of connective tissue, grouped as fibrillinopathies. Fibrillin is a 350 kD, cysteine-rich glycoprotein that exists in three homologous forms [Keene et al., 1991], of which fibrillin-1 and fibrillin-2, respectively encoded by the FBN1 gene on 15q21 and the FBN2 gene on 5q23 [Lee et al., 1995] are the best characterized. Fibrillin-1 is the main constituent of extracellular microfibrils, which can exist as individual structures or associate with elastin to form elastic fibers [Dietz & Pyeritz, 1995; Dietz & Pyeritz, 2001].

The diagnosis of MFS is largely clinical and relies on a set of diagnostic criteria known as the Ghent Nosology [De Paepe et al., 1996]. These criteria require the presence of a combination of clinical manifestations in different organ systems (skeletal, ocular, cardiovascular, pulmonary system, skin and integumentum, and dura). “Major” manifestations are highly specific for the condition and include ectopia lentis, aortic root dilatation/dissection, dural ectasia, and at least 4 of 8
specific skeletal features [De Paepe et al., 1996]. In addition, several “minor” clinical manifestations are observed in MFS such as tall stature, scoliosis, mitral valve prolapse, pectus excavatum, joint hypermobility, and myopia. According to the Ghent Nosology, the diagnosis in an individual requires the presence of two major manifestations in two different organ systems and the involvement of a third organ system. If a positive family history is present, a major manifestation in one organ system and the involvement of a second organ system are required to establish the diagnosis. The age-related nature of some clinical manifestations and the variable phenotypic expression may hinder the clinical diagnosis of MFS, particularly in children. A laboratory diagnosis of FBN1 mutation may be established in the majority of affected individuals.

Various craniofacial abnormalities have been described in patients with MFS, although the literature on this topic has consisted predominantly of case reports. A number of oral manifestations, such as a high caries experience, tooth root deformity and a high susceptibility to periodontal pathologies, have been reported to be closely related to the syndrome [De Coster et al., 2002]. Craniofacial abnormalities include dolichocephaly, maxillary constriction with a highly arched palate (i.e. a narrow and high palatal vault), maxillary and mandibular retrognatia, prognathia, and macrocephaly, which have been reported with variable frequencies [Gazit & Lieberman, 1981; Crosher & Homes, 1988; Motohashi, 1985; Poole, 1989; Gorlin et al., 1990; Westling et al., 1998; Cistulli et al., 2001]. A number of craniofacial characteristics, mainly comprising retrognathia and a high and narrowly arched palate, have been assigned to the minor diagnostic manifestations [Beighton et al. 1988; Pyeritz 1993; De Paepe et al. 1996]. However, there is yet no agreement in literature concerning the diagnostic validity of these features in MFS. Data on the morphogenetic aspects of the palatal vault, the position of the craniofacial bones, and the typical “long face” appearance in relation to the genetic defects of the extracellular microfibrils (fibrillin), which are causal in the syndrome, are also lacking. In addition, patient selection may have been considerably biased in previous studies by a lack of molecular diagnosis of the participants.

V.2 Aims of the Study

The study aimed to analyze the structure of the craniofacial complex in a group of patients with a clinical and molecular diagnosis of MFS (n=26), by comparing cephalometric measurements to age- and sex-matched population standards. The occurrence of a number of reported craniofacial manifestations with assumed ‘minor’ diagnostic specificity (maxillary and mandibular retrognathia, long face, and a highly arched palate) was analyzed in adult MFS subjects (n=17) and compared with matched controls (n=32).

The null hypothesis stated that MFS is not characterized by craniofacial manifestations that are specific to the syndrome.
V.3  Material & Methods

V.3.1  Patient Selection

The study population consisted of 26 individuals (n=26) responding to an invitation for cephalometric screening at the Centre for Special Care, Dental School, Ghent University Hospital, Belgium. All participants had previously been diagnosed with MFS according to the Ghent Nosology [De Paepe et al., 1996] at the Centre for Medical Genetics, Ghent University Hospital (see also Chapter III.2.1). Mean age was 24.5 ± 14 years (age range 3 – 57 yrs) and gender distribution was 32% males to 68% females. Among these individuals, an adult group (n=17) was selected by age to compare for palatal morphology with controls. Cut-off age values were set at 17 years in females and 18 years in males, since at these ages the location of cephalometric landmarks in general, and palatal vault morphology in particular, are accepted to be constant [Solow, 1966; Snodell et al., 1993]. Mean age of this adult group was 31.4 ± 11.4 yrs, comprising 23% males to 77% females. For each adult subject, a diagnostic analysis of the cephalogram according to Sassouni [Sassouni V, 1969] and Steiner [Steiner CC, 1969] was performed in order to assess sagittal and vertical skeletal characteristics. Assessment of sagittal skeletal characteristics comprised evaluation of the position of the maxilla relative to the cranial base (S-N-A = sella to nasion to A point angle; 82° = normal, <82° = maxillar retrognathia, >82° = maxillar prognathia), the position of the mandibula with respect to the cranial base (S-N-B = sella to nasion to B point angle; 80° = normal, <80°= mandibular retrognathia, >80° = mandibular prognathia), and the intermaxillar position (A-N-B = A point to nasion to B point angle; 2°-4° = normal or Angle Class I, >4° = mandible retrognatism or Angle Class II, <2° = mandibular prognatism or Angle Class III). Assessment of the vertical skeletal characteristics was performed by evaluating the angulation of the mandible relative to the anterior cranial base (S-N/ Go-Gn = the angulation between the anterior cranial base plane, SN, and the mandibular plane, Go-Gn; 32° = normal, <32° = deep growth pattern or short face, >32° = open growth pattern or long face) [Steiner CC, 1969]. For comparison of palatal landmarks, a control group of 32 patients (n=32) was selected from the files of the Department of Orthodontics, Ghent University Hospital, to match individually the adult MFS group patients (n=17) by sex, age, and sagittal and vertical skeletal characteristics. As a rule, for each MFS subject two controls were individually matched; for two cases, only one control subject could be found.

V.3.2  Methods

Lateral cephalometric radiographs were taken with the same X-ray device (Omnix Tele, Trophy Radiologie, Vincennes, France) using a standardized technique [Broadbent et al., 1975]. Subjects were seated with their heads positioned in a cephalostat and oriented to the Frankfort plane, with teeth in maximal occlusion. The distance from the mid-sagittal plane was standardized to 23 cm, and the distance from the source to the midsagittal plane was fixed at 380 cm,
producing a magnification of 6.1%. The cephalograms were digitized with an Agfa Snapscan 1236U flatbed scanner (Agfa Gevaert, Mortsel, Belgium) at 300 dpi, and imported in OrthView 6.0 software for cephalometric analysis (American Orthodontics, Sheboygan, Wisconsin, USA). Fourteen landmarks were identified from the MFS cephalograms according to Broadbent et al. [1975] (Figure V.1), and 24 linear and angular measurements were calculated (Table V.1). The data were compared to population norms of the Bolton Standards [Broadbent et al., 1975], which were collected from over 5,000 caucasian youngsters at Case Western Reserve School of Dentistry in Cleveland, Ohio. Radiographic enlargement of the midsagittal plane, i.e. 6.1% in the MFS sample and 5.5% in the Bolton population, was corrected before statistical analysis [Dibbets & Nolte, 2002].

Three original cephalometric landmarks were defined (P, M1 and M2). The palato-alveolar point (P), the point of intersection or contact point between the palatal maxillary cortex and the maxillary central incisor, allowed for linear and angular measurements of the maxillo-palatal complex dimensions.

Figure V.1 Definitions for the landmarks used in the cephalometric analysis according to Broadbent et al. (1975).
From an anatomical point of view, \( P \) is concomitant with the frontal boundary of the palatal vault, and its location with respect to the caudal cortex of the maxilla is determining palatal height and palatal vault skewness. The relative position of \( P \) to the palatal plane (ANS-PNS line) was defined as the maxillo-alveolar height (\( P \) to ANS-PNS). Palatal height in the mid-sagittal plane was defined as the distance of the most caudal point of the mesial cusp of the maxillary first molar (\( M1 \)) to the caudal cortex of the maxilla (\( M2 \)), measured perpendicularly to the occlusal plane (i.e. the plane defined by the tip of the central incisor and the tip of the mesial cusp of the maxillary first molar). Palatal length was defined as the distance between \( P \) point and the posterior nasal spine (\( P-PNS \)). Four additional measurements were performed for studying palatal morphology in the adult MFS group and controls (\( P \) to ANS-PNS, \( M1-M2 \), PNS-P, U1 to ANS-PNS) (Figure V.2). In addition, five angular (\( N-S-Bn, S-Ar-Go, S-N/ANS-PNS, S-N-ANS, PNS-ANS-central maxillary incisor edge \)) and five linear measurements (\( N-A, N-B, S-Ar, Go-Me, N-Pg \)) were recorded to allow for superimposing of average tracings in adult MFS and controls.

### Table V.1  List of cephalometric variables according to Broadbent et al. (1975)

<table>
<thead>
<tr>
<th>Cranial base</th>
<th>Linear</th>
<th>S-N</th>
<th>Anterior cranial base length</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S-Bn</td>
<td></td>
<td>Posterior cranial base length</td>
</tr>
<tr>
<td>Maxillary relations</td>
<td>Angular</td>
<td>S-N-A</td>
<td>Degree of maxillary prognatism</td>
</tr>
<tr>
<td>Mandibular relations</td>
<td>Angular</td>
<td>S-N-B</td>
<td>Degree of mandibular prognatism</td>
</tr>
<tr>
<td></td>
<td>S-N-Pg</td>
<td></td>
<td>Degree of chin prognatism</td>
</tr>
<tr>
<td></td>
<td>Ar-Go-Gn</td>
<td></td>
<td>Jaw angle</td>
</tr>
<tr>
<td></td>
<td>S-N/Go-Gn</td>
<td></td>
<td>Angulation of the mandible relative to the anterior cranial base</td>
</tr>
<tr>
<td></td>
<td>N-S-Ar</td>
<td></td>
<td>Angle between articulare and the anterior cranial base</td>
</tr>
<tr>
<td>Linear</td>
<td>Go-Pg</td>
<td></td>
<td>Mandibular body length</td>
</tr>
<tr>
<td></td>
<td>S-Gn</td>
<td></td>
<td>Position of the mandible relative to the cranial base</td>
</tr>
<tr>
<td></td>
<td>Ar-Go</td>
<td></td>
<td>Mandibular ramus height</td>
</tr>
<tr>
<td></td>
<td>ANS-Me</td>
<td></td>
<td>Anterior lower face height</td>
</tr>
<tr>
<td>Maxillomandibular relations</td>
<td>Angular</td>
<td>A-N-B</td>
<td>Basal sagittal jaw relationship</td>
</tr>
<tr>
<td></td>
<td>ANS-PNS/Go-Gn</td>
<td></td>
<td>Angle between maxillary and mandibular planes</td>
</tr>
<tr>
<td>Linear</td>
<td>N-Me</td>
<td></td>
<td>Anterior face height</td>
</tr>
<tr>
<td>Dental relations</td>
<td>Angular</td>
<td>U1-L1</td>
<td>Interincisal angle</td>
</tr>
<tr>
<td></td>
<td>U1/S-N</td>
<td></td>
<td>Angulation of the maxillary incisors relative to the cranial base</td>
</tr>
<tr>
<td></td>
<td>L1/S-N</td>
<td></td>
<td>Angulation of the mandibular incisors relative to the cranial base</td>
</tr>
<tr>
<td></td>
<td>U1/N-A</td>
<td></td>
<td>Proclination of the maxillary incisors</td>
</tr>
<tr>
<td></td>
<td>L1/N-B</td>
<td></td>
<td>Proclination of the mandibular incisors</td>
</tr>
<tr>
<td></td>
<td>L1/Go-Gn</td>
<td></td>
<td>Angulation of the mandibular incisors relative to the mandibular plane</td>
</tr>
<tr>
<td></td>
<td>U1/ANS-PNS</td>
<td></td>
<td>Angulation of the maxillary incisors relative to the maxillar plane</td>
</tr>
</tbody>
</table>
Interrater reliability analysis for landmark identification yielded an average measure ICC of 0.937 (95% C.I.: 0.824-0.997) for linear and 0.998 (95% C.I.: 0.997-0.999) for angular measurements. Intra-observer error variance, calculated between paired tracings (Dahlberg's formula), was 0.3 mm for linear measurements (average measure ICC = 0.998; 95%C.I.: 0.990-0.999) and 0.3° (average measure ICC = 0.997; 95%C.I.: 0.988-0.999) for angular measurements. These errors of measurement were considered acceptable when compared to other cephalometric studies.

Statistical analysis was performed by use of SPSS for Windows, version 11.0.1 (SPSS Inc., Chicago, Illinois, USA). Comparison between patient data and normative data were carried out by calculating the individual Z-score compared to the Bolton value of the matching age- and sex-stratum (i.e. \( Z = [\text{patient value} - \text{reference value}] / \text{SD of reference stratum} \)). For each measurement, the mean Z-score and SD were calculated, and one-sample statistics (Z-test) were performed. Mann-Whitney U-test (unpaired Wilcoxon test) was performed to compare data from palatal vault analysis between adult MFS group and controls. Spearman’s Rank Correlation Coefficient was used to test correlations between data from palatal landmark measurements among adult MFS and controls. Differences at \( P<0.05 \) level were considered statistically significant [Clayton & Hills, 1993].

**Figure V.2** Cephalometric measurements of palatal landmarks according to Broadbent et al. (1975); landmarks indicated with an asterisk (*) are original.

\[
\begin{align*}
\text{ANS} &= \text{anterior nasal spine, sharp median process formed by the forward prolongation of the two maxillae at the lower margin of the anterior aperture of the nose;} \\
\text{PNS} &= \text{posterior nasal spine, process formed by the united projecting medial ends of the posterior borders of the two palatine bones;} \\
\text{OP} &= \text{occlusal plane, plane defined by the tip of the maxillary central incisor and the tip of the mesial cusp of the maxillary first molar;} \\
\text{P}^* &= \text{palatal or alveolar point, intersection of the caudal cortex of the maxilla and the distal aspect of the central maxillary incisor root;} \\
M_1^* &= \text{the most caudal point of the mesial cusp of the maxillary first molar;} \\
M_2^* &= \text{intersection of the caudal cortex of the maxilla and the line, perpendicular to the occlusal plane (OP) through } M_1; \\
U_1 &= \text{upper incisor axis, the line through the upper incisor edge and the upper incisor apex.}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Linear measurements (mm)</th>
<th></th>
<th>Angular measurements (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P to ANS-PNS</td>
<td>M_1-M_2</td>
<td>U_1 to ANS-PNS</td>
</tr>
<tr>
<td>Maxillo-alveolar height</td>
<td>Palatal height</td>
<td>Palatal length</td>
</tr>
<tr>
<td>PNS-P</td>
<td></td>
<td>Angulation of maxillary central incisor axis relative to the palatal plane (ANS-PNS)</td>
</tr>
</tbody>
</table>
The study had previously been approved by the Ethical Committee of Ghent University Hospital (ref. 2000/308). A written consent was obtained from all participants prior to examination.

V.4 Results

V.4.1 Diagnostic Tracings in MFS group

Mean values and standard deviations of sagittal and vertical skeletal characteristics of the MFS study population (n=26) are listed in Table V.2. Frequency analysis showed the respective characteristics to be heterogeneously distributed in MFS group. The position of the maxilla relative to the cranial base was normal in 8% (S-N-A = 82°), maxillary retrognathia was found in 84% (S-N-A < 82°), and maxillary prognathia in 8% (S-N-A > 82°). The mandible was normally positioned in 12% (S-N-B = 80°), and mandibular retrognathia was found in 88% of MFS subjects (S-N-B < 80°). Concurrent presence of both maxillary and mandibular retrognathia was found in 81%. Intermaxillary relation (A-N-B angle) was normal in 44% (2° < A-N-B < 4°), whereas mandibular retrognathism was present in 48% (A-N-B > 4°) and mandibular prognathism in 8% of the cases (A-N-B < 2°). Analysis of vertical characteristics (S-N/Go-Gn) yielded normal mandibular plane angulation in 12% (S-N/Go-Gn = 32°), long face or open growth pattern in 72% (S-N/Go-Gn > 32°) and a deep growth pattern or short face in 16% (S-N/Go-Gn < 32°).

Table V.2 Sagittal and vertical skeletal characteristics among MFS subjects (n=26)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANB</td>
<td>4.6</td>
<td>3.2</td>
<td>0.1</td>
<td>10.2</td>
</tr>
<tr>
<td>S-N/Go-Gn</td>
<td>36.2</td>
<td>6.9</td>
<td>25.5</td>
<td>50.0</td>
</tr>
<tr>
<td>SNA</td>
<td>79.4</td>
<td>4.8</td>
<td>71.2</td>
<td>88.1</td>
</tr>
<tr>
<td>SNB</td>
<td>75.1</td>
<td>4.3</td>
<td>66.6</td>
<td>81.9</td>
</tr>
</tbody>
</table>

V.4.2 Cranial Base

The distribution and one-sample statistics of Z-values of cephalometric data in MFS (n=26) are listed in Table V.3. Both the anterior (S-N) and posterior cranial base length (S-Bn) were short in MFS compared to population norms (One-sample Z-test; resp. P=0.044 and P=0.043).

V.4.3 Facial Height

The overall anterior facial height (N-Me) was greater in MFS (P<0.001), reflecting a marked reduction in anterior upper facial height (N-ANS) (P=0.012)
and an increase in anterior lower facial height (ANS-Me) (P<0.001) in the syndrome (Figure V.3). These results confirmed the preliminary diagnostic finding of a long face profile in the majority of patients with MFS.

**Table V.3** Mean z-values, 95% confidence intervals (CI) and test significance (One Sample test) for the cephalometric measurements of MFS (n=26), compared to normative data (Bolton Standards : Broadbent et al. 1975)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Test Value = 0</th>
<th>95% CI of the Difference</th>
<th>Significance **</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Angular (°)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-N-A</td>
<td>-1.76</td>
<td>-2.736 -0.784</td>
<td>0.001</td>
</tr>
<tr>
<td>S-N-B</td>
<td>-2.493</td>
<td>-3.356 -1.630</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A-N-B</td>
<td>1.347</td>
<td>0.326 2.368</td>
<td>0.012</td>
</tr>
<tr>
<td>N-A-Pg</td>
<td>-1.440</td>
<td>-2.498 -0.382</td>
<td>0.010</td>
</tr>
<tr>
<td>N-S-Ar</td>
<td>0.143</td>
<td>-1.133 1.412</td>
<td>0.820</td>
</tr>
<tr>
<td>Ar-Go-Gn</td>
<td>1.523</td>
<td>0.606 2.439</td>
<td>0.002</td>
</tr>
<tr>
<td>S-N-Pg</td>
<td>-2.615</td>
<td>-3.361 -1.868</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S-N/Go-Gn</td>
<td>2.295</td>
<td>1.252 3.337</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANS-PNS/Go-Gn</td>
<td>3.097</td>
<td>1.884 4.309</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U1/S-N</td>
<td>0.082</td>
<td>-0.419 0.582</td>
<td>0.739</td>
</tr>
<tr>
<td>L1/S-N</td>
<td>-1.225</td>
<td>-2.045 -0.405</td>
<td>0.005</td>
</tr>
<tr>
<td>U1/N-A</td>
<td>0.662</td>
<td>0.189 1.135</td>
<td>0.008</td>
</tr>
<tr>
<td>L1/N-B</td>
<td>0.794</td>
<td>0.489 1.100</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>L1/Go-Gn</td>
<td>0.646</td>
<td>0.057 1.235</td>
<td>0.033</td>
</tr>
<tr>
<td>U1/L1</td>
<td>-1.100</td>
<td>-1.464 -0.734</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U1/ANS-PNS</td>
<td>-0.390</td>
<td>-0.898 0.117</td>
<td>0.126</td>
</tr>
<tr>
<td><strong>Linear (mm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-N</td>
<td>-0.709</td>
<td>-1.394 -0.091</td>
<td>0.044</td>
</tr>
<tr>
<td>S-Bn</td>
<td>-0.735</td>
<td>-1.446 -0.023</td>
<td>0.043</td>
</tr>
<tr>
<td>S-Gn</td>
<td>-0.828</td>
<td>-1.379 -0.278</td>
<td>0.005</td>
</tr>
<tr>
<td>N-ANS</td>
<td>-1.279</td>
<td>-2.249 -0.309</td>
<td>0.012</td>
</tr>
<tr>
<td>N-Me</td>
<td>1.242</td>
<td>0.606 1.878</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANS-Me</td>
<td>2.786</td>
<td>2.038 3.534</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Go-Pg</td>
<td>-2.442</td>
<td>-2.974 -1.910</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ar-Go</td>
<td>-0.888</td>
<td>-1.790 0.013</td>
<td>0.053</td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>-0.830</td>
<td>-1.481 -0.179</td>
<td>0.014</td>
</tr>
</tbody>
</table>

* Mean difference = (measured value in Marfan - value of matching Bolton Standards) / SD of matching Bolton Standards; values adjusted for radiographic enlargement at baseline

** One-Sample test (Z-test); P<0.05 are considered statistically significant

**V.4.4 Maxilla**

The maxilla (ANS-PNS) was short compared to normative data (P=0.014), and was situated more posteriorly with respect to the anterior cranial base (maxillary retrognathia), shown by a significantly reduced SN-A angle (P=0.001) (Figure V.4).
V.4.5 Mandibula

The mandibula showed deviations in sagittal and vertical positions, as well as in size and in proportions. The mandibular body (Go-Pg) was especially short ($P<0.001$). The gonial angle (Ar-Go-Gn) was increased ($P=0.002$), and the significantly greater SN/Go-Gn angle ($P<0.001$) indicated a posterior rotation of the mandibular plane. The mandible was also positioned more posteriorly with respect to the cranial base (mandible retrognathia): the distance from gnathion to sella (S-Gn) was short ($P=0.005$), and both SN-B and SN-Pg angles were small compared to population norms ($P<0.001$ in both cases). The mandibular ramus height (Ar-Go) and the position of the ramus with respect to the cranial base (S-N-Ar angle) did not differ significantly from normative data (resp. $P=0.053$ and $P=0.820$) (Figure V.4).

Figure V.3 Boxplots of Z-values (median, range and extremes) of basal craniofacial measurements in MFS (n=26), expressed as standard deviations as compared to population norms (value=0) (Broadbent et al., 1975)

$S-N =$ anterior cranial base length; $S-Bn =$ posterior cranial base length; $N-Me =$ anterior face height; $N-ANS =$ anterior upper face height; $ANS-Me =$ lower anterior face height

V.4.6 Jaw Relations

Relations of the jaws with respect to each other differed significantly from population norms. The basal sagittal jaw angle (A-N-B) was significantly greater and the interincisal angle (U1/L1) was significantly smaller in MFS (resp. $P=0.012$ and $P<0.001$). The interbasal angle between the maxillary and mandibular planes (ANS-PNS/Go-Gn) was increased in MFS ($P<0.001$). The angle of the maxillary central incisors both with respect to the cranial base (U1/ S-N) and the maxillary
Boxplots of Z-values (median, range and extremes) of cephalometric measurements of maxilla and mandible in MFS (n=26), expressed as standard deviations as compared to population norms (value=0) (Broadbent et al., 1975)

ANS-PNS = length of the maxilla along the nasal floor; S-N-A = degree of maxillary prognatism; Go-Pg = mandibular body length; A r-Go-Gn = mandibular jaw angle; SN/Go-Gn = angulation of the mandible relative to the anterior cranial base; SGn = position of the mandible relative to the cranial base; S-N-B = degree of mandibular prognatism; SN-Pg = degree of prognatism of the chin; Ar-Go = mandibular ramus height; N-S-A r = angle between articulare and the anterior cranial base

plane (U1/ANS-PNS) did not differ significantly from normative data (resp. P=0.739 and P=0.126). Maxillary central incisors were rotated more anteriorly (U1/ N-A angle) with respect to the anteroposterior position of the maxilla in MFS (eversion of central maxillary incisors) (P=0.008). The mandibular incisors were tipped more posteriorly with respect to the cranial base (L1/ S-N angle) (P=0.005) compared to population norms. Significant greater L1/ N-B (P<0.001) and L1/ Go-Gn angles (P=0.033) indicated a more anteriorly rotated position of the mandibular central incisors with respect to the anteroposterior position of the mandible (eversion of central mandibular incisors) and to the mandibular plane (Figure V.5).

V.4.7 Comparison of Skeletal Characteristics between Adult MFS and Controls

Mann-Whitney U-test yielded no significant differences for A-N-B (P=0.539), SN/ Go-Gn (P=0.291), SN-A (P=0.676) and SN-B (P=0.697) between adult MFS group and controls, confirming that both groups had similar sagittal and vertical skeletal characteristics. One-sample statistics of Z-values of other relevant cephalometric measurements in the control group (n=32) showed no significant differences compared to population standards (Table V.4).
Figure V.5  Boxplots of Z-values (median, range and extremes) of cephalometric measurements of jaw and incisor interrelations in MFS (n=26), expressed as standard deviations as compared to population norms (value=0) (Broadbent et al., 1975)

A-N-B = basal sagittal jaw interrelationship; U1/ L1 = interincisal angle; ANS-PNS/ Go-Gn = angle between maxillary and mandibular planes; U1/ S-N = angulation of the maxillary incisors relative to the cranial base; U1/ ANS-PNS = angulation of maxillary incisors relative to the maxillary plane; U1/ N-A = proclination of the maxillary incisors; L1/ S-N = angulation of the mandibular incisors relative to the cranial base; L1/ N-B = proclination of the mandibular incisors; L1/ Go-Gn = angulation of the mandible incisors relative to the mandibular plane.

There was a slight tendency (P=0.052) towards an increased gonial angle (Ar-Go-Gn), i.e. an angular value for the posterior lower facial height. The linear variables indicative of the anterior facial height (N-ANS, N-Me) likewise did not differ significantly, however the lower anterior facial height (ANS-Me) was slightly, but not significantly, increased (P=0.057). These findings may be related to the specific (matched) sagittal and vertical skeletal characteristics of both the study and control population.

V.4.8  Palate

Mean values and test statistics of cephalometric measurements of palatal landmarks in adult MFS and controls are listed in Table V.5. The maxilla (ANS-PNS) was significantly shorter in adult MFS (P=0.005) compared to controls. Maxillo-alveolar height (P to ANS-PNS) (P<0.001) and both palatal length (P-PNS) (P=0.005) and palatal heigth (M1-M2) (P<0.001) were significantly greater in MFS. Maxillo-alveolar height was found to be an important determining factor for both palatal height and palatal length in MFS. There was a high correlation between palatal height (M1-M2) and maxillo-alveolar height (P to ANS-PNS) in both adult
MFS (Spearman’s Rank Correlation Coefficient $r_s=0.728$, $P=0.001$) and controls ($r_s =0.787$, $P<0.001$). Palatal length and maxillo-alveolar height were significantly correlated in MFS ($r_s =0.541$, $P=0.025$) but not in controls ($r_s =0.224$, $P=0.185$). Palatal height (M1-M2) and palatal length (PNS-P) were significantly correlated in MFS ($r_s =0.500$, $P=0.041$), which was different from controls ($r_s =0.179$, $P=0.335$).

V.4.9 Superimposing of Tracings

The differences in mean craniofacial morphology between adult MFS (n=17) and controls (n=32) are visualized in Figure V.6. Average tracings of the adult MFS and controls, based on the mean cephalometric variables for each group, were superimposed on the sella-nasion plane (anterior cranial base) and registered at the sella point. Adult MFS subjects were characterized by a prolonged face with anterior displacement of the major bone structures (frontonasal bone, maxilla and mandible). Both maxilla and mandible were rotated more posteriorly with respect to the anterior cranial base (S-N).
Table V.5  Mean values and SD of cephalometric measurements of palatal landmarks among adult MFS (n=26) and controls (n=32)

<table>
<thead>
<tr>
<th>Variable*</th>
<th>MFS (n=17)</th>
<th>Controls (n=32)</th>
<th>P-value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANS-PNS</td>
<td>49.6</td>
<td>52.5</td>
<td>0.005</td>
</tr>
<tr>
<td>P to ANS-PNS</td>
<td>23.9</td>
<td>18.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>M1-M2</td>
<td>27.8</td>
<td>24.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PNS-P</td>
<td>48.8</td>
<td>45.4</td>
<td>0.005</td>
</tr>
<tr>
<td>Angular (degrees)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U1 to ANS-PNS</td>
<td>76.0</td>
<td>67.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* ANS-PNS = maxillary length; P to ANS-PNS = maxillo-alveolar height; M1-M2 = palatal height; PNS-P = palatal length; U1 to ANS-PNS = angulation of maxillary central incisor axis relative to maxillary plane

** Mann-Whitney U-test; P<0.05 is considered statistically significant

Figure V.6  Average tracings of adult MFS (n=17) and matched controls (n=32) based on the mean cephalometric variables for each group, superimposed on the sella-nasion line (anterior cranial base) and registered at the sella point.

MFS group was characterized by a prolonged face with anterior displacement of the major bone structures, and a more posteriorly rotated position of both maxilla and mandible.
V.4.10 Power Analysis

Techniques measuring the statistical power of a study estimate the probability that a study could establish a statistically significant difference if a specified size-true difference actually exists in the larger population (see also Chapter III.4.6). A post-hoc power analysis was performed on selected outcome variables with assumed high diagnostic relevance for the different items being investigated [Power Calculator, http://calculators.stat.ucla.edu/powercalc]. According to the plural objective of the present study, a series of variables characterizing the craniofacial complex was selected. With regard to cephalometric measurements of the craniofacial structure in MFS (n=26) as compared to population norms (one sample test), excellent power levels (0.99 – 1.0) were found for the anterior face height (N-Me), degree of maxillary prognathia (S-N-A), degree of mandibular prognathia (S-N-B), basal sagittal jaw relationship (A-N-B), and angulation of the mandible relative to the cranial base (S-N/Go-Gn). With respect to additional linear measurements of sagittal palatal dimensions in adult MFS (n=17), acceptable to excellent levels of power were computed for maxillar length (ANS-PNS) (0.75), maxillo-alveolar height (P to ANS-PNS) (0.99), palatal height (M1-M2) (0.99), and palatal length (PNS-P) (0.82).

V.5 Discussion

The cephalometric analysis of MFS subjects in this study supported only part of previously published data. This may be dependent on the sample size, which was considerably larger in the present study, on regional differences, and/or on a thorough patient selection based on both clinical and laboratory diagnoses. Previous studies assigned MFS diagnosis on the basis of clinical signs [Westling et al., 1998; Cistulli et al., 2001], which may have produced biased results. Both anterior and posterior cranial base lengths were short in MFS, which was different from other studies [Westling et al., 1998; Cistulli et al., 2001]. Since the postnatal growth of the anterior cranial base is dependent on growth in three suture sites (fronto-ethmoidal, sphenofrontal, and spheno-ethmoidal sutures) [Thilander, 1995], and sutures in MFS are believed to have a decreased growth potential caused by improper response to tension [Gigante et al., 1996; Gigante et al., 1999], deficient sutural growth probably may account for shortened cranial base length. MFS group was characterized by an increased overall anterior face height (N-Me), as a result of a reduction in anterior upper face height (N-ANS) and an increased anterior lower face height (ANS-Me). The latter may, amongst others, be closely associated with vertical midface hypoplasia. In contrast with the present data, the anterior upper face height was previously reported to be significantly greater in MFS [Westling et al., 1998; Cistulli et al., 2001].

Although sella to nasion to A point angle (S-N-A) on average was decreased in MFS (n=26) compared to population norms, maxillary retrognathia (S-N-A < 82°) was only found in 84.7% of the subjects. This finding could be
maintained by a short anterior cranial base length and short maxilla, which was
different from previous reports [Westling et al., 1998]. The findings on mandibular
size and position largely confirmed previous data [Westling et al., 1998; Cistulli et
al., 2001]. Mandibular retrognathia, indicated by a decreased sella to nasion to B
point angle (S-N-B < 80°), was present in 88% of MFS patients. Concurrent
presence of both maxillary and mandibular retrognathia was found in 81% of MFS
subjects, while a posterior or clockwise rotation of the mandibular plane (S-N/Go-
Gn > 32°) was present in 72%. These findings, along with Z-statistics outcome, are
indicative of a strong correlation between maxillary and mandibular retrognathia,
long face and MFS.

In the literature, a highly arched palate has been associated with a number
of connective tissue disorders and syndromes [McKusick, 1972; Gazit &
Lieberman, 1981; Poole, 1989; Gorlin et al., 1990; Pyeritz, 1993]. In medical
examinations, the registration of high and narrow palatal vaults has primarily been
based on clinical evaluations. At present, generally accepted instructions for the
assessment of palatal morphology are still lacking. A number of methods for
measuring and evaluating palatal dimensions have been proposed by use of a
palatal index (palatal height / palatal width) [Shapiro et al., 1963; Westling &
Mohlin, 1996]. Controversy exists, however, in defining palatal height as well as
palatal length [Shapiro et al., 1963; Westling et al., 1993]. Clinical interpretation of
the palatal vault, moreover, may be confounded by mucosal and gingival
structures, often leading to assessment of soft tissue contours rather than palatal
bone morphology. For these reasons, in this study palatal morphology and palatal
dimensions in the mid-sagittal plane were evaluated by means of specific
cephalometric measurements of dento-palatal landmarks. The definition of the P
point or palato-alveolar point was based on the clinical finding that high palates
have a steep and high frontal slope, characterized intraorally by a low-placed
insertion of the palatal mucosa to the central incisors. Maxillo-alveolar height (P to
ANS-PNS) was found to co-determine palatal heighth both in MFS (P=0.001) and
controls (P<0.001), and may thus represent a useful tool in evaluating the palatal
vault shape. Palatal height (M1-M2) was significantly greater in adult MFS
compared to controls (P<0.001). This finding may considerably contribute to an
increased lower anterior face height (ANS-Me), which in part may account for a
long face profile. The length of the maxilla (ANS-PNS) in MFS was short compared
to both normative data (P=0.014) and controls (P=0.005). Palatal length (PNS-P)
showed significantly larger in MFS compared to controls (P=0.005), and was
significantly correlated to palatal height in MFS (P=0.041). Since controls were
matched for both gender and vertical and sagittal skeletal characteristics of the
adult MFS subgroup, these findings suggest that the maxillary complex and the
palatal vault in MFS may be characterized by a different growth and development
than observed in the general population. However, since both adult MFS and
control groups were small and other physical features influencing palatal
dimensions, such as tall stature [Westling & Mohling, 1996] and nasal obstruction
[Solow et al., 1984], were not included as variables in this study, diagnostic
specificity of the clinical manifestation of a high and narrow palate cannot be judged properly.

The cause of the craniofacial abnormalities observed in MFS is not yet clear. In particular, it is not clear whether they are intrinsic to the genetic abnormality or whether environmental influences play a role. The patterns of craniofacial bone alterations observed in this study are known to be associated with chronic airway obstruction during childhood and are collectively termed “long face syndrome” in the orthodontic literature [Linder-Aronson et al., 1960; Solow et al., 1984]. Oral breathing consequent to nasal obstruction has been implicated to cause modification of head posture, which may influence facial development and dentofacial growth [Solow et al., 1984; Warren, 1990; Cistulli et al., 2001]. Animal studies further supported the important role of airway patency on facial development [Timms, 1974]. Hence, the observed craniofacial abnormalities may be the result of chronic airway obstruction, which is associated with mouth breathing. This is supported by the observation that a considerable number of these patients have high nasal resistance [Cistulli et al., 1996], which has been suggested to be causally related to the characteristic maxillary constriction and high-arched palate [Cistulli & Sullivan, 2000].

Craniofacial growth represents a complex enlargement and differentiation of hard and soft tissues, characterized by cephalocaudal and allometric patterns. The basic phenomena involved in bone growth mechanisms are conversion of cartilage (synchondroses, nasal septal cartilage, condylar cartilage), appositional bone growth (also called sutural deposition), and periostal remodeling [Thilander, 1995; Rönning, 1995; Persson, 1995]. The cranial base matures earlier than the face [Snodell et al., 1993] and is used cephalometrically as a relatively stable reference area. The maxilla, although intimately associated with the cranium, exhibits some independent growth, particularly in the vertical dimension (displacement in forward and downward direction) [Thilander, 1995; Enlow, 1990; Ranly, 2000]. The factor responsible for bone displacement in the midface is controversial. Although a number of studies support the hypothesis that nasal cartilage has an independent growth potential driving the nasomaxillary complex [Scott, 1962; Grewe et al., 1971; Grymer & Bosch, 1997; Sugarawa et al., 1999], others conclude that its growth is secondary to and compensatory for a prior passive displacement of the midfacial bones, playing a significant biomechanical role in maintaining normal midfacial form [Björk & Skieller, 1976; Björk & Skieller, 1977]. The mandible, hanging in a sling of muscles, and articulating only at the temporomandibular fossae and with the opposing dentition, is purportedly able to adapt its shape and position in space to some extent as a function of condylar growth [Enlow, 1990; Thilander, 1995; Rönning, 1995; Ranly, 2000].

The influence of perichondral-periosteal membranes, which consist of collagen and elastic fibers, is of greatest importance for the change in size and shape of the cranial bones. Facial morphogenesis is controlled by growth remodeling, that is both modeling (apposition and resorption as surface-specific
activities) and remodeling (reconstruction of previously existing osseous tissue and rebuilding at the molecular level) [Thilander, 1995; Ranly, 2000]. Basal modeling forces are generated by function of inserting (masticatory) musculature [Kiliaridis, 1995]. A periostal cell layer, the inner cambrium layer, is established with the initiation of the intramembraneous ossification of bone, and the surrounding mesenchymal cells acquire the character and the potential of osteoblasts. As bone growth presupposes a continuous replacement of matrix-producing cells via cell division in this cambium layer, both matrix-producing and proliferating cells are subject to mechanical influence. In this way the periosteum responds with bone deposition when exposed to muscular tension, and continues to function as an osteogenic zone throughout life [Enlow, 1990; Kiliaridis, 1995]. Changes in elastic fibers in musculoskeletal tissues of Marfan syndrome have been linked to joint laxity [Giganti et al., 1999a; Giganti et al., 1999b], and the decrease in number of elastic fibers in the perichondral-periostal membranes was suggested to result in reduced restraint of skeletal growth (skeletal overgrowth) [Giganti et al., 1999a; Giganti et al., 2001].

VI.6 Conclusion

A combination of both intrinsic genetic factors (fibrillin deficiency in periosteum and inserting musculature), and environmental factors (improper response to deficient tension on periosteum and sutures leading to “long face” growth) can provide a plausible explanation for the specific morphogenetic aspects of the craniofacial complex in Marfan syndrome. These skeletal abnormalities may be considered intrinsic to the causal mutation in FBN1.

On the basis of the present findings in a relatively small group of subjects (n=26), generalizing conclusions as to the association between specific craniofacial features and MFS cannot be formulated. To test the null-hypothesis that MFS is not characterized by craniofacial abnormalities, as already rejected by previous reports [Gazit & Lieberman, 1981; Crosher & Homes, 1988; Motohashi, 1985; Poole, 1989; Gorlin et al., 1990; Westling et al., 1998; Cistulli et al., 2001], an attempt must be made to examine families with both affected and healthy subjects of equal gender, and to include stature and airway patency as physical variables. Future studies on the craniofacial structure in MFS, including cephalometrics as well as anthropometric analysis, must address this issue.
Chapter VI

Generalized Joint Hypermobility and Temporomandibular Disorders

Inherited Connective Tissue Disorder as a Model of Disease with Maximum Expression of Capsular Joint Laxity

ABSTRACT

VI.1 INTRODUCTION

VI.2 AIMS

VI.3 MATERIAL AND METHODS

VI.4 RESULTS

VI.5 DISCUSSION

VI.6 CONCLUSION

Parts of this chapter have been accepted for publication as:


1Prof.dr. Linda Van den Berghe (DDS, PhD), Unit for Orofacial Pain and Temporomandibular Disorders, Centre for Special Care, Ghent University Hospital
The objective of this study was to analyse the relationship between generalized joint hypermobility (GJH) and temporomandibular disorders (TMD) by assessing prevalence and characteristics of TMD in a population of patients with maximum expression of generalized joint hypermobility (GJH) as a symptom of inherited connective tissue disease. In addition, the diagnostic reliability of a series of clinical signs indicative of TMJ hypermobility was tested.

The study sample consisted of 42 subjects with GJH, i.e. 24 with Marfan syndrome, and 18 with Ehlers-Danlos syndrome. An adult subgroup of 27 individuals was selected by age (≥18 yrs) and was compared to 40 controls with TMD and normal peripheral joint mobility. TMD diagnoses were assigned to each subject according to the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) [LeResche, 1992]. The RDC/TMD interview and clinical examination were extended with structured queries on pain and TMJ dislocation characteristics.

In the GJH sample (n=42), 71% of the subjects were symptomatic for TMD, of whom 13.3% were seeking treatment. Sixty-nine % were assigned a myofascial pain diagnosis, whereas disk dislocation with reduction was diagnosed in 85.7 % and TMJ arthralgia in 61.9 %. Multiple individual TMD diagnoses were assigned in 69 %, of which 57 % had three or more subgroups diagnoses. Joint noises (P<0.01) and recurrent TMJ dislocations (P<0.01) were a frequent finding in an adult GJH subgroup (n=27) compared to controls, with symptomatic GJH subjects presenting more and prolonged dislocation events than asymptomatic (P<0.001). High diagnostic reliability as clinical signs indicative of condylar hypermobility was computed for a large endfeel distance in the absence of muscular pain (sensitivity 92.9, specificity 88.0), large linear measurements of lateral border positions (sens. 90.5, spec. 92.0), reproducible 'jumps' during mandibular movement (sens. 100, spec. 82.0), a preauricular depression at the end of the opening cycle (sens. 95.2, spec. 84.0), and recurrent TMJ dislocations (sens. 97.6, spec. 90.0). These TMJ hypermobility signs were significantly more expressed in GJH (n=42) compared to controls with TMD and normal joint mobility.

This study suggests a positive relationship between GJH and TMD, indicating that GJH may represent a risk factor for the development of TMD. When examining a patient with TMD and hypermobility characteristics, the practitioner should be suspicious of a connective tissue involvement.

VI.1 Introduction
Generalized joint hypermobility (GJH) is a cardinal feature of inherited connective tissue disease, which encompasses a group of disorders that are considered to be the result of laxity of supporting ligaments. Failure of the structural components of these ligaments is caused by a defective metabolism of collagen or fibrillin, which is caused by mutations in the genes coding for these two extracellular matrix proteins [Beighton et al., 1999]. Joint laxity also occurs without involvement of other structures and has been suggested to predispose to the development of temporomandibular disorders (TMD) and osteoarthritis. To assess conflicting evidence in the literature for association between TMD and generalized joint hypermobility (GJH), and to study the effects of structural joint component laxity on TMD, a model of a disease that has maximum expression should be evaluated [Dijkstra et al., 2002; Perrini et al., 1997]. This is true in the hypermobility type and the classical type of Ehlers-Danlos syndromes (EDS) and in the Marfan syndrome (MFS), both of which diseases share joint laxity as a cardinal feature.

The EDS are a heterogeneous group of heritable connective tissue disorders characterized by articular hypermobility, skin hyperextensibility, increased tissue fragility, and chronic joint and limb pain (see also Chapter I.2.2). The condition comprises 6 major types, each with different molecular basis, and is reflected in mainly structural aberrations of collagen. Most prevalent are the hypermobility EDS type (1:10,000) and the classical EDS type (1:15,000 to 25,000). These conditions account for 85-90% of EDS syndromes, and share an autosomal dominant inheritance, skin hyperextensibility and generalized joint hypermobility as major diagnostic criteria. Whereas extensive tissue fragility and moderate joint hypermobility (causing sprains, recurrent subluxations of the shoulder and patella, and pes planus) are characteristic for the classical EDS type, severe joint hypermobility (displaying most frequently dislocations of the shoulder and patella) is the dominant clinical manifestation in the hypermobility EDS type. Laboratory differential diagnosis includes detection of biochemical abnormalities of collagen type V and type I, and genetic linkage to the genes encoding the pro?1 or pro?2 chains of these collagen types [Beighton et al., 1997]. Instability and recurrent subluxation of the temporomandibular joints (TMJ) have been reported as a prevalent joint sign in hypermobility and classical EDS types by various authors [McKusick, 1972; Norton, 1984; Sadeghi et al., 1989; Fridrich et al., 1990; Sacks et al., 1990; Norton & Assael, 1997; Miller et al., 1997; Letourneau et al., 2001].

The MFS is an autosomal dominant multisystem disorder with a variable phenotype, caused by mutation in FBN1 gene on 15q21 coding for fibrillin-1, an extracellular matrix protein associated with tissues displaying elastic properties (see also Chapter I.2.3). Multiple organ systems are affected, with most features being age-related. Most prominent are skeletal overgrowth, joint hypermobility, subluxation of the eye lens, mitral valve prolapse and dilatation and/or dissection of the ascending aorta. Prevalences of 5-8:100,000 [Gorlin et al., 1990] to 3-4:10,000 have been reported [Dietz & Pyeritz, 2001].
The term TMD embraces a number of clinical problems that involve the masticatory musculature, the TMJ and associated structures, or both. The most frequently presenting symptom is pain, usually localized in the muscles of mastication, the preauricular area, and/or the TMJ. The classification of TMD is hampered by limited knowledge of the cause and progression of these disorders [Von Korff et al., 1988; Oke son, 1996a; List et al., 1999]. Clinical diagnoses of TMD apply criteria identifying abnormalities of structure and function of the muscles of mastication and/or the TMJs, and are divided into 3 groups. Muscle disorders include both painful and non-painful disorders. The common painful muscle disorders comprise myofascial pain (pain of muscle origin, including a complaint of pain as well as pain associated with localized areas of tenderness to palpation in muscle) with or without limited jaw opening (limited jaw movement and stiffness of the muscle during stretching in the presence of myofascial pain). Disc displacements may occur with or without reduction, the latter presenting with or without limited jaw opening. A third group embraces arthralgia (pain and tenderness in the joint capsule and/or the synovial lining of the TMJ), osteoarthritis (an inflammatory condition within the joint resulting from a degenerative condition of the joint structures), and osteoarthrosis (a degenerative disorder of the joint in which joint form and structure are abnormal). To make diagnoses of these groups, it is first necessary to rule out some specific muscle conditions (muscle spasm, myositis and contracture), as well as joint conditions (polyarthrides, acute traumatic injuries and infections in the joint) [LeResche, 1992; Widmer, 1992].

Several studies have been performed to analyze the association between generalized joint hypermobility and TMD [Katzberg et al., 1982; Wright & Hopkins, 1982; Bates et al., 1984; McCarroll et al., 1987; Blasberg & Chalmers, 1989; Crun & Koskines, 1990; Westling & Mattiasson, 1991; Paesaini et al., 1992; Westling et al., 1992; Perrini et al., 1997; Conti et al., 2000; Dijkstra et al., 2002]. The results of these studies are conflicting: some studies yielded an association between TMD and generalized joint laxity, while others could not demonstrate an interrelation. Furthermore, none of these studies did analyze the clinical signs and symptoms of TMJ hypermobility, nor did they address possible underlying connective tissue alterations which might account for elongation of the collateral TMJ ligaments or disk displacement.

VI.2 Aims of the Study

The aim of this study was to assess the prevalence and characteristics of TMD in a population of patients with maximum expression of GJH as a symptom of inherited connective tissue disease. In addition, measurement reliability and the diagnostic validity of a series of clinical signs indicative of TMJ hypermobility were tested. The null hypothesis stated that patients with inherited disorders of
connective tissues, i.e. conditions caused by deficiency of collagen or fibrillin, are not at a higher risk for TMD development compared to the normal population.

VI.3 Material and Methods

VI.3.1 Patient Selection

The study group (hypermobility group, HG) comprised 42 patients with inherited connective tissue disease (15 subjects with hypermobility EDS type, 3 with classical EDS type, and 24 with MFS). Mean age was 27.4 ± 15.5 yrs (age range 6 - 61 yrs) and gender distribution was 26.2% male to 73.8% female. All individuals had been diagnosed clinically and biochemically at the Centre for Medical Genetics, Ghent University Hospital, according to the above mentioned criteria [De Paepe et al., 1996; Beighton et al., 1998]. For comparison with controls (see below) an adult subgroup (n=27) was selected; this subgroup comprised 12 patients with hypermobility EDS type and 15 with MFS (mean age 36.5 ± 11.4 yrs; age range 18-61 yrs; 20.6% male to 79.4% female). Selection of the adult subgroup was made by age (> 18 yrs), since the pool of available control subjects seeking TMD treatment at the Ghent University Hospital exclusively consisted of adults. Forty subjects (n=40) with normal peripheral joint mobility (control group, CG) were individually matched to the adult HG subgroup (n=27) for age and gender, and were included as controls (mean age 36.4 ± 11.8 yrs; age range 19-68; 22.4% male to 77.6% female). To minimize confounding of facial pain assessment, individuals with a history of orofacial trauma, rheumatoid arthritis or whiplash were excluded. The distribution of TMD diagnoses in both adult hypermobile subjects (aged > 18 yrs; n=27) and controls (aged > 18 yrs; n=40) are displayed in Figure VI.1. Definitions of TMD diagnostic groups are listed in Table VI.1.

Figure VI.1. Distribution of individual TMD diagnoses in adult hypermobile population (aged > 18 yrs; n=27) and controls (aged > 18 yrs; n=40)

![Distribution of individual TMD diagnoses in adult hypermobile population](image)

VI.3.2 Methods
Joint mobility was assessed in each individual by determining the mobility score as proposed by Beighton et al. [1973]. The maneuvers used in this scoring system are as follows:

a) passive dorsiflexion of the little fingers beyond 90° (1 point for each hand), 2 points
b) hyperextension of the elbows beyond 10° (1 point for each elbow), 2 points
c) passive apposition of the thumbs to the flexor aspect of the forearm (1 point for each thumb), 2 points
d) hyperextension of the knees beyond 10° (1 point for each knee), 2 points
e) forward flexion of the trunk with the knees fully extended so that the palms of the hands rest flat on the floor, 1 point.

Measurements were made by means of a protractor. The range of scoring was 0–9, with the higher score denoting greater joint laxity. A score of ≥ 3 indicated widespread hypermobility of the peripheral joints [Beighton et al., 1973; Beighton et al., 1999].

The clinical examination and patient interview were based on the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) [LeResche, 1992; Widmer, 1992]. The RDC/TMD provide clinical researchers with a standardized system of methods for recording the history and the clinical signs of functional disturbances in the masticatory system. A dual-axis system is used with known reliability and validity of the applied examination methods [Wahlund et al., 1998; Dworkin et al., 2002]. Axis I measures record clinical physical findings, whereas Axis II scale records behavioral, psychologic and psychosocial status. The diagnostic system as proposed in RDC/TMD is nonhierarchical and allows for the possibility of multiple diagnoses for a given subject. TMD diagnoses are divided into three groups (Table VI.1).

**Table VI.1 Temporomandibular Joint Disorder Diagnoses, according to the RDC/TMD [Dworkin & LeResche 1992]**

<table>
<thead>
<tr>
<th>Group I : Muscle diagnoses (muscle disorders)</th>
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<tbody>
<tr>
<td>I.b. Myofascial pain with limited opening</td>
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<table>
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<tr>
<th>Group II : Disc displacements (disk disorders)</th>
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</thead>
<tbody>
<tr>
<td>II.a Disc displacement with reduction</td>
</tr>
<tr>
<td>II.b Disc displacement without reduction, with limited opening</td>
</tr>
<tr>
<td>II.c Disc displacement without reduction, without limited opening</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group III : Arthralgia, arthritis, arthrosis (joint disorders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.a Arthralgia</td>
</tr>
<tr>
<td>III.b Osteoarthritis of TMJ</td>
</tr>
<tr>
<td>III.c Osteoarthrosis of TMJ</td>
</tr>
</tbody>
</table>

A subject can be assigned at most one muscle (Group I) diagnosis, whereas each joint may be assigned at most one diagnosis of Group II and one diagnosis of Group III. This means that, in principle, a subject can be assigned from zero to five
diagnoses, however cases with more than three diagnoses appear to be rare [LeResche, 1992].

The RDC/TMD patient interview was extended with questions on pain (pain zone, character, pattern frequency, onset, and modifying factors) and TMJ dislocation characteristics, which were scored on a ordinal scale. The latter characteristics comprised frequency of dislocation events (once or more a day, once or more a week, and less than once a week), provoking factors for dislocation onset (spontaneous, random mandibular movement, chewing, speaking, laughing), dislocation pattern (generally starting at the right side, at the left side, no pattern), duration of dislocations (very short, few seconds, more than one minute), reduction of dislocations (spontaneous, manipulative repositioning, assisted repositioning), and consequences of dislocation events (no consequence, stiffness, pain, stiffness and pain). A TMJ dislocation, also known as open lock or subluxation, was defined as a condition in which the condyle is positioned anterior to the articular eminence, and is unable to return to a closed position. It is manifested clinically as an inability to close the mouth without a specific manipulative maneuver. There is usually a clinical history of excessive range of motion that is not painful, but pain can occur at the time of dislocation with residual pain following the episode. Dislocation may be the result of a physical jamming of the disc-condyle complex beyond the articular eminence that is maintained by muscle activity or a true hyperextension of the disc-condyle complex beyond its normal translation position [Okeson, 1996b]. The capsular condition was assessed clinically by means of additional registration of reproducible incoordination and jumps during mandibular movement, evaluation of the quality of TMJ endfeel (normal, hard, soft or stiff, with or without pain) and joint play under distraction (normal, hypomobile or hypermobile, with or without pain) [Ohrbach, 1994], and the presence of a preauricular depression at the end of mandibular opening. Joint endfeel, appearing to be a means for assessing condylar function during the range of motion testing, was assessed during assisted maximal opening by noting the quality of the movement at the end of the assisted opening. Its quality was scored either as normal, hard, soft or stiff, with or without pain. Joint play was performed to test the capsular ligaments by applying caudal force on the joint, permitting a discrimination between joint and muscle as sources for restriction. The quality of the movement on caudal joint distraction was classified either as normal, hypomobile or hypermobile, with or without pain [Ohrbach et al., 1990]. A preauricular depression was defined as the clinical presence of an extra-oral depression in front of the external auditory meatus and situated at the lateral pole of the condyle, presenting at the end of the mandibular opening cycle, and was scored positive if assessed by combined observation and palpation [Conti et al., 2000]. Radiological assessment of condyle hypertranslation, i.e. the condyle excessively passing the eminentia at the translation phase of mandibular opening [Katzberg et al., 1982; Dworkin et al., 2002] was not performed due to the extent of the examined population.
Inter-examiner agreement was tested using Kappa statistics (Cohen’s Kappa or ?) [Dworkin et al., 1990] for nominal and ordinal variables, such as palpation for muscle (at 16 different sites; ? = 0.60) and joint tenderness (lateral and posterior pole; ? = 0.78), evaluation of the quality and occurrence of joint sounds on vertical opening (? = 0.69) and with excursive movements (? = 0.65), and registration of the jaw opening pattern (? = 0.78). All Kappa’s guaranteed an acceptable level of examiner agreement. Intraclass correlation coefficients (ICC) were computed for continuous variables, such as linear measurements of mandibular border positions, and ranged from 0.92 to 0.99, indicating excellent reliability between the calibrated examiners. The obtained values for both reliability analysis methods proved acceptable compared to previously reported values (Table VI.2) [Dworkin et al., 1990; Wahlund et al., 1998; Goulet et al., 1998].

Table VI.2 Inter-examiner reliability for measurements of TMD clinical signs

<table>
<thead>
<tr>
<th>Clinical sign</th>
<th>Statistics*</th>
<th>Tested</th>
<th>95% CI</th>
<th>Reported**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical dimension</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unassisted opening without pain (mm)</td>
<td>ICC</td>
<td>0.97</td>
<td>0.82 - 1.10</td>
<td>0.90 - 0.94</td>
</tr>
<tr>
<td>Maximum unassisted opening (mm)</td>
<td>ICC</td>
<td>0.99</td>
<td>0.86 - 1.12</td>
<td>0.96 - 0.98</td>
</tr>
<tr>
<td>Maximum assisted opening (mm)</td>
<td>ICC</td>
<td>0.99</td>
<td>0.87 - 1.15</td>
<td>0.94 - 0.98</td>
</tr>
<tr>
<td>Jaw opening pattern</td>
<td>K</td>
<td>0.78</td>
<td>0.64 - 0.91</td>
<td>0.56 - 0.7c</td>
</tr>
<tr>
<td>Jaw excursions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral excursions (mm)</td>
<td>ICC</td>
<td>0.98</td>
<td>0.81 - 1.12</td>
<td>0.67 - 0.97</td>
</tr>
<tr>
<td>Protruded movement (mm)</td>
<td>ICC</td>
<td>0.92</td>
<td>0.78 - 1.03</td>
<td>0.30 - 0.66</td>
</tr>
<tr>
<td>Joint sounds on vertical opening (on palpation)</td>
<td>K</td>
<td>0.69</td>
<td>0.46 - 0.89</td>
<td>0.62 - 0.75</td>
</tr>
<tr>
<td>Joint sounds with excursive movements (on palpation)</td>
<td>K</td>
<td>0.65</td>
<td>0.52 - 0.78</td>
<td>0.37 - 0.75</td>
</tr>
<tr>
<td>Pain with function/movement (mean)</td>
<td>K</td>
<td>0.80</td>
<td>0.69 - 0.97</td>
<td>0.63 - 0.83</td>
</tr>
<tr>
<td>Pain on palpation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Masticatory muscles (mean of 16 palpation sites)</td>
<td>K</td>
<td>0.60</td>
<td>0.49 - 0.74</td>
<td>0.52 - 0.86</td>
</tr>
<tr>
<td>TMJ (2 palpation sites)</td>
<td>K</td>
<td>0.78</td>
<td>0.67 - 0.89</td>
<td>0.52 - 0.84</td>
</tr>
</tbody>
</table>

* ICC : intraclass correlation coefficient (> 0.90 excellent; 0.80 - 0.89 good; 0.70 - 0.79 acceptable; < 0.70 not acceptable); K = Cohen’s Kappa (> 0.8 excellent; 0.6 - 0.8 good; 0.4 - 0.6 acceptable; < 0.4 not acceptable)

** after Wahlund et al.(1998); Dworkin et al.(1990); Goulet et al.(1998)

Statistics were performed with Fischer’s exact test and chi-square test for comparison of proportions, Mann-Whitney U-test (unpaired Wilcoxon test) to analyze the effect of qualitative factors on continuous variables, and rank correlation analysis to describe the relationship between two continuous variables. Receiver operating curve analysis (ROC) was used to compute sensitivity and specificity of a series of clinical TMJ hypermobility signs. Differences at the P≤0.05 level were considered statistically significant [Clayton & Hills, 1993; Petrie & Sabin, 2000].

The study design had previously been approved by the Ethical Committee of Ghent University Hospital (ref. 2000/ 308-309). A written informed consent was obtained from all enrolled subjects.
VI.4 Results

VI.4.1 Clinical assessment of hypermobility of the peripheral joints

A Beighton sum score ≥ 3 was found in 88.1% of the hypermobile study group (n=42), of which 73.8% presented with a score ≥ 5 (median 5.0 ± 0.37). In 11.9% the Beighton criteria for GJH were not met (< 3) because of chronic joint pain, oedema or chronic stiffness. In the adult GJH subgroup (n=27), a score ≥ 3 was recorded in 88.9% of the subjects (median 5.8 ± 2.7). No interrelations could be assessed between Beighton sum score and gender, age or disease subtype. No individual in CG (n=40) had a Beighton score ≥ 3 (0%), which was indicative of normal peripheral joint mobility.

Table VI.3 Individual TMD subgroup diagnoses in hypermobile population (n=42)

<table>
<thead>
<tr>
<th>Individual diagnosis</th>
<th>n</th>
<th>Proportion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No TMD diagnosis</td>
<td>3</td>
<td>7.1%</td>
</tr>
<tr>
<td>Single TMD group diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group Ia</td>
<td>0</td>
<td>0.0%</td>
</tr>
<tr>
<td>Group IIa (uni- or bilateral)</td>
<td>9</td>
<td>21.4%</td>
</tr>
<tr>
<td>Group IIIa (uni- or bilateral)</td>
<td>1</td>
<td>2.4%</td>
</tr>
<tr>
<td>Combined TMD group diagnosis</td>
<td>29</td>
<td>69.1%</td>
</tr>
<tr>
<td>Group I + II</td>
<td>5</td>
<td>11.9%</td>
</tr>
<tr>
<td>Group I + II + III</td>
<td>24</td>
<td>57.2%</td>
</tr>
</tbody>
</table>

* A subject can be assigned at most one muscle diagnosis (Group I), whereas each joint may be assigned at most one diagnosis from Group II and one diagnosis from Group III (RDC/TMD) (after LeResche, 1992)

VI.4.3 TMD prevalence in hypermobile population

Table VI.3 shows the distribution of TMD in the hypermobile study population (HG, n=42). Sixty-nine percent of the subjects were assigned myofascial pain diagnosis (Group I.a), whereas unilateral and bilateral disc displacement with reduction (Group II.a) were diagnosed in resp. 9.5% and 76.2%. TMJ arthralgia (Group III.a) was assigned unilaterally or bilaterally in resp. 23.8% and 38.1%. When individual TMD diagnoses were analyzed, 7.1% of the individuals were found to have no diagnosis, and 23.8% were assigned a single diagnosis (i.e. 7.1% unilateral and 14.3% bilateral disc displacement with reduction, and 2.4% bilateral TMJ arthralgia). Multiple TMD diagnoses were assigned in 69.1% of the individuals, of which 57.1% presented with three or more different RDC/TMD group diagnoses. 71.5% of the cases were symptomatic (i.e. 30:42) (i.e. reporting pain or tenderness of masticatory muscles and TMJ, joint sounds and/or limitation or disturbance of mandibular movement), indicating a strong tendency
for development of TMD in patients with GJH. Of these symptomatic subjects 13.3\% (i.e. 4:30) were asking for treatment.

In the adult HG subgroup (n=27), 70.4\% was assigned myofascial pain diagnosis (I.a), 96.3\% disc displacement with reduction (II.a) (14.8\% unilaterally and 81.5\% bilaterally), and 59.2\% TMJ arthralgia (III.a) (18.5\% unilaterally and 40.7\% bilaterally). All subjects had TMD (100\%), whereas 92\% was assigned multiple TMD diagnoses. In CG (n=40), 85\% had a muscle diagnosis (Group I), i.e. 35\% had myofascial pain (I.a) and 50\% myofascial pain with limited jaw opening (I.b). Disc displacement diagnoses were assigned bilaterally in 60\% of CG: 52.5\% had disc displacement with reduction (II.a) and 7\% had disc displacement without reduction and limited jaw opening (II.b). TMJ arthralgia diagnosis (III.a) was assigned in 37.5\%. Eighty-five \% had multiple TMD diagnosis (Figure VI.1).

VI.4.4 Pain history and pain characteristics in symptomatic hypermobile population

Single myofascial pain diagnosis (MP) was significantly less prevalent in adult HG subgroup (11\%) compared to CG (50\%) (P=0.001). Myofascial pain with unilateral or bilateral arthralgia (MPA) occurred in a higher rate in adult HG (59\% vs. 35\% in CG), but was not significantly different (P=0.071) (Table VI.4).

Table VI.4 Individual TMD pain diagnosis in adult hypermobile (HG) and control groups (CG)

<table>
<thead>
<tr>
<th></th>
<th>HG (n=27)</th>
<th></th>
<th>CG (n=40)</th>
<th></th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>myofascial pain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>myofascial w/ arthralgia</td>
<td>3</td>
<td>11</td>
<td>20</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>59</td>
<td>14</td>
<td>35</td>
<td>0.071</td>
</tr>
</tbody>
</table>

* Fischer's exact test; differences at P<0.05 level are considered significant

Joint noises (96\% in HG vs. 60\% in CG) and TMJ subluxations (100\% in HG vs. 0\% in CG) were significant findings (both P<0.001) in adult HG (n=27). For psychosocial history being recorded, interpersonal factors (i.e. social or family problems; P=0.013) and emotional status (i.e. depression, anxiety; P=0.046) were assessed significantly more frequently as contributing psychosocial factors in TMD etiology in HG.

There were no significant differences in pain character (i.e. localized, migrating, spreading or irradiating character) between adult HG, CG and TMD subgroups (P=0.455 for MP; P=0.595 for MPA). With regard to pain onset (i.e. spontaneous, provoked or triggered onset) and pain frequency no differences were established between the distinct groups (P=0.811 for MP; P=0.333 for MPA).
However a diurnal pain pattern was frequently reported in symptomatic HG (67% among MP, and 75% among MPA), no significant differences were found compared to symptomatic controls (P=0.602 for MP; P=0.540 for MPA). The findings on pain history and pain characteristics in HG (n=42) were comparable to those in the adult HG subgroup (n=27).

VI.4.5 TMJ dislocation characteristics in hypermobile population

When comparing adult symptomatic and asymptomatic subjects with GJH (HG, n=42), i.e. individuals with or without a report of pain or tenderness in the masticatory system, a significant difference was found in occurrence of TMJ dislocations: 73% of the symptomatic cases reported dislocations to occur once or more a day, whereas pain-free subjects reported an occurrence of once or more per week in 58% (P<0.001) (Table VI.5). There was no significant difference concerning the factors provoking dislocations between symptomatic and asymptomatic subjects (P=0.777). Duration of the dislocations was generally longer in symptomatic HG (P<0.001). No interrelation was found between these dislocation characteristics and age, other clinical observations (e.g. hypermobility score, range of mouth opening), or pain history records (e.g. system involvement, pain character, pain onset) among GJH subjects. TMJ dislocation was not recorded in controls.

Table VI.5 TMJ dislocation characteristics among symptomatic and asymptomatic hypermobile subjects (n=42): TMJ dislocation occurrence

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic (n=12)</th>
<th>Symptomatic (n=30)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>once or more a day</td>
<td>1</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>once or more a week</td>
<td>7</td>
<td>58</td>
<td>5</td>
</tr>
<tr>
<td>less frequent</td>
<td>4</td>
<td>34</td>
<td>3</td>
</tr>
</tbody>
</table>

* Chi-square test (2 x 3 table); differences at P<0.05 level are considered significant

VI.4.6 Evaluation of clinical TMJ hypermobility signs

A series of clinical observations, indicative of the character and the extent of condylar mobility, was tested for measurement reliability and diagnostic validity. Although linear measurement of mandibular border positions is not considered as a highly reliable method for assessing condylar (hyper)mobility [Westling & Helkimo, 1992; Szenpétery, 1993; Winocur et al., 2000], active range of opening movement (AROM) showed significantly greater in both sexes in HG (n=42) (HG males 52.5 ± 5.2 mm vs. male controls 41.4 ± 7.4 mm: P<0.001; HG females 47.4 ± 6.1 mm vs. female controls 36.9 ± 9.1 mm: P<0.001). Unpaired
Wilcoxon tests calculated AROM to be significantly influenced by gender in HG (P<0.001), and by the presence of self-reported pain (P<0.001), pain on excursion (P<0.001), and pain on palpation (P=0.048) (Table VI.6). Rank correlation analysis yielded no association between age (Spearmann’s coefficient $r_s = -0.113$, $P=0.469$), body height ($r_s = 0.183$, $P=0.183$) or hypermobility score ($r_s = 0.189$, $P=0.225$) and AROM.

Table VI.6  **Mann-Whitney U-test (unpaired Wilcoxon test) for qualitative factors influencing the active range of mandible opening (AROM) in hypermobile population (n=42)**

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Qualitative variable</th>
<th>Subgroup</th>
<th>Mean</th>
<th>SD</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal opening (mm)</td>
<td>Gender</td>
<td>Male</td>
<td>52.50</td>
<td>1.56</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td>47.50</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Self-reported pain</td>
<td>Pain</td>
<td>48.04</td>
<td>1.23</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>No pain</td>
<td>50.52</td>
<td>1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain on excursion</td>
<td>Pain</td>
<td>48.10</td>
<td>1.22</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>No pain</td>
<td>50.39</td>
<td>1.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain on movement</td>
<td>Pain</td>
<td>48.67</td>
<td>1.06</td>
<td></td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>No pain</td>
<td>49.84</td>
<td>2.11</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Significant at P<0.05 level

Intra-examiner consistency showed good to excellent for all variables (Table VI.7). Since there were no significant differences in the expression of these clinical signs or symptoms between adults and children in HG, the data were pooled. Sensitivity and specificity, and the positive (PLR) and negative likelihood ratio (NLR) of the clinical observations are displayed in Table VII.8. A large endfeel distance in the absence of muscular pain (sensit. 92.9; specif. 88.0), large linear measurements of lateral border positions (sensit. 90.5; specif. 92.0), reproducible ‘jumps’ during mandibular movement (sens. 100; specif. 82.0), a preauricular depression (sens. 95.2; specif. 84.0), and recurrent TMJ dislocations (sens. 97.6; specif. 90.0) seem to be useful as reliable clinical signs indicative of increased condyle mobility (as a result of structural capsular laxity). These variables had a high ‘area under ROC curve’-value (AUC ? 0.900) indicative of excellent discriminatory diagnostic capacities [Clayton & Hills, 1993; Petrie & Sabin, 2000].

VI.4.7  **Power of the Study**

Techniques measuring the statistical power of a study estimate the probability that a study could establish a statistically significant difference if a specified size-true difference actually exists in the larger population (see also Chapter III.4.6). A post-hoc power analysis was performed on selected outcome variables with assumed high diagnostic relevance for the different items being
investigated [Power Calculator, http://calculators.stat.ucla.edu/powercalc]. According to the plural objective of the present study, the statistical power of three main findings with assumed high clinical and/or diagnostic relevance was tested.

### Table VI.7 Intra-examiner reliability for measurements and clinical observations indicative of TMJ (hyper)mobility

<table>
<thead>
<tr>
<th>Clinical observation</th>
<th>Statistics*</th>
<th>Intra-examiner Reliability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximal unassisted mandibular opening (mm)</td>
<td>ICC</td>
<td>0.96</td>
</tr>
<tr>
<td>Endfeel distance</td>
<td>ICC</td>
<td>0.92</td>
</tr>
<tr>
<td>Hyperelastic endfeel quality</td>
<td>K</td>
<td>0.90</td>
</tr>
<tr>
<td>Lateral excursions (mm)</td>
<td>ICC</td>
<td>0.89</td>
</tr>
<tr>
<td>Reproducible incoordination of mandibular movement</td>
<td>K</td>
<td>0.89</td>
</tr>
<tr>
<td>Reproducible jumps during mandibular excursions</td>
<td>K</td>
<td>0.88</td>
</tr>
<tr>
<td>Preauricular depression at end of opening cycle</td>
<td>K</td>
<td>0.83</td>
</tr>
</tbody>
</table>

* ICC: intraclass correlation coefficient (> 0.90 excellent; 0.80 - 0.89 good; 0.70 - 0.79 acceptable; < 0.70 not acceptable); K = Cohen’s Kappa (> 0.80 excellent; 0.6 - 0.8 good; 0.4 - 0.6 acceptable; < 0.4 not acceptable)

### Table VI.8 Sensitivity and specificity of TMJ (hyper)mobility clinical signs and symptoms (hypermobiles n=42; controls n=40)

<table>
<thead>
<tr>
<th>Clinical observation</th>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PLR</th>
<th>NLR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active range of opening movement (AROM-males)</td>
<td>50.1 mm*</td>
<td>72.7</td>
<td>89.0</td>
<td>6.55</td>
<td>0.31</td>
</tr>
<tr>
<td>Active range of opening movement (AROM-females)</td>
<td>45.0 mm*</td>
<td>71.0</td>
<td>87.1</td>
<td>5.50</td>
<td>0.33</td>
</tr>
<tr>
<td>Endfeel distance</td>
<td>3.6 mm*</td>
<td>92.9</td>
<td>88.0</td>
<td>7.74</td>
<td>0.08</td>
</tr>
<tr>
<td>Endfeel hyperelasticity</td>
<td>present</td>
<td>97.6</td>
<td>38.0</td>
<td>1.57</td>
<td>0.06</td>
</tr>
<tr>
<td>Lateral excursions (sum; mm)</td>
<td>19.5 mm*</td>
<td>90.5</td>
<td>92.0</td>
<td>11.31</td>
<td>0.10</td>
</tr>
<tr>
<td>Reproducible incoordination of mandibular movement</td>
<td>&gt;3 trials</td>
<td>95.2</td>
<td>62.0</td>
<td>2.51</td>
<td>0.08</td>
</tr>
<tr>
<td>Reproducible jumps during mandibular excursions</td>
<td>&gt;3 trials</td>
<td>100.0</td>
<td>82.0</td>
<td>5.56</td>
<td>0.00</td>
</tr>
<tr>
<td>Preauricular depression at end of opening cycle</td>
<td>present</td>
<td>95.2</td>
<td>84.0</td>
<td>5.95</td>
<td>0.06</td>
</tr>
<tr>
<td>Recurrent TMJ dislocations</td>
<td>&gt;once a week</td>
<td>97.6</td>
<td>90.0</td>
<td>9.76</td>
<td>0.03</td>
</tr>
</tbody>
</table>

PLR: positive likelihood ratio: true positive rate / false positive rate; NLR: negative likelihood ratio: false negative rate / true negative rate.

* The cutoff values for mandibular border positions were calculated for the measurements obtained in this specific population (males-females and young-adults pooled, except for AROM).

With regard to the postulation that TMJ dislocations are pathognomonic for GJH, as observed in adult GJH (n=27) compared to matched controls (n=40) (see also VI.4.4), the power of the test was found to be 1.0. As for the postulation that TMJ dislocations have high clinical significance as to the predisposition for TMD development, as observed among symptomatic and non-symptomatic individuals with GJH.
(n=42) (Table VI.5), the power was 0.99. With respect to the postulation that adult 
subjects with GJH may develop more often multiple TMDs than controls with normal 
peripheral joint mobility, as observed in adult GJH (n=27) compared to matched 
controls (n=40) (Table VI.4), the power of the test was 0.95.

VI.5 Discussion

Conflicting evidence exists in the literature on the role of generalized joint 
hypermobility (GJH) as a potential risk factor for TMD development [Katzberg et 
al., 1982; Wright & Hopkins, 1982; Bates et al., 1984; McCarroll et al., 1987; 
Blasberg & Chalmers, 1989; Crun & Koskines, 1990; Westling & Mattiasson, 1991; 
Paesaini et al., 1992; Westling, 1992; Perrini et al., 1997; Conti et al., 2000; Dijkstra et 
al., 2002]. An important aspect contributing to inconsistency in literature, is the 
problem how to define and to assess GJH. First, there is major controversy on the 
reported prevalence of GJH, which is entirely determined by the study population, 
reflecting dependence of hypermobility on age, gender, family and ethnic 
background [Dijkstra et al., 2002]. Previous studies have exclusively documented 
on the incidence of signs and symptoms of TMD in heterogeneous populations 
with assumed benign GJH [Katzberg et al., 1982; Wright & Hopkins, 1982; Bates et 
al., 1984; McCarroll et al., 1987; Blasberg & Chalmers, 1989; Crun & Koskines, 1990; 
Westling & Mattiasson, 1001; Paesaini et al., 1992; Westling, 1992; Perrini et al., 
1997; Conti et al., 2000; Dijkstra et al., 2002], a disorder where joint laxity occurs 
without a known underlying collagen or fibrillin defect. Both benign GJH, benign 
joint hypermobility syndrome (BJS), and GJH as a symptom of connective tissue 
disease, are supposed to result from capsular laxity, which is generally caused by 
structural alterations in the supporting ligaments [Wahlund et al., 1998]. By 
definition, BJHS is not synonymous to (benign) GJH: BJHS is said to exist when 
hypermobility becomes symptomatic, and as a rule has more serious (poly-
articular) joint involvement [Kirk et al., 1967; Beighton et al., 1999]. There is no 
agreement on the fact whether the molecular cause of joint hypermobility is 
different between individuals having GJH and those developing BJHS. Little is still 
known about the biochemical cause and the evolution of joint pathology in BJHS 
[Grahame, 1999; Baum & Larsson, 2000; Grahame et al., 2000; Magnusson et al., 
2001]. Therefore, BJHS should not be evaluated as a model of disease in order to 
study the effects of structural joint component laxity on TMD.

Generalized capsular laxity usually is assessed clinically using the 
Beighton scale [Beighton et al., 1973; Beighton, 1993; Beighton et al., 1999]. The 
score is obtained by measurement of the mobility of five peripheral joints, with a 
sum score of 3/9 or greater defining hypermobility [Beighton et al., 1973]. Previous 
studies have set their cut-off value at score 3 [McCarroll et al., 1987; Westling, 1989; 
Westling & Helkimo, 1992] or 4 [Perrini et al., 1997; Winocur et al., 2000], or have 
only assessed 1 or 2 joints on the dominant side [Crun & Koskines, 1990; Harkins et 
al., 1995], which has produced confusing evidence. The cut-off value of Beighton ? 
3 allows for refinement of GJH diagnosis in terms of poly- and pauci-articular (by 
definition, less than five joints involved) varieties [Beighton et al., 1973; Beighton et
Epidemiological studies have shown that joint hypermobility (depending on the population and the criteria used) is seen in 3% in Western [Larsson et al., 1987] up to 10% in Middle-Eastern [Al-Rawi et al., 1985] and 25% in African populations [Birrell et al., 1994], with a rapid decrease in the first decade of life [Beighton et al., 1973; Wahlund et al., 1998]. The majority of these studies looked at generalized joint hypermobility (GJH), but it is known that pauci-articular hypermobility is more prevalent than the poly-articular variety [Larsson et al., 1987; Grahame, 2000]. The overall methodological quality of studies on GJH and TMD also varied considerably, thus influencing the possible association between GJH and TMD [Dijkstra et al., 2002]. Therefore, some authors suggested selection bias could be minimized by studying populations with inherited connective tissue disease as a model of disease with maximum expression of GJH [Perrini et al., 1997; Dijkstra et al., 2002]. As TMJ ligaments and disc basically consist of a fibrous network of collagen types I, II and V, and elastin [Gage et al., 1990], EDS and MFS were selected as models of disease with known molecular alterations in connective tissue [Perrini et al., 1997; Letourneau et al., 2001].

Second, assessment of GJH may be confounded by a number of factors, including pain of myofascial origin, joint oedema and stiffness, often manifesting in a circadian pattern. For these reasons, the Beighton sum score should be seen as a random indication of peripheral joint mobility standing for the moment of assessment. This explains the presence of a low GJH score in 11.9% of the study subjects.

Third, there is no substantial evidence to assess an interrelation between TMJ hypermobility and hypermobility of the five peripheral joints evaluated in the Beighton sum-score [Perrini et al., 1997; Dijkstra et al., 2002]. Consequently, when looking for potential etiologic factors for TMD, restraint is called for assignment of GJH diagnosis in subjects not affected with an inherited connective tissue disorder.

The present study indicates a positive association between structural GJH and TMD. It also confirms reported associations of GJH with a variety of complaints of the general locomotor system, such as myalgia, arthralgia (up to 38% in HG), dislocation of major and peripheral joints (TMJ dislocations in 100% of subjects with GJH), and soft tissue lesions [Dijkstra et al., 2002]. There were no significant differences in patient and pain characteristics among the symptomatic HG and CG, except for psychosocial factors (P=0.013). The low treatment need in the symptomatic HG (13.3% of all subjects reporting of pain) may be the result of major somatic problems crowding out TMD-related discomfort in the syndromes. Social problems and emotional instability may be the result of the debilitating nature of inherited connective tissue disorders, and may substantially contribute to TMD development.

Fifty-nine percent of HG subjects were diagnosed with myofascial pain with unilateral or bilateral arthralgia (MPA), compared to 35% in CG. However not statistically significant (P=0.071), this finding is consistent with former reports of arthralgia being associated with joint instability [Westling & Helkimo, 1992].
Occurrence of joint noises (96% in HG vs. 60% in CG) and dislocations (100% in HG vs. 0% in CG) were statistically significant findings (P<0.001), confirming previous reports on interrelation between joint clicks and dislocations [Widmer, 1992; Wahlund et al., 1998]. In HG, AROM was significantly dependent on gender (greater in males; P<0.001), and the presence of self-reported pain (P<0.001), pain on excursion (P<0.001), and pain on palpation (P=0.048) (Table VII.6), as was previously reported in healthy subjects [Westling & Helkimo, 1992]. The AROM in HG, contrary to the expectations in GJH, was low compared to previous reports on AROM in healthy individuals [Katzberg et al., 1982; Wright & Hopkins, 1982; McCarroll et al., 1987; Westling & Helkimo, 1992; Szenpétery, 1993; Conti et al., 2000], but this might be explained by their TMD.

Although a high occurrence of osteoarthrosis was reported in hypermobile TMJ of healthy patients [Stegenga et al., 1993], no degenerative disorders of this nature were found in the study group. Since degenerative TMJ disorders are known to occur with increasing age, the latter finding may be influenced by the age distribution in the sample group.

At present, there is no general agreement on which level the TMJ should be classified as hypermobile. Radiographically, TMJ hypermobility has been postulated when the condyle is excessively passing the articular eminence at the translation phase of mandibular opening [Katzberg et al., 1982; Stegenga et al., 1993]. Epidemiological surveys in healthy populations with normal TMJ mobility, however, yielded prevalences of radiographically assessed condylar hypertranslation up to 39%, contesting the diagnostic validity of this criterion [Meng et al., 1987]. Despite of a considerable number of reports on the subject, there are yet no validated criteria for clinical assessment of TMJ hypermobility. Since the range of mandibular movements is reported to be closely related to facial morphology [Ingervall, 1971; Agerberg, 1974], linear measurement of mandibular border positions generally is not considered as a highly reliable method for assessing condylar (hyper)mobility [Westling & Helkimo, 1992; Szenpétery, 1993; Winocur et al., 2000]. Only a few weak correlations were found between linear measurement of maximal mandibular opening capacity and peripheral joint mobility either at active or assisted range of motion [McCarroll, 1987; Plunkett & West, 1988; Westling & Helkimo, 1992].

Recurrent TMJ dislocations were commonly recorded during the structured patient interview as a consequence of capsular laxity. This study also shows that the occurrence and duration of dislocations are higher, respectively longer, in symptomatic GJH individuals compared to asymptomatic. Further research is needed to elucidate the contribution of dislocations to the development of TMD. Table VII.8 displays a series of TMJ hypermobility clinical signs or symptoms which are more expressed in (adult) GJH compared to (adult) subjects with TMD and normal joint mobility. A hyperelastic endfeel with large endfeel distance, and a preauricular depression on maximum opening have been suggested as clinical indications of condyle hypertranslation [Conti et al., 2000],...
but these signs lacked epidemiological validation. Moreover, joint endfeel quality and a preauricular depression may be hard to establish in the presence of pain. Two other frequent clinical observations in patients with capsular laxity, such as reproducible incoordination of mandibular movement (on three or more consecutive trials and occurring during both vertical and horizontal excursions), and ‘jumps’ during these movements [Conti et al., 2000], also yielded an acceptable diagnostic reliability in this study. However, since the cut-off values for clinical TMJ hypermobility measurements are entirely dependent on the population, it is recommended to rely rather on a combination of these indicative signs than to use them as absolute criteria for assignment of condylar hypermobility diagnosis. This is certainly true in the linear measurement of AROM in the presence of myofascial pain. Future analysis of potential risk factors and confounders in larger hypermobile populations (children vs. adults) may provide the clinician with odds ratios of risk factors and a better insight into the role of structural capsular defects for TMD development.

The present study analyses signs and symptoms of TMD in a population consisting of patients with a clear clinical, biochemical and genetical diagnosis of EDS or MFS. Both disorders are intrinsically characterized by GJH, which is caused by aberrations in the biosynthesis of connective tissue components, leading to structural alterations of joint components. A recommendation to examine the peripheral joints in any TMD patients cannot be offered on the basis of this study. However, since a high score on the Beighton scale may indicate severe connective tissue involvement, an additional exam needs to be performed in any patient presenting with TMD and hypermobility characteristics. In such cases, the examiner should be suspicious of a connective tissue involvement.

VI.6 Conclusion

The present study aimed to assess TMD in a population with inherited connective tissue disorder as a model of disease with maximum expression of GJH. The data on prevalence and patient characteristics of TMD in this population indicate a positive association between GJH and TMD, the greater part presenting individually a combination of myofascial pain and disk displacement, associated with uni- or bilateral TMJ arthralgia. A series of clinical signs indicative of condylar hypermobility is presented, together with computed values for sensitivity and specificity. Reliable discriminatory diagnostic capacities for assessment of endfeel distance in the absence of muscular pain, linear measurement of lateral border positions, reproducible ‘jumps’ during mandibular movement, a preauricular depression at the end of the opening cycle, and recurrent TMJ dislocations, are also shown. Linear measurement of maximal unassisted mouth opening in GJH is significantly greater compared to controls, lacks ‘normal’ association with body height and masticatory or TMJ pain symptoms, but proves to be related to gender. The findings suggest that, as in healthy subjects, the vertical range of mandibular movement in GJH is not a reliable instrument for assessing condylar (hyper)mobility. Recurrent TMJ dislocations (once or more a
day, with a duration of several seconds) are a frequent finding in symptomatic GJH compared to asymptomatic, but their contribution to TMD development remains elusive.

Chapter VII
Dentin Structure in Abnormal Collagen I Metabolism

Abstract

Dentin matrix of teeth from three patients with different types of abnormal collagen I metabolism and unaffected controls was compared by light and transmission electron microscopy. Ultrastructure of the pathological samples was commonly characterized by great variation between different areas. In both OI III with DI, and EDS
VII.1C, the dentinal tubules appeared more scattered or clustered with increasing irregularity of the dentin matrix. They had an irregular cross-sectional form, surrounded by sparse and wavy collagen fibers. Giant tubules/canal-like structures were seen, reflecting inclusion of capillaries during pathological dentinogenesis. In EDS I with unusual COL1A1 mutation, the size and distribution of tubules was less uniform than in controls. Giant tubules were scattered among normal ones throughout the dentin matrix. The present histological and ultrastructural findings reflect the different abnormalities in the organization of the collagen I meshwork, serving as a scaffold for dentin mineralization.

The present findings indicate that different types of collagen I deficiency, resulting from different gene mutations, may cause similar abnormalities of dentin matrix with distinct varieties of structures. Ultrastructural evidence of disrupted dentinogenesis may also be found in clinically unaffected teeth in these patients. Histological analysis of dentin matrix may be considered a valuable diagnostic tool in the assessment of collagen I deficiency. On the basis of this study, it can be recommended to examine the connective tissues in any patients presenting with clinical and/or radiographical features of dentin defects.

Abbreviations

DI dentinogenesis imperfecta; DD dentin dysplasia; OI Osteogenesis Imperfecta; EDS Ehlers-Danlos syndromes; COL1A1* gene encoding the pro-a1(I) chain of type I collagen; COL1A2* gene encoding the pro-a2(I) chain of type I collagen; ADAMTS2* gene encoding the enzyme procollagen-I-N-proteinase; DSPP* gene encoding dentin sialoprotein and dentin phosphoprotein

* By international convention, human gene symbols should be written in italicized capital letters and Arabic numerals [HUGO Gene Nomenclature Committee; http://www.gene.ucl.ac.uk/nomenclature].

VII.1 Introduction

The collagens comprise a diverse family of structurally related extracellular matrix proteins, having a varied set of functions involving structural, developmental (organogenesis, cell adhesion, cell movement) and physiological
(wound healing, hemostasis) processes. Type I collagen (collagen I) is the most abundant and ubiquitously distributed of the collagen proteins. It is a heterotrimer composed of two different polypeptide chains (pro-a chains), i.e. two pro-a1(I) chains and one pro-a2(I) chain. Collagen I provides the structural framework for a number of mineralized (bone, dentin) and unmineralized tissues (skin, muscle, tendon), giving them strength and resilience [Berkovitz, 2002]. Mutations in the genes encoding the two pro-a chains (COL1A1 for the pro-a1(I) chain, COL1A2 for the pro-a2(I) chain) or in genes coding for enzymes essential to collagen I synthesis, result in deficiency of the biosynthetic end product, i.e. the collagen I molecule. Hence, the association of aberrant collagen I molecules into fibrils may be disrupted, leading to a wide variety of clinical manifestations, such as brittle bones, blue sclerae, joint hypermobility and increased tissue fragility. Mutations in COL1A1 or COL1A2 result in the heritable connective tissue disorders Osteogenesis Imperfecta (OI) and Ehlers-Danlos syndromes (EDS) types VIIA and VIB [Dalgleish, 1997]. Mutation in ADAMTS2, the gene encoding procollagen-I-N-proteinase, the enzyme that excises the N-terminal propeptide in procollagen type I and II, results in Ehlers-Danlos syndrome type VIIIC (human dermatosparaxis). As a consequence, there is accumulation of pN-procollagen I (type I procollagen that still contains the N- but not the C-propeptide) (Nusgens et al., 1992), resulting in polymerization of abnormal collagen fibers.

Collagen I makes up the bulk of dentin, providing a scaffold for intra- and interfibrillar deposition of hydroxyapatite crystals during late dentinogenesis. Both aberrations in collagen fibril formation and mineralization of pre dentin matrix may result in structural defects of dentin. In the current classification of human heritable dentin defects two main groups are recognized: dentinogenesis imperfecta (DI) and dentin dysplasia (DD). DI is divided into types I-III and DD into types I and II [Shields et al., 1973]. Several syndromes are associated with dental findings which are clinically and radiographically similar to those in type II DI [Kantapoutra, 2001]. The classification of heritable dentin defects is essentially based on clinical and morphological findings. DI and DD share many clinical, radiographic and histopathological features [Ranta et al., 1993]. Type I DI has been linked to collagen I deficiency in OI, whereas both type II DI and type I DD, occurring as a single trait, have been linked to mutations in DSPP. The latter gene on chromosome 4 has been reported to code for two dentin-specific proteins, i.e. dentin sialoprotein and dentin phosphoprotein, which are both crucial in initiation of mineralization and crystal organization [Ritchie et al., 1998].

VII.2 Aims of the Study

The purpose of this study was to examine and compare the ultrastructure of dentin matrix of teeth from three patients with different types of collagen I deficiency and controls. In addition, it was assessed whether histological analysis
of dentin matrix may represent a valuable tool in diagnostics of collagen I deficiency.

VII.3 Material and Methods

VII.3.1 Patients and Mutational Analysis of Abnormal Type I Collagen Metabolism

Patient 1 - Osteogenesis Imperfecta Type III with Dentinogenesis Imperfecta. Osteogenesis Imperfecta (OI) [OMIM Entry 166220] [Online Inheritance in Man; http://www3.ncbi.nlm.nih.gov/Omim] is a clinically and genetically heterogeneous group of connective tissue diseases, which is divided into four main types based on clinical grounds [Sillence, 1988]. However, molecular/genetic evidence suggests that OI is far more complex, comprising a whole range of mutations which do not necessarily fit into the Sillence classification [Korkko et al., 1998]. OI affects tissues rich in type I collagen, primarily bone, but clinical, radiographic and histological findings suggest that dentin, the organic matrix which mainly consists of type I collagen, is abnormal only in one third of patients. The dental involvement has been used to divide OI types into a subgroup with normal teeth and another with abnormal teeth [Sillence, 1988; Byers et al., 1992]. The dental defects associated with OI are known as dentinogenesis imperfecta (DI) and are specified as type I DI in distinction from type II DI, where dentin is the only tissue affected [Ranta et al., 1993]. Dentin is often affected in type III and type IV OI, whereas DI is rather rare in conjunction with type I OI [Lukinmaa et al., 1987a; Sillence et al., 1979].

Figure VII.1 Patient 1 presenting dentinogenesis imperfecta (DI) as a symptom of Type III osteogenesis imperfecta (OI).

Patient 1 is the only child of unaffected parents and has been diagnosed clinically with OI III. No data on the genetic defect were yet available. In the literature, both an autosomal dominant form (caused by mutation in COL1A1 or COL1A2) and an autosomal recessive form (COL1A2 null allele) of OI type III have been reported [OMIM 259420]. Patient 1 suffered from numerous fractures and presents severe bone deformity with short stature and a large skull. The permanent dentition of this adult Caucasian female, aged 34 years, displays all clinical and radiographic features of DI, i.e. an opalescent tooth discoloration, root dysplasia,
bulbous crowns, and pulp calcification. A mandibular first molar was extracted for reasons of crown fracture consequent to decay. Both coronal and radicular dentin were available for examination.

Patient 2 - Ehlers-Danlos Syndrome Type I (classical type) with unusual COL1A1 mutation. The Ehlers-Danlos syndromes (EDS) comprise a heterogeneous group of heritable disorders of connective tissue, characterized by joint hypermobility, skin hyperextensibility, and tissue fragility [Beighton et al., 1998]. The clinical features, modes of inheritance, and molecular bases differ according to the type. EDS are caused by a genetic defect causing an error in the synthesis or processing of collagen types I, III or V. At present, at least six subtypes are delineated according to clinical features, mode of inheritance, and underlying biochemical and molecular defects [Beighton et al., 1988; Beighton et al., 1998].

Patient 2, a Caucasian 12-year-old girl, was diagnosed clinically with type I EDS (EDS I) on the basis of increased tissue fragility with easy bruising and hypertrophic scarring, a soft and velvety skin, moderate hypermobility of the metacarpophalangeal joints and the patellae, and blue sclerae. A mutation in COL5A1, the gene coding for type V collagen, can be demonstrated in at least one-third of patients affected with EDS I (classical type) [OMIM Entry 130000-130010] [De Paepe et al., 1997; Beighton et al., 1998]. However, in patient 2 a missense mutation was found in COL1A1 (C>T transition at nucleotide 934 exon 14, resulting in the substitution of arginine for cysteine in position 134 of the pro-a1(I) chain). This mutation has so far been described in only two subjects with EDS I [Nuytinck et al., 2000]. On subsequent oral examinations, no clinical or radiographic abnormalities had been found in the patient’s dentition. Two deciduous teeth, i.e. a canine and a mandibular molar, were obtained. Since these teeth had been naturally shed and the roots were more or less resorbed, only coronal dentin was available for examination.

Patient 3 - Ehlers-Danlos Syndrome Type VIIC (human dermatosparaxis). EDS VIIC is a recessively inherited disorder, characterized clinically by premature rupture of fetal membranes, extreme skin fragility and laxity, characteristic facies, blue sclerae, short stature, short fingers, and easy bruising [OMIM Entry 225410] (Fujimoto et al., 1997; Beighton et al., 1998). At present, only ten patients have been diagnosed with EDS VIIC [Malfait et al., in press]. Oral features have been described in three patients and include micrognathia, gingival hyperplasia, and tooth agenesia, whereas tooth discoloration, dysplastic roots and pulp obliteration may occur in a localized fashion in the permanent dentition [De Coster et al., 2003; Malfait et al., in press].

In the present proband, a Caucasian 13-year-old girl, the condition was caused by a nonsense mutation (G>A transition at nucleotide 2384, changing TGG tryptophan codon at position 795 to a TAG stopcodon) in ADAMTS2. A shed deciduous mandibular molar was obtained for analysis, showing no evidence of pathologic dentinal involvement. The patient’s deciduous teeth had been found
unaffected on subsequent clinical and radiographic examinations. Although a great part of the tooth crown was missing, the mesial root was still almost complete and could be used for examination.

Figure VII.2 Clinical and tomographic image of patient 3 with EDS VIIC presenting (A) severe gingival hyperplasia, tooth spacing, open bite, and discoloration of the permanent dentition. (B) Multiple agenesia, localized root dysplasia, and partial pulp obliteration are seen in the permanent dentition.

A shed deciduous canine and a mandibular molar from unaffected subjects served as controls.

The study project had previously been approved by the Ethical Committee of Ghent University Hospital (ref. 2000/ 308-309). A written consent was obtained from the participants prior to examination. The work was supported by grant B/ 05088 from the Special Research Fund of Ghent University, Section IV1.

VII.3.2 Histological and Ultrastructural Methods

The shed deciduous teeth had been kept air-dried, whereas the permanent teeth were immediately immersed in 10% formalin and fixed for one week. All teeth were demineralized with aqueous ethyldiaminetetra-acetic acid (EDTA; 0.33 mol/ L) at room temperature for about four weeks, and bisected bucco-lingually to facilitate both the histological and ultrastructural analysis. For each specimen, one tooth half was dehydrated with ascending ethanol series and toluol, and embedded in paraffin. A series of longitudinal sections was cut from each tooth in a bucco-lingual plane at 5 µm. The sections were stained with hematoxyline and eosine, by Gordon and Sweets’ silverstaining method for reticuline, and by Masson’s trichrome method for collagen.

Rectangular pieces with a cross-sectional area of about one to two square millimeter were cut with a scalpel from selected parts of the primary dentin of the remaining tooth halves. Care was taken to avoid the mantle dentin (the first formed layer of dentin), zones of resorption (adjacent to caries), and reactionary dentin (also known as tertiary dentin) at the pulpal side. The samples were rinsed with distilled water and soaked in 0.1 M cacodylate buffer (pH 7.4). They were post-fixed with 2% osmium tetroxide in cacodylate buffer for 90 min, dehydrated
in ascending aceton series, and embedded in ERL 4206 (vinyl cyclohexene dioxide epoxy resin). Halfthin sections (1-2 µm) were cut with a glass knife in two planes, i.e. one expected to cross-cut the dentinal tubules, and another in a longitudinal plane. The sections were first stained with hematoxyline and eosine, by Gomori’s method for reticuline and by Grimley’s basic trichrome method. The paraffin and halfthin sections were examined by transmitted light microscope. Ultrathin sections (60-70 nm) were then cut with a diamond knife from selected areas, and, after staining on the grid with uranyl acetate and lead citrate, were examined in a Jeol JEM-1200 EX II transmission electron microscope at 80 kV.

VII.4 Results

The histological appearances of circumpulpal dentin matrix varied among the three pathological tooth samples. Light microscopy of dentin of teeth from both patient 1 (OI type III with DI) and patient 3 (EDS VIIc) differed greatly from one area to another: among dentin closely resembling that seen in normal teeth there were various abnormalities (Figure VII.3B-D).

Figure VII.3. Histological appearances of cross-sectioned dentin matrix (mid-third of circumpulpal dentin) from three patients with different types of abnormal collagen I metabolism compared to a control specimen (A). Sparse tubuli and clustered canal-like structures appear in pathological dentin areas of patients 1 (B) and 3 (C). Throughout dentin matrix from patient 2 (D), abnormally enlarged tubules of varying diameter are scattered among normal ones. Hematoxylin and eosin stain on paraffin sections; x 400.

Figure VII.4. Electron micrographs of central sections of demineralized circumpulpal dentin matrix of a control tooth (A), and clinically unaffected teeth from patient 2 (B) and patient 3 (C). Dentinal tubules are indicated by T. In patient 2 (B), collagen fibers are condensed around the irregularly shaped tubules. Loosely arranged fibers of varying thickness hazardly criss-cross the intertubular matrix. In patient 3 (C), the margins of the
sparse dentinal tubules are poorly defined. Extreme thin fibers are haphazardly arranged among normal-sized ones. (A,B) x 1200; (C) x 10,000.

There were apparently normal areas with a collagen-rich matrix surrounding dentin tubules, which were well demarcated with quite uniform tubule-to-tubule distance, equal size, and approximately circular cross-section. The tubules appeared empty or showed intratubular contents varying from delicate granular material to dense, amorphous masses. The thickness of the collagen fibers was fairly uniform. The fibers criss-crossed between the tubules without apparent bundle formation or preferable direction (Figures VII.4A and VII.5A).

With increasing irregularity of the dentin matrix, the dentinal tubules became more scattered and/or clustered and had an irregular cross-sectional form. Their structure varied from normal to coarse and branched, and giant tubules/canal-like structures, often arranged in clusters surrounded by ill-defined lacunae, were seen (Figure VII.3B-D).

Tubular areas alternated with atubular ones, and areas with sparse collagen became frequent (Figure VII.6B-C). The collagen fibers appeared short and curvy, and intruded into the tubules (Figures VII.4B-C, VII.5B and VII.5D). In the most aberrant areas, where dentinal tubules could not be distinguished, parallel collagen fibers formed thick bundles with a slightly wavy course (Figures VII.4C and VII.5C-D).

The spreading and morphological appearances of abnormal structures presented differently depending on the underlying collagen I abnormality. Abnormal areas were the most prominent and showed more extensive morphological variety in dentin matrix from patient 1 (OI type III and DI), who was the only subject showing clinical/radiographical manifestations of (type I) DI (Figure VII.3B). This finding was independent on the location (crown or root) and

Figure VII.5. Electron micrographs of central sections of demineralized circumpulpal dentin matrix. Dentinal tubules are indicated by T. In contrast with the fairly uniform and densely packed appearance of collagen fibers in a control specimen (A), fiber organisation around ill-defined dentinal tubules may vary from either loosely arranged in patient 1 (B) and 3 (C) to abnormally condensed in patient 2 (D). Thick (C, arrowed) or
short and curvy (D, arrowed) collagen fibers may co-distribute with normal ones. (A) x 12,000; (B) x 20,000; (C) x 25,000; (D) x 30,000.

orientation of the examined section. Histologically, dentin matrix of a clinically unaffected tooth from patient 2 (EDS I with unusual COL1A1 mutation) displayed the least of morphological aberrations, i.e. a number of dentinal tubules were enlarged and ill-defined, often displaying irregular branches, and were scattered between normal ones. However, tubule-to-tubule distance, size and dimensions on cross-section of the ‘normal’ dentinal tubules were slightly less uniform than in controls. There was no delineation between normal and abnormal dentin areas in patient 2 on light microscopy, i.e. the morphological abnormalities presented throughout the dentin matrix (Figure VII.3D). On electron microscopy, dentin matrix of patient 2 was characterized by short and irregularly shaped collagen fibers, somehow more condensed around abnormal and ill-defined tubules, demonstrating topological segregation of morphological aberrations at ultrastructural level (Figures VII.4B and VII.5D). Although in patient 3 (EDS VIIC) aberrant areas were seen less frequently than in patient 1, these were characterized by the presence of more and greater clusters of canal-like structures than in both patients 1 and 2. Transition from normal to abnormal areas was most clearly demarcated in patient 3 (Figure VII.3C).

Figure VII.6. Trichrome staining for collagen of demineralized dentin matrix shows a less dense and heterogeneously stained clew of bundles in patient 1 (B) and 3 (C) compared to a control specimen (A,E), indicating that collagen is sparse in pathological areas. Staining is uniform throughout the dentin from patient 2 (D). Cytoplasmatic remnants of vessel wall endothelium and erythrocytes are characteristic findings in some of the large and irregularly shaped canals (arrowed) in dentin from all three patients.
(D,F). (A-D) Masson's trichrome method on paraffin sections: collagen stains blue; (E,F) Grimley's basic trichrome method on halfthin sections: collagen stains red; (A-D) x 400; (E,F) x 1000.

The pattern of trichrome staining has been reported to be consistent with that of immuno-staining for type I collagen [Sauk et al., 1980; Berkovitz, 2002]. On sections of all three pathological dentin samples stained by Masson's or Grimley's trichrome method, the collagen fiber meshwork appeared in different patterns according to the severity of local dentinal involvement (Figure VII.6). In patient 1 and 3, tubular areas showed a dense and homogeneous wavy organization of collagen, whereas abnormal areas were characterized by a less dense, irregularly branched and heterogeneously stained clew of bundles, indicating that collagen may be sparse in atubular areas (Figure VII.6B-C). Cytoplasmatic remnants of vessel walls and erythrocyte cells, found in the large and irregularly shaped tubules. Gordon and Sweets' silverstaining method for reticulin on paraffin sections; x 400.
from a fine reticular pattern to more amorphous and/or granular clusters of fibers, somehow denser around abnormal tubular structures and in atubular areas. In normal areas, dentinal tubules were occasionally filled with granular or amorphous stained material. In dentin matrix from patient 2, staining displayed a fine reticular pattern throughout the dentin, which was more condensed around the large tubules. As was previously reported, reticulin staining was absent in the control teeth [Sauk et al., 1980; Takita et al., 1987]. Since the pattern of staining for reticulin fibers has been reported to coincide with immunostaining for type III collagen [Sauk et al., 1980; Ranta et al., 1990], these findings may reflect the abnormal presence of collagen III in the pathological dentin samples, as was previously demonstrated in teeth from patients with type I DI [Sauk et al., 1980] and type II DD [Ranta et al., 1990].

VII.5 Discussion

The present study demonstrates that clinically unaffected teeth from patients with collagen I deficiency, i.e. patients 2 and 3, may present ultrastructural abnormalities. Given the small sample size, however, the generalizability of this finding is rather low. Previous studies already demonstrated that clinically and radiographically normal appearing teeth in OI can show microscopic changes in dentin [Witkop & Rao, 1971; Sillence et al., 1979a; Sillence et al., 1979b; Waltimo et al., 1996], which can be assigned to cell-type specific differences in the transcription of the mutated gene [Schwartz et al., 1990]. Further investigation, however, is needed to elucidate the diagnostic specificity of the present morphological features by using larger samples, and to assess the validity of these features as a diagnostic tool.
Ultrastructure of the pathological dentin samples in this study was characterized by a great variability between different areas and by the presence of similar morphological anomalies. A number of ultrastructural dissimilarities suggest that the nature of the underlying collagen I deficiency may account for specific morphological features of dentin. The present light microscopy and ultrastructural findings reflect the different abnormalities in the organization of the collagen I meshwork, serving as a scaffold for dentin mineralization. For example, the telopeptide domain disponibility on the assembled collagen molecules, needed for initiation of mineral deposition, may be specifically altered as a function of the particular biosynthesis error of collagen I, hence resulting in a variety of mineralization defects. Previous studies indicated that the telopeptide domains of collagen I play a role in the interaction with phosphophoryn, which is critical for the mineral nucleation process [Saito et al., 1998; Saito et al., 2000]. Therefore, it can be postulated that dentin of teeth from patients with abnormal collagen I is characterized by structural aberrations resulting from both a defective collagen framework and a consequent failure of mineral deposition at specific telopeptide domains on the abnormal collagen fibrils. Previous studies also demonstrated that changes in the concentrations of noncollagenous proteins occur in OI and may interfere with complete mineralization and/or normal tissue architecture [Vetter et al., 1991; Fedarko et al., 1992].

The alternating pattern of abnormal and normal areas in dentin may be linked to defective production of dentin matrix by successive odontoblast generations, hence reflecting selective expression of the causal (collagen I) pathology at the odontoblast level [Schwartz et al., 1990]. In all pathological dentin samples, the morphological continuity presenting in consecutive sections (i.e. sectioned and examined in an axial order), is in line with previous postulations that defective dentin of teeth affected by type I DI [Aldred, 1992] and by type II DI [Ivancie, 1954] might be deposited by successive cell generations. This may explain the ‘lamellated’ or alternated arrangement of pathological dentin areas [Waltimo, 1994]. Godfrey [1973] studied odontoblast differentiation in developing deciduous teeth in OI presenting with DI. The odontoblasts differentiated normally and assumed a columnar form with regular arrangement while predentine was formed. After tooth formation was initiated, normal development deteriorated. During late matrix formation and with the onset of mineralization, the odontoblasts changed to polyhedral cells with no definite orientation [Godfrey, 1973]. The present findings sustain the hypothesis that, as a result of the loss of cell polarization, odontoblasts in heritable defects of dentin mineralization may fail to arrange in cell lines (‘drifting’), and start secretion of mineralizing matrix proteins in a multidirectional fashion. Although the nature of this aberrant matrix calcification is still poorly understood, a striking similarity exists with the formation and mineralization of bone matrix. Like osteoblasts, the ‘drifted’ polyhedral odontoblasts are likely to get gradually incorporated in the mineralized dentin matrix, resulting in the presence of irregularly shaped lacunae. On the basis of the present microscopic findings, smaller lacunae in atubular areas are likely to arise from (longer) continuous secretion activity of the ‘drifted’ odontoblasts.
On the other hand, larger and more regularly shaped lacunae, often including remnants of blood vessels, are suggestive to originate from premature cell degeneration following axial occlusion of the blood vessel by irregular and selective mineralization of the predentin [Lindau et al., 1999]. The inclusion of small arterial structures in these lacunae are suggestive of a rapid formation of dentin matrix [Ooshima et al., 1990; Lindau et al., 1999]. The present findings of cytoplasmatic remnants of vessel walls and erythrocyte cells in the large and irregularly shaped canals in the atubular areas, sustain previous postulations of inclusion of pulpal capillaries in pathological dentin (Figure VII.6F) [Rushton, 1939; Godfrey, 1973]. These canals may be interpreted as the calcified outlines of embedded blood vessels.

Occasionally, at the center of the denser stained parts of atubular areas, remnants of cellular material were found. Since this cell material roughly resembled degenerated osteocytes in bone, it can be postulated that these remnants might reflect the presence of odontoblasts embedded in the pathological dentin. This finding supports the concept that during early dentinogenesis pathological odontoblasts might behave like osteoblasts, i.e. secrete their matrix in an unpolarized and random fashion and not at their proximal end. As a consequence, odontoblasts may become incorporated in the dentin matrix and degenerate. However, despite recent insights into the complexity of genes controlling dentinogenesis [Thesleff, 2000], the pathological pathways of aberrant dentin formation mainly remain unclear. Further investigation is needed to establish phenotype-genotype correlations in different forms of abnormal dentinogenesis.

Previous studies demonstrated the absence of reticulin staining in normal dentin [Godfrey, 1973], and reported that a relationship may exist between the severity of dentinal involvement and the presence of type III collagen (or reticulin fibers) [Sauk et al., 1980]. Studies on normal dentin formation have indicated that the synthesis of type III collagen (collagen III) occurs during the early stages of normal dentinogenesis. On subsequent odontoblast differentiation, this collagen is replaced entirely by collagen I. Hence, collagen III is not a normal component of mineralized dentin: the demonstration of small amounts of collagen III in defective dentin would suggest a deficiency in the normal development of the dentin matrix [Lesot et al., 1978; Lesot et al., 1979; Thesleff et al., 1979]. Yet, it has been found to be a constant finding in dentin of patients with DI associated with OI (DI Type I) [Sauk et al., 1980; Gage, 1985; Lukinmaa, 1988; Waltimo, 1994]. In other types of hereditary dentin defects, however, collagen III has been reported to display a variable presence, e.g. in DI Type II [Sauk et al., 1980], and to be absent in coronal dentin dysplasia (DD Type II) [Ranta et al., 1990]. On the other hand, the presence of collagen III in normal predentin (i.e. unmineralized dentin matrix) remains controversial [Thesleff et al., 1979; Cournil et al., 1979; Wright & Leblond, 1980; Becker et al., 1986; Takita et al., 1987; Anjudar et al., 1988]. Collagen III molecules retain the N-terminal propeptide as shown both in non-mineralized tissues and in the dentin matrix of patients with Type I DI [Lukinmaa, 1988]. The failure to
demonstrate the occurrence of collagen III in some families affected with Type II DI and in Type II DD suggests that the structural irregularity of dentin is not necessarily associated with incorporation of collagen III in the matrix. The results of the present study, showing the presence of reticulin fibers in dentin from patients with different types of collagen I deficiency, correlate well with observations for collagen III in other tissues in OI [Sauk et al., 1980; Gay & Miller, 1978]. In EDS types I and VIIC, however, no reports of this nature have yet been made. The above postulations about the presence of collagen III in the pathological samples, however, remain to be confirmed by immunostaining.

Although collagen I deficiency in patients 1 and 2 was caused by a mutation in one of the genes encoding procollagen I, ultrastructural aberrations of dentin were less similar than between patient 1 (OI III and DI) and patient 3 (EDS VIIC), the latter disorder being caused by mutation in ADAMTS2 gene. The morphological dentin abnormalities in patient 2 largely coincide with those found in a patient with unspecified EDS VII (subtype A, B or C unknown) as documented by Ooshima [Ooshima et al., 1990]. The unique phenotype-genotype correlation in patient 2 may well account for a number of dissimilarities at the ultrastructural level, as compared to the genetically-related (i.e. related on mutational level) findings in patient 1. In addition, there were no convincing similarities in histological appearances of the clinically unaffected teeth from patients 2 and 3. To date, it remains unclear which factors contribute to the expression of these defects at the clinical level. As a result, no conclusions can yet be made as to the effect of any alterations, occurring at different stages of collagen I biosynthesis, on the tissue architecture of human dentin. Further investigations should also address the non-collagenous protein components in pathological dentin samples.

VII.6 Conclusion

Different types of collagen I deficiency, resulting from different gene mutations, may cause similar abnormalities of dentin matrix with distinct varieties of structures. Ultrastructural evidence of disrupted dentinogenesis was found in clinically unaffected teeth of two patients with collagen I deficiency other than OI. Histological analysis of dentin matrix may hence be considered a valuable diagnostic tool in the assessment of qualitative collagen I defects. On the basis of this study, it can be recommended to examine the histological/ultrastructural appearances of dentin of teeth from patients suspected of a collagen I disorder. On the other hand, it can be recommended to examine the connective tissues in any patients presenting with clinical and/or radiographical features of dentin defects.
General Discussion and Conclusion

Collagens and fibrillin are two major protein components of the extracellular matrix (ECM), mainly providing somatic tissues with structure (collagen), mechanical strength (collagen, fibrillin) and elastic properties (collagen, fibrillin). Collagen I makes up the bulk of the organic components of the orofacial hard (craniofacial skeleton, alveolar bone, dentin) and soft (mucosae, periodontium, TMJ ligaments, pulp) tissues. Collagen V is involved in fibril formation of collagen I, whereas collagen III accounts for the formation of scar
tissue, elasticity of blood vessel walls, and the transition of calcified tissue matrices into their mineralized phase. Fibrilline is the major component of the microfibrillar networks and elastin-associated fibrils, which provide different organs and tissues (skeleton, joints, blood vessels etc.) with specific elastic capacities. From clinical experience, it is clear that abnormal metabolism of these ECM proteins may be expressed as specific oral manifestations and/or an increased liability for the development of functional and/or infectious oral disease. At present, testable data on epidemiological, morphometrical and histological aspects of these conditions are still lacking.

The Ehlers-Danlos syndromes (EDS) and Marfan syndrome (MFS) were selected as models of disease with maximum expression of deficiency of the ECM proteins in focus. Prevalence estimates vary from 1:10,000–100,000 for different EDS subtypes [McKusick, 1972; Gorlin et al., 1990a], and from 5–8:100,000 [Gorlin et al., 1990a] to 3–4:10,000 [Dietz & Pyeritz, 2001] for MFS. The EDS have a different etiology according to the EDS type. Mutations may occur in genes coding for collagen types I, III or V, or in genes encoding enzymes essential to biosynthesis of these proteins. All EDS types share an increased tissue fragility, generalized joint hypermobility with chronic joint/limb pain, and an increased bleeding tendency as major features. MFS is caused by mutation in FBN1, the gene encoding fibrilline, and is characterized by skeletal overgrowth, generalized joint hypermobility, and dilatation/dissection of the aorta. An early diagnosis and treatment of oral manifestations, and an appropriate anticipation to specific tissue responses may prove important elements to be integrated in dental treatment strategies. However, only few data, most of which having poor methodological qualities, are yet available from literature to substantiate these strategy concepts.

The null hypothesis of this study stated that patients affected with inherited deficiency of collagens or fibrillin do not present orofacial symptoms with (high) diagnostic specificity, and that oral health in these patients is not compromised.

The following aims of the study were postulated:

1. Analysis of the orofacial (oral soft tissues, dental hard tissues, craniofacial skeleton, and temporomandibular joints) manifestations of patients affected with EDS or MFS.

2. Assessment of oral health (caries experience, periodontal health, condition of the oral soft tissues, and temporomandibular disorders) in patients affected with EDS or MFS.

3. Formulation of therapeutical guidelines for dental treatment of patients affected with EDS or MFS.

4. Assessment of the value of ultrastructural analysis of dentin as a diagnostic tool for the determination of collagen I deficiency.
Distributed over the different parts of the study, a total number of 33 patients with one of the three most prevalent EDS types (hypermobility EDS, classical EDS or vascular EDS) and 51 affected with MFS were examined. All participants were selected on the basis of the most recent criteria for clinical and molecular-genetical diagnosis [De Paepe et al., 1996; Beighton et al., 1998]. The control populations were matched for age, gender and dental attendance (Chapter III-IV), vertical and sagittal skeletal characteristics of the craniofacial complex (Chapter V), or signs and symptoms of temporomandibular disorders (TMD) (Chapter VI). The total number of control subjects was 172. Since prevalences of both syndromes are low, the sample sizes were found to be representative for the Belgian EDS/MFS populations and, hence, can be considered epidemiologically valid. This was confirmed by a post-hoc power analysis for the most relevant outcome variables.

In a first section (Chapter III-IV), oral manifestations and oral health were analyzed in 31 patients with EDS and 23 with MFS, using standardized protocols and indices as recommended by the World Health Organisation [WHO, 1997]. In Chapter III, patients affected with EDS were found to have a significantly higher dmf/DMF-score, more dental plaque and a greater mean pocket depth as compared to controls. Sixty-two % of EDS, mostly patients with hypermobility EDS, had high periodontal treatment needs, i.e. CPITN =III in at least one sextant. In these cases, a statistically significant association was found between pocket depth, dental plaque, gingival inflammation, and restraint of joint mobility of the wrists. An increased mucosal fragility was recorded in 74% of EDS, which showed interrelated with a low brushing frequency, a high plaque index and a high dmf/DMF-score. Morphological aberrations of dentin, such as root dysplasia and pulp obliteration, were frequent findings in patients with classical EDS. In Chapter IV, subjects affected with MFS were found to have a significantly higher caries experience (higher d/D scores) and a lower level of dental care (on surface level) than unaffected controls. The gingival condition in MFS on average was poor as compared to controls. These findings, however, may be influenced by socio-economic and/or emotional factors, which are closely related to the physical restraints and complications that are characteristic to MFS. About one-third of MFS persons presented with specific morphological abnormalities of the dental hard tissues, i.e. long and tapered roots with obliteration of the pulp chambers.

In a second section (Chapter V), a comparative cephalometric analysis was conducted in 26 subjects with MFS. Vertical and sagittal skeletal characteristics of the craniofacial complex were analyzed and compared to population norms (Bolton Standards) [Broadbent et al., 1975]. Apart from a number of significant differences in ‘classical’ cephalometric variables, mandibular retroposition and a long face with an increased anterior face heigh were found to be highly characteristic for MFS population. Additional cephalometric points and variables were designed to define the sagittal palatal dimensions in adult MFS (n=17) and matched controls (n=32). As a result, sagittal skeletal dimensions of the (high and narrow) palate in MFS were found to be significantly different from controls.
In a third section (Chapter VI), signs and symptoms, as well as individual
diagnoses of TMD were analyzed in a population comprising 42 patients with
generalized joint hypermobility (GjH) as a symptom of inherited connective tissue
disease. The study population consisted of 18 patients with EDS and 24 with MFS.
The clinical symptoms of TMD, and the signs and consequences of hypermobility
of the temporomandibular joints (TMJs) were analyzed in adult study subjects
(n=27) and contemporary controls (n=40). Subjects with GjH and symptoms of
TMD more frequently had multiple TMD diagnoses as compared to controls with
TMD and normal joint mobility. These patients significantly more frequently
reported of recurrent TMJ dislocation than subjects with TMD and normal joint
mobility. From these findings, it was concluded that recurrent TMJ dislocation are
pathognomic for GjH, which, in turn, may predispose to the development of TMD.
A number of clinical signs/functional parameters were found to have a high
diagnostic specificity for hypermobility of the TMJs, such as a large endfeel
distance in the absence of pain, a preauricular depression at the end of the opening
cycle, and recurrent TMJ dislocations.

In a fourth section (Chapter VII), histological/ultrastructural characteristics
of dentin samples from three patients with different types of collagen I deficiency
(i.e. type III OI with DI, classical EDS and dermatosparaxis EDS) and healthy
controls were compared by light and transmission electron microscopy. In the
pathological dentin samples, several similarities were found with respect to dentin
morphology, although presenting with a variety in morphological structures. In all
samples, normal areas alternated with pathological ones, the latter being
characterized by a decreased number of dentinal tubules with irregular form and
dimensions. Enlarged and canal-like structures presented in clusters and had
inclusions of endothelial remnants (capillary vessel wall). It was postulated that
the morphology of pathological dentin samples largely reflects abnormal secretory
function of the odontoblasts. Although the dentinal aberrations, found in all three
pathological samples, resulted from different mutations, no morphological
differences with high diagnostic specificity were found. Another significant
finding was that clinically unaffected teeth of patients with collagen I deficiency
presented with structural abnormalities at the ultrastructural level. On the basis of
these findings, it was concluded that histological/ultrastructural analysis of dentin
matrix may be a valuable diagnostic tool in diagnosis of deficiency of collagen I.

The aims of the study can be ascertained from the former findings. The
first objective, analyzing orofacial manifestations as assumed clinical expressions
of the underlying ECM protein deficiency, was met for both syndromes. In patients
with EDS, an increased mucosal fragility and TMJ hypermobility were significantly
frequent findings. A number of additional oral manifestations were found to be
associated with specific EDS types: pulp chambers had an abnormal shape in
classical EDS, and progressive pulp obliteration was found significantly more
frequently in hypermobility EDS. Short, dysplastic roots were an occasional
finding in a small number of EDS subjects. In patients affected with MFS, abnormal
tooth morphology (long and tapered roots with pulp obliteration), an increased
mucosal fragility, a high and narrow palate, mandibular retrognathia, a ‘long face’, and hypermobility of the TMJs appeared to be related to the syndrome.

With respect to the **second objective**, analyzing different aspects of oral health in EDS/ MFS, the incidence and epidemiological-clinical characteristics of oral disease (decay, gingivitis and periodontitis) and TMD were assessed and compared to matched controls. In both syndromes, the liability for development of decay and/or inflammation/breakdown of periodontal tissues, and mucosal fragility were significantly increased. A high incidence of multiple TMD was found to be related to patient characteristics of TMJ hypermobility.

On the basis of the former clinical findings and the syndromes’ medical risk profiles, therapeutical guidelines for dental treatment of patients affected with EDS or MFS were formulated (Chapter III.6 and IV.6). As a result, the **third objective** of the study was met.

With regard to the **fourth objective**, assessing the value of histological/ultrastructural analysis of dentin as a diagnostic tool for the determination of collagen I deficiency, a number of highly specific, morphological characteristics were delineated for pathological dentin samples from patients with three different types of collagen I deficiency, as compared to unaffected controls. On the basis of these findings, a recommendation to perform a histological/ultrastructural analysis of dentin in any patient with e.g. brittle bones, skeletal malformation, blue eye sclerae, GJH and/or an increased fragility of the soft tissues, can be offered. A histological analysis of dentin may confirm a clinical diagnosis of collagen I deficiency, and hence may represent a valuable diagnostic tool in these cases.

On the basis of the above conclusions, the null hypothesis is rejected showing that the underlying molecular defect may indeed produce syndrome-specific symptoms in different orofacial structures, such as teeth, oral soft tissues, alveolar bone, craniofacial skeleton and TMJs. However, these symptoms may not always be perceptible at the clinical level.

In patients with a qualitative defect of collagen I, it follows, as a rule, that even in clinically unaffected teeth dentine is always aberrant at the ultrastructural level. At present, it remains elusive whether excessive formation of reactionary dentin (progressive obliteration of pulp chambers), being a candidate symptom of a number of connective tissue disorders, is part of the phenotypic continuum of defective dentinogenesis. The present knowledge on dentinogenesis sustains the postulation that this feature may result from disturbed odontoblast metabolism that may not exclusively be associated with deficient collagen biosynthesis. On the basis of both histopathologic evidence (Chapter VII) and former postulations from the literature [Waltimo, 1999], further investigation should focus on how accumulation of prematurely degraded procollagen molecules in the rough endoplasmatic reticulum may disturb metabolism of the odontoblast in patients
with a qualitative collagen defect. This might provide a plausible explanation for a common pathogenetic pathway resulting in strikingly similar phenotypes between solitary forms of generalized dentin defects (DI type II and dentin dysplasia) and dentinogenesis imperfecta as a symptom of heritable collagen I disorders. This may also explain the presence of abnormal high amounts of collagen III and other extracellular matrix proteins in the majority of these dentin defects as a result of a disrupted homeostasis of the production of these proteins. As a consequence, the secreting odontoblasts may lose their polarity and produce abnormal dentin matrix in various directions and in a non-concerted manner. An early apoptosis may follow and the odontoblasts may become incorporated into the dentin [Godfrey, 1973]. Consequent generations of “new” odontoblasts may differentiate from the proximal mesenchymal pulp cells, and formation of irregular dentinal structures continues. In dentin from patients with a qualitative collagen I defect, nucleation and growth of apatite crystals in and around the abnormal collagen fibrils will be disrupted. In contrast, in DI type II and dentine dysplasia, disrupted mineralisation of predentin results from a defective production of dentin-specific proteins, such as dentin sialoprotein and dentin phosphoprotein (promoting nucleation and growth of dentin apatite). Further research, however, is needed at the odontoblast level to elucidate the specific pathogenetic aspects of defective dentinogenesis.

On the basis of the above findings, it remains elusive whether these structural dentin defects might affect caries experience and caries progression in these populations. Previous reports on altered mechanical properties of dentin I DI type II [Kinney et al., 2001; Kinney et al., 2003] may lead to the assumption that dentin breakdown at carious sites might be influenced (i.e. increased) by these structural alterations. However, at present scientific data are lacking to sustain this postulation.

Taking into account the former findings, it may be clear that qualitative defects of collagen or fibrillin may alter both the mechanical properties (strength and resilience) and the regenerative capacities of a number of soft tissues, such as muscle, skin, mucosa, gingiva and the periodontal ligament. This may result in hypotonia with increased tissue fragility (skin, mucosa and gingiva) and a defective regeneration after traumatic and/or infectious assaults. In infectious oral disease, the periodontal soft tissues in particular may display a variety of tissue reactions. Both the primary (swelling and bleeding) and secondary (tissue regeneration) clinical symptoms will be negatively influenced by the underlying molecular defect. As a consequence, appropriate preventive measures and atraumatic treatment strategies are highly recommended in these populations.

Since different aspects of the pathogenesis of both syndromes yet remain unclear, further research is still needed to validate the diagnostic specificity of these findings. Further research should focus on documenting on genotype-phenotype correlation in these populations.
It can also be concluded that patients affected with heritable collagen or fibrillin disorders may be at high risk for oral problems, such as caries, gingivitis and periodontitis, and TMDs. However, it is most likely that the development of these diseases may also be influenced by epigenetic and environmental factors, such as metabolic processes (hormones), psychological profile, socio-economic status, and physical restrictions.

At last, it is clear that a number of specific preventive and therapeutical measures should be integrated in dental treatment strategies of patients affected with EDS or MFS. Since cardiovascular structures may be involved, it is highly recommended to inquire about the presence of cardiovascular risks during patient interview. In patients affected with MFS, functional valvular problems and cardiovascular surgery are frequent findings, compelling one to administer appropriate antibiotic prophylaxis before starting dental treatment. In patients with aortic dilatation, both the administration of appropriate local anesthetics and monitoring of cardiac output is recommended during long or invasive dental treatments. On the other hand, in patients with collagen disorders (OI, EDS) cardiovascular risks are equal to those of the normal population. In case of doubt, these risks can easily be assessed by routine echocardiographic examination.

On the basis of the above conclusions, a detailed protocol can be offered to the general practitioner to be used for diagnosis and therapy of oral disease in patients affected with EDS or MFS. Moreover, these guidelines may lead to an early diagnosis of mild phenotypes and may provide the outlines for an appropriate and successful dental treatment of these patients.
Algemene Discussie en Conclusie

(C General Discussion and Conclusion in Dutch)

Collageen en fibrilline zijn twee belangrijke extracellulaire matrixeiwitten die structuur (collageen), mechanische sterkte (collageen, fibrilline) en elastische eigenschappen (collageen, fibrilline) verlenen aan de verschillende weefsels van het lichaam. Collageen I vertegenwoordigt het grootste organische volume van de harde (aangezichtsskelet, alveolair bot en dentine) en zachte (mucosa, parodontium, kaakgewrichtskapsel en pulpa) weefsels in de orofaciale zone, en wordt in de associatie tot fibrillen voor een aanzienlijk deel gestuurd door collageen V. Voor de voorbereiding van de mineralisatie van de harde weefsels, de vorming van littekenweefsel, en de elasticiteit van bloedvatwanden, is collageen III mede verantwoordelijk. Fibrilline vormt de bouwsteen van het microfibrillair
network en de elastinevezels, die in verschillende orgaansystemen (skelet, gewrichten, bloedvaten enz.) voor aangepaste (elastisch-)functionele eigenschappen zorgen. Klinische ervaring leert dat een abnormaal metabolisme van deze matrixeiwitten het optreden van specifieke orale symptomen kan veroorzaken en dat het individu aanzienlijk vatbaarder kan zijn voor een aantal functionele en infectieuze aandoeningen. Epidemiologische, morfometrische en histologische gegevens ontbreken echter om deze stelling te toetsen.

Als ziektemodel met maximale expressie van deficiëntie van deze structurele matrixeiwitten werden de syndromen van Ehlers-Danlos (EDS) en het syndroom van Marfan (MFS) geselecteerd, welke, afhankelijk van de bron, respectievelijk een prevalentiebereik van 1:10,000–100,000 voor de verschillende EDS subtypes [McKusick, 1972; Gorlin et al., 1990a], en van 5-8:100,000 [Gorlin et al., 1990a] tot 34:10,000 [Dietz & Pyeritz, 2001] voor MFS, worden toegekend. De EDS kennen een verschillende etiologie naargelang het subtype; oorzakelijke mutaties doen zich voor in de genen die coderen voor de collageentypes I, III en V, evenals in enzymes die essentieel zijn voor de biosynthese van deze collagenen. Gemeenschappelijke kenmerken zijn o.m. een verhoogde weefselkwetsbaarheid, veralgemeende gewrichts-hypermobiliteit met chronische gewrichtspijnen, en broze bloedvaten. Het MFS wordt veroorzaakt door mutatie in FBN1, het gen dat codeert voor fibrilline, en is o.m. gekenmerkt door overmatige skeletale lengtegroei, veralgemeende gewrichtshypermobiliteit, en verbreding en scheurvorming in de aorta. Het tijdig herkennen/behandelen van de orale symptomen en het doelgericht anticiperen op syndroom-specifieke weefselreacties vertegenwoordigen belangrijke elementen in de tandheelkundige behandelings-strategie voor deze populaties. De beschikbare literatuurgegevens om deze strategieën te onderbouwen zijn echter zeldzaam en dikwijls methodologisch slecht onderbouwd.

De nulhypothese van het huidige onderzoek stelde enerzijds dat patiënten met deficiëntie van collageen of fibrilline geen orale symptomen met (grote) diagnostische specificiteit vertonen. Anderzijds werd gesteld dat de mondgezondheid in deze patiënten niet gecompromitteerd is.

Dedoelstellingen van het onderzoek waren:

1. Het analyseren van de orale symptomen (in de zachte en harde orale weefsels, het aangezichtsskelet en kaakgewrichten) bij patiënten met EDS of MFS, als intrinsieke klinische expressie van de onderliggende eiwitdeficiëntie.

2. Het bepalen van de verschillende aspecten van de mondgezondheid (cariesvoorkomen, gezondheid van het parodontium en de zachte mondweefsels, en functie van de kaakgewrichten) bij patiënten met EDS of MFS.
3. Het opstellen van richtlijnen voor tandheelkundige behandeling van patiënten met EDS of MFS.

4. Het bepalen van de diagnostische waarde van histologisch onderzoek van het dentine voor het vaststellen van collageen I deficiëntie.

Gespreid over de verschillende onderzoeksgebieden werden in totaal 33 personen met één der meest frequente EDS types (hypermobiele EDS, klassieke EDS of vasculaire EDS) en 51 met MFS onderzocht. De selectie van de proefpersonen gebeurde op basis van de meest recente richtlijnen voor klinische en moleculair-genetische diagnostiek [De Paepe et al., 1996; Beighton et al., 1998]. Per onderzoeksliuk werd een controlegroep samengesteld op grond van overeenkomsten qua geslacht en leeftijd, en frequentie van tandartsbezoeken (Hfdst.III-IV), verticale en sagittale skeletale kenmerken van het aangezichtskelet (Hfdst.V), of symptomen van temporomandibulaire dysfunctie (Hfdst.VI). Het totaal aantal onderzochte controlepersonen bedroeg 172. Omwille van de lage prevalentie van beide syndromen waren de onderzoekspopulaties voldoende groot om als epidemiologisch valabel en representatief voor de Belgische EDS/MFS populaties worden beschouwd. Dit werd bevestigd via een post-hoc power analyse van de meest relevante outcome variables.

In een eerste liuk (Hfdst.III-IV) werden bij 31 patiënten met EDS 23 met MFS de orale symptomen en de mondgezondheid onderzocht volgens epidemiologische protocols gebaseerd op recente richtlijnen van de Wereld Gezondheidsorganisatie [WHO, 1997]. In Hfdst.III werd vastgesteld dat patiënten met EDS algemeen een significant hogere dmf/DMF-score, meer tandplaque en diepere pockets hadden dan controlepersonen. In 62% was een hoge parodontale behandelingsnood (CPITN = III in minstens één sextant) aanwezig, vooral in hypermobiele EDS. In deze gevallen kon een significant verband worden gelegd tussen verdiepte pockets, de aanwezigheid van tandplaque en bloedend tandvlees, en beperkte gewrichtsmobiliteit van de pols. Een verhoogde mucosale kwetsbaarheid werd vastgesteld bij 74% van EDS patiënten, wat gerelateerd kon worden aan een lage poetsfrequentie, een hoge plaque index en hoge dmf/DMF-score. Structurele afwijkingen van het dentine, hoofdzakelijk misvormde tandwortels en obliteratie van de pulpa bij klassieke EDS, bleken een hoge diagnostische specificiteit te vertonen. In Hfdst.IV werd vastgesteld dat patiënten met MFS significant meer cariës (hogere d/D scores) vertoonden dan gezonde leeftijdgenoten, en dat de graad van mondverzorging (dental care level on surface level) algemeen lager was in MFS. Patiënten met MFS vertoonden algemeen een slechtere gingivale gezondheidstoestand dan controlepersonen. Deze resultaten kunnen echter negatief beïnvloed zijn door socio-economische en/ of emotionele factoren, die ontstaan uit de fysieke beperkingen eigen aan het syndroom. Ongeveer een derde van de proefpersonen vertoonden morfologische afwijkingen van de harde tandweefsels, meer bepaald lange en smalle tandwortels met obliteratie van de pulpakamers, die specifiek bleken voor de MFS populatie.
In een tweede luik (Hfdst.V) werden door cefalometrische analyse van gestandardiseerde laterale schedelradiografieën de karakteristieken van het aangezichtsskelet van een populatie van 26 patiënten met MFS gemeten in vertikale en sagittale zin, en vergeleken met populatinormen [Broadbent et al., 1975]. Naast een aanzienlijk aantal afwijkende cefalometrische variabelen bleken vooral mandibulaire retropositie en een lang en smal gelaat met verlengde voorste aangezichtshoogte (long face) typisch voor de MFS populatie. Bijkomende cefalometrische punten en meetwaarden werden ontwikkeld voor het beschrijven van de sagittale dimensies van het palatum bij de volwassenen uit deze groep (n=17) en getoetst aan deze van een controlegroep (n=32). Aan de hand van deze sagittale variabelen konden de typische skelettale dimensies van het hoge en diepe verhemelte in MFS worden aangetoond.

In een derde luik (Hfdst.VI) werden de symptomen en individuele diagnose van temporomandibulaire dysfunctie (TMD) geanalyseerd bij een populatie van 42 patiënten met veralgemeende gewrichtshypermobiliti et (VGH) als intrinsiek symptoom van erfelijke bindweefselaandoeningen. Deze populatie was samengesteld uit 18 personen met EDS en 24 met MFS. In deze groep werden de klinische symptomen van TMD en de gevolgen van hypermobiliti et van de kaakgewrichten vergeleken tussen de volwassen proefpersonen (n=27) en een gematchte controlegroep (n=40). Patiënten met VGH en symptomen van TMD vertoonden frequenter een meervoudige TMD diagnose dan symptomatische controlegroepen. Deze patiënten rapporteerden ook frequenter het optreden van onderkaakdislocaties dan symptomatische individu’s met VGH. Uit het geheel van data kon worden geconcludeerd dat frequent terugkerende onderkaakdislocaties pathognoom zijn voor VGH en dat VGH in se predisponeert tot het ontstaan van TMD. Van een aantal klinische symptomen en functionele parameters kon worden aangetoond dat zij een hoge diagnostische specificiteit bezitten voor het vaststellen van hypermobiliti et van de kaakgewrichten (o.m. een grote pijnvrije endfeel afstand, reproduceerbare instabiliteit van de kaakgewrichten tijdens functie, het ontstaan van een huiddepressie frontaal van de buitenste gehoorgang bij maximale opening, en frequent optredende onderkaakdislocaties).

In een vierde luik (Hfdst.VII) werden aan de hand van lichtmicroscopie en transmissie-elektronenmicroscopie de histologische/ ultrastructurele kenmerken vergeleken van dentinestenen van patiënten met drie verschillende storingen in de biosynthese van collageen I (OI type III met DI, klassieke EDS of type I, en deramtosparaxis EDS of type VIIC) en van gezonde controlegroepen. Tussen de verschillende pathologische stenen bleek een groot aantal overeenkomsten te bestaan wat betreft de structuur van het dentineeweefsel, evenwel met mineure, typische morfologische varianten. In alle stenen wisselden zones met normale morfologie af met pathologische zones, die gekarakteriseerd werden door een verminderd aantal dentinetubuli met variabele vorm en afmeting. Typerend waren ook vergrote, kanaalvormige structuren, die in groepjes voorkwamen en inclusie veroorzaakt van endotheelresten (bloedvatwand). Op grond van deze
vaststellingen werd aangenomen dat de morfologie van de pathologische dentinestenalen algemeen een afwijkend secretoir gedrag van de odontoblasten reflecteert. Hoewel de dentinestoornissen in de drie stalen het resultaat waren van verschillende mutaties, bleken er geen hoog-specifieke morfologische kenmerken te bestaan. Een significante vaststelling was echter dat deze afwijkingen ook voorkwamen bij klinisch normale tanden van deze proefpersonen. Uit dit onderzoek kon worden geconcludeerd dat histologisch dentine-onderzoek een valabile methode kan zijn voor het diagnosticeren van collageen I deficiëntie.

Aan de hand van voorgaande onderzoeksresultaten kunnen de doelstellingen van de studie worden getoetst. De eerste doelstelling, het analyseren van orale symptomen (in de zachte en harde orale weefsels, het aangezichtsskelet en kaakgewrichten) als intrinsieke klinische expressie van de onderliggende eiwitdeficiëntie, leverde betekenisvolle resultaten op voor beide syndromen. Bij EDS waren een verhoogde weefselkwetsbaarheid van de mucosa en hypermobilitiet van de kaakgewrichten veralgemeende verschijnselen (>70%). Een aantal orale symptomen bleken geassocieerd te zijn met bepaalde syndroomtypes: een abnormale vorm van de pulp kamers kwam voor in klassieke EDS, terwijl progressieve pulpaoberliteratie werd vastgesteld in klassieke en hypermobile EDS. Verkorte, misvormde tandwortels werden opgemerkt in een klein aantal EDS patiënten. Bij MFS bleken morfologische tandafwijkingen (lange en smalle wortels met progressieve pulpaoberliteratie), verhoogde kwetsbaarheid van de mucosa, een hoog en diep verhemelte, mandibulaire retrognatie, 'long face', en hypermobilitiet van de kaakgewrichten gerelateerd te zijn aan het syndroom.

Wat betreft de tweede doelstelling, het bepalen van de verschillende aspecten van de mondgezondheid, werd het voorkomen van orale infectieziekten (cariës, gingivitis en parodontitis) en kaakgewrichtsdysfunctie bepaald bij patiënten met EDS of MFS, en getoetst aan een gezonde controlepopulatie. In beide syndromen werd een significant hogere vatbaarheid voor cariës en inflammatief/abbraak van de parodontale weefsels vastgesteld, en vertoonde de mucosa een verhoogde kwetsbaarheid. Een hoge incidentie van meervoudige kaakgewrichtsaandoeningen kon worden gerelateerd aan symptomen van verhoogde mobilitiet van de kaakgewrichten.

Aan de hand van de voorgaande vaststellingen en van de algemeen-medische risico's, eigen aan de verschillende syndromen, werden een aantal richtlijnen opgesteld voor tandheelkundige behandeling van patiënten met EDS of MFS, zodat voldaan werd aan de derde doelstelling (zie Hfdst.III.6 en IV.6).

De vierde doelstelling, het bepalen van de diagnostische waarde van histologisch onderzoek van het dentine voor het vaststellen van collageen I deficiëntie, werd verwezenlijkt door het beschrijven van een aantal typische morfologische weefselkarakteristieken van pathologische dentinenstenalen. Indien algemene symptomen, zoals botbroosheid met misvorming, blauwe oogsclerae, gewrichtshypermobilitiet en/ of verhoogde weefselkwetsbaarheid, aanwezig zijn in
een proefpersoon, kan een diagnose van collageen I deficiëntie worden gestaafd met histologisch dentineonderzoek. In deze gevallen kan dentineonderzoek dus een valable bijdrage vormen tot de medische diagnostiek.

De voorgaande conclusies verwerpen de nulhypothese en tonen aan dat de onderliggende moleculaire storing specifieke symptomen in de verschillende orofaciale structuren (tanden, orale zachte weefsels, alveolair bot, craniofaciale skelet en kaakgewrichten) kan produceren, die echter niet steeds klinisch waarneembaar zijn.

In het geval van een kwalitatief defect van collageen I houdt dit onder meer in dat er in de regel altijd (ultra)structurele afwijkingen van het dentine aanwezig zijn, zelfs indien de tanden klinisch en radiografisch normaal zijn. Het is heden onbekend welke plaats de overmatige vorming van reactionair dentine (progressieve obliteratie van de pulpakamers), aanwezig in de dentitie van een aantal patiënten met erfelijke bindweefselaandoeningen, inneemt in het continuum van formatieve dentinestoornissen. Op grond van histogenetische gegevens lijkt het aanneembaar dat deze tekenen van abnormale odontoblastenactiviteit niet uitsluitend het resultaat kunnen zijn van een gestoorde collageensynthese. Aansluitend bij de resultaten van het histologisch onderzoek in Hfdst.VII en pathogenetische hypothesen uit de literatuur [Waltimo, 1999], lijkt het zinvol verder te onderzoeken hoe het metabolisme van de odontoblasten kan verstoord raken door accumulatie van vroegtijdig gedegradeerde procollageenmoleculen in het ruw endoplasmatisch reticulum bij patiënten met een aangeboren kwalitatief defect van deze proteïnen. Dit zou, als gemeenschappelijk pathogenetisch principe, de fenotypische gelijkenissen kunnen verklaren tussen solitaire vormen van veralgemeende dentinestoornissen (dentinogenesis imperfecta type II) en dentinestoornissen als onderdeel van erfelijke collagenaandoeningen. Mogelijk ligt hier ook de oorzaak van de aanwezigheid van abnormaal hoge concentraties van collageen III en andere extracellulaire matrixproteïnen in een aantal van deze dentinestoornissen, ten gevolge van het “verstoren” van het intracellulaire productie-evenwicht van deze proteïnen. Als gevolg van het verstoorde metabolisme kunnen de actieve odontoblasten tevens hun polarisatie verliezen en multidirectioneel een abnormale dentinematrix beginnen afscheiden. Dit kan vervolgens leiden tot vroegtijdige apoptose, waarbij de cel ingesloten wordt in de dentinmatrix (zie Hfdst. VII), en tot de vorming van onregelmatige dentinetubuli door nieuwe generaties odontoblasten, ontstaan uit reservoireellen van het proximale pulpamesenchym. In het geval van gestoorde collageen-I-synthese zal er een verstoorde inbouw van apatiekristallen plaatsvinden en rond de abnormale collageenfibrillen. Bij niet-collageengebonden dentinestoornissen (dentinogenesis imperfecta type II en dentine dysplasie) zal de mineralisatie van de dentinematrisz eerder verstoord zijn ten gevolge van een abnormale synthese van dentine-specificieke proteïnen zoals dentine sialoproteïne en dentine fosfoproteïne, die verantwoordelijk zijn voor de nucleatie en de groei van de apatiekristallen. Verder onderzoek van hogervermelde pathogenetische aspecten van verstoorde
dentinevorming, al dan niet als onderdeel van een ruimere pathologie, blijft nodig op het niveau van de odontoblast.

Op grond van hogervermelde gegevens is het onduidelijk wat de invloed van deze dentinestoornissen is op het voorkomen van cariës, en meer in het bijzonder op de cariësprogressie. Naar analogie met de gerapporteerde verminderde mechanische eigenschappen van dentine in DI type II [Kinney et al., 2001; Kinney et al., 2003], lijkt het aannemelijk dat dentineafbraak ten gevolge van bacteriële aanval (cariës) in deze patiënten sneller voorloopt. Heden ontbreken echter wetenschappelijke data om deze stelling te onderbouwen.

Zoals blijkt uit voorgaande resultaten, hebben kwalitatieve collageen- en fibrillinestoomnissen een belangrijke invloed op de mechanische eigenschappen (stevigheid en weerstand tegen druk- of trekkrachten) en de regeneratiedrapaciteiteinen van een aantal zachte weefsels, zoals spieren, huid, mucosa, gingiva en het parodontale ligament. Dit kan resulteren in hypotonie (spieren) met een sterk verhoogde weefselkwaalbaarheid (huid, mucosa en gingiva) en een gebrekkig herstelvermogen na mechanische of chemische (infectieuze) trauma's. Bij infectieuze mondzieken kunnen bij uitstek de zachte parodontale weefsels een opvallende variatie van weefselreacties vertonen, waarbij zowel de primaire (zwelling en bloeding) en secundaire (weefselherstel) klinische symptomen negatief beïnvloed worden door de onderliggende bindweefselawandoening. Een adequate preventie en atraumatische behandeling van deze infecties verdient bijgevolg de voorkeur.

Aangezien tot heden verschillende aspecten van de pathogenese van beide syndromen grotendeels onbekend blijven, dient verder onderzoek te bepalen of de vermelde orale symptomen voldoende specificiteit bezitten om een valabile aanvulling van het diagnostisch areaal te vormen, onder meer door gedetailleerd te documenteren over de genotype-phenotype correlatie in deze populaties.

Verder tonen bovengaannde conclusies eveneens aan dat personen met aangeboren stoornissen van collageen of fibrillin vanuit tandheelkundig perspectief beschouwd kunnen worden als een belangrijke risicogroep. Dit houdt in dat deze populaties een verhoogd risico vertonen op het ontwikkelen van een aantal mondaandoeningen, zoals cariës, gingivitis, parodontitis en kaakgewrichtsproblemen. Het is echter waarschijnlijk dat een aantal epigenetische en omgevingsfactoren, zoals metabole processen (hormonen), psychologisch profiel, socio-economische status en fysieke beperkingen, deze risico's voor een aanzienlijk deel mee bepalen.

Uit deze conclusies volgt eveneens dat zowel op het vlak van preventie en behandeling een aantal aangepaste maatregelen in acht dienen genomen te worden (zie Hfdst. III.6 en IV.6). Gezien de cardiovasculaire structuren frequent in het algemene ziektebeeld van bepaalde bindweefselandaarden betrokken zijn, dient de practicus tijdens de behandelingsplanning te informeren naar het bestaan
van dergelijke risico's. In de regel zullen vooral bij MFS (functionele klepafwijkingen en/of cardiovasculaire chirurgie) profylactische maatregelen dienen genomen te worden ter preventie van endocarditis. Bij patiënten met gekende dilatatie van de aorta geniet het de voorkeur aangepaste lokale anesthesietao te dienen en hartbewaking te voorzien tijdens lange of invasieve tandheelkundige behandelingen. Bij collageenstoornissen (OI, EDS) is het vóórkomen van deze afwijkingen, die via een routine echocardiografisch onderzoek kunnen worden vastgesteld, in de regel zeer gering.

Uit bovenstaande conclusies wordt aan de algemene tandarts een bruikbaar protocol aangeboden voor de diagnose en behandeling van mondaandoeningen bij patiënten met EDS of MFS. Deze richtlijnen kunnen een nuttige bijdrage betekenen zowel voor het (vroegtijdig) diagnosticeren van milde bindweefseloordeningen, als voor het adequaat en succesvol behandelen van deze patiëntengroepen.
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