ORAL HEALTH IN HEREDITARY GELSOLIN AMYLOIDOSIS

Pirjo Juusela

ACADEMIC DISSERTATION

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To all the AGel amyloidosis patients.
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ABSTRACT

Hereditary gelsolin amyloidosis (AGel amyloidosis) is an autosomally dominantly inherited disease, mostly found in Finland, but also with worldwide distribution. Its most characteristic clinical signs are corneal lattice dystrophy, polyneuropathy, and cutis laxa, which after onset in the thirties to forties slowly progress.

AGel amyloidosis is caused by a point mutation c.640G>A/T, formerly known as c.654G>A/T, in the gene coding for both cytosolic and secretory gelsolin. Cytosolic gelsolin has many roles in cellular activities. Most importantly, it modulates actin formation and participates in cell shape alterations, motility, phagocytosis, and other functions. The c.640G>A/T gene defect causes abnormal cleavage of gelsolin and eventually leads to accumulation of aberrant secretory gelsolin as amyloid fibrils. The systemic nature of this disease has been considered to result mainly from amyloid deposits accumulating in many tissues of AGel amyloidosis patients, but the pathogenesis of the disease is not yet fully understood.

Patients with AGel amyloidosis reported to their physician oral problems such as sense of dry mouth and loose or cracked teeth. This information served as a starting point for this study in which we elucidated the impact of this systemic disease on oral condition, including salivary function and periodontal health. Further, oral fibroblasts and vascular smooth muscle cells were examined in vitro to clarify whether mutated cytosolic gelsolin affects their function, thus contributing to the pathogenesis of AGel amyloidosis in general and/or in relation to periodontal health. Patients were invited to the study through their patient organization and forty patients volunteered.

We found that patients frequently exhibit subjective mouth dryness, i.e. xerostomia, and also decreased saliva secretion, i.e. hyposalivation. The saliva composition was also altered. Especially, secretion rate of salivary IgA was decreased, further increasing the risk for oral diseases such as oral candidiasis and caries. Histopathological analyses in minor labial salivary gland (LSG) biopsies showed gelsolin amyloid deposits, as well as atrophy of the glands, and minor inflammation. These
novel histopathological LSG findings could explain at least partly the alterations in saliva secretion and composition. In one case sicca symptoms (dry eyes and mouth, i.e. xerophthalmia and xerostomia, respectively) and LSG findings had misled to the diagnosis of Sjögren’s syndrome, which was later substituted with an AGel amyloidosis diagnosis.

According to this study, AGel amyloidosis, on average, does not present a generally increased risk for periodontitis. However, some patients presented a high rate of disease progression, indicating that AGel amyloidosis might in some cases be associated with periodontal problems. Because both AGel amyloidosis and periodontitis progress with age, this association appears to be more common in older patients, who had lost especially their molar teeth quite commonly. In general, however, non-specific oral microbiota and common periodontal status prevail in this disease.

*In vitro* cell studies showed that oral fibroblasts and vascular smooth muscle cells of heterozygote AGel amyloidosis patients had similar actin cytoskeleton morphology and cytosolic gelsolin distribution, migration rate, and collagen type I metabolism as control cells. Only the reaction to staurosporine, an inhibitor of protein kinases, induced minor differences in the shape change rate between the patient and control oral fibroblasts. The altered reaction of oral fibroblasts of the patients to staurosporine should be further evaluated. These results suggest that the patient oral fibroblasts and vascular smooth muscle cells mainly function normally *in vitro* and may not, at least via cytosolic gelsolin-associated dysfunction, contribute to the pathogenesis of AGel amyloidosis.

Thus, patients with AGel amyloidosis due to their systemic disease have greater risk for oral diseases and benefit from preventive oral hygiene procedures, such as diet advice, extra fluoride, lubricants, and regular dental check-ups.

KEY WORDS: AGel amyloidosis, oral health, hyposalivation, salivary IgA, small salivary glands, periodontitis, fibroblasts, vascular smooth muscle cells.
TIIVISTELMÄ

Meretojan tauti, nykyiseltä nimityksestä perinnöllinen gelsoliiniamyloidoosi (AGel-amyloidoosi) on autosomissa vallitsevasti periytyvä systeemisairaus, joka on yksi yleisimmistä suomalaisen tautiperinnön sairauksista. Suomessa on arvioitu olevan 400–1000 potilasta, joista liki kaikki ovat heterotsygootteja geenivirheen suhteen. Tautia esiintyy yksittäisissä suvuissa myös maailmanlaajuisesti. Tyyppillisä sairauden oireita ovat silmän verkkomainen rappeuma (corneal lattice dystrophy), polyneuropatia ja poikkeava ihon löystyminen (cutis laxa), jotka ilmenevät usein potilaiden ollessa 30-40-vuotiaita. Sairaudelle on ominaista oireiden hidas eteneminen eliniänodotteen ollessa liki sama kuin muulla väestöllä.


AGel-amyloidoosipotilaat olivat kertoneet lääkärilleen suun alueen ongelmistaa, kuten kuivan suun tunteesta sekä heiluvoista ja lohkeilevistä hampaista. Tämä tieto toimi lähtökohtana tälle tutkimukselle, jonka tarkoituksena oli kliinisin, biokemiallisin, histologisen ja mikrobiologisen menetelmin selvittää tämän systeemisairauden vaikutusta potilaiden suun terveyden tilaan. Vastaavanlaista tutkimusta ei ole aiemmin tehty. Syljenerityksen mitattaminen, syljen proteiinkonsentraation määrittäminen sekä hampaisten kiinnityskudosten eli parodontiumin tilan kartoittaminen, kliinisesti ja mikrobiologisesti, olivat päätutkimuskohteita. Lisäksi tutkimme suun alueen sidekudossoluja ja verisuonten sileitä lihassoluja in vitro arvioidaksemme geenimutaation

Tutkimus osoitti, että potilailla ilmeni usein kuivan suun tunnetta (xerostomia) ja alentunutta syljeneritystä (hyposalivaatio). Kontrollihenkilöihin verrattuna potilailla todettiin alentunutta syljen IgA:n eritystä ja kohonneita suun Candida albicans -määriä. Tässä tutkimuksessa ei havaittu epätavallisen runsasta reikiintymistä, vaikka aiemman kirjallisuuden perusteella tiedetään hyposalivaation ja alentuneen syljen IgA pitoisuuden lisäävät riskiä kariekselle ja suun limakalvojen hiivatulehduselle.


In vitro-solututkimukset osoittivat, että AGel-amyloidoosipotilaiden suun sidekudossoluilla ja verisuonien sileäiliassoluilla oli verrokkisoluja vastaava aktiinitukiranka, solunsisäisen gelsoliinin


AVAINSANAT: AGel amyloidoosi, suun terveys, hyposalivaatio, syljen IgA, pienet sylkirauhaset, parodontiitii, sidekudossolut, sileäläihassolut.
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LIST OF ORIGINAL PUBLICATIONS

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I  

II  
Xerostomia in hereditary gelsolin amyloidosis. 

III  
Relation of gelsolin amyloidosis and periodontal health. 

IV  
Gelsolin c.640G>A mutation in oral fibroblasts and vascular smooth muscle cells of hereditary gelsolin (AGel) amyloidosis patients. 
Amyloid, submitted.  
* The authors contributed equally to this work.

This thesis also contains unpublished data.

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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AA</td>
<td>amyloid A</td>
</tr>
<tr>
<td>AApoAI</td>
<td>amyloid apolipoprotein AI</td>
</tr>
<tr>
<td>AApoAII</td>
<td>amyloid apolipoprotein AII</td>
</tr>
<tr>
<td>Aβ2M</td>
<td>amyloid β2 microglobulin</td>
</tr>
<tr>
<td>ABri</td>
<td>amyloid ABriPP</td>
</tr>
<tr>
<td>ACys</td>
<td>amyloid cystatin C</td>
</tr>
<tr>
<td>AFib</td>
<td>amyloid fibrinogen α</td>
</tr>
<tr>
<td>AGel</td>
<td>amyloid gelsolin</td>
</tr>
<tr>
<td>AIAPP</td>
<td>amyloid islet amyloid polypeptide</td>
</tr>
<tr>
<td>AL</td>
<td>amyloid light chain</td>
</tr>
<tr>
<td>ALECT</td>
<td>amyloid leukocyte chemotactic factor-2</td>
</tr>
<tr>
<td>ALys</td>
<td>amyloid lysozyme</td>
</tr>
<tr>
<td>APECED</td>
<td>autoimmune polyendocrinopathy candidiasis ectodermal dystrophy</td>
</tr>
<tr>
<td>Apo E</td>
<td>apolipoprotein E</td>
</tr>
<tr>
<td>ATTR</td>
<td>amyloid transthyretin</td>
</tr>
<tr>
<td>ATTRwt</td>
<td>amyloid transthyretin, wild type</td>
</tr>
<tr>
<td>AV</td>
<td>absorbance value</td>
</tr>
<tr>
<td>BOP</td>
<td>bleeding on probing</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium ion</td>
</tr>
<tr>
<td>CAL</td>
<td>clinical attachment level</td>
</tr>
<tr>
<td>cGSN</td>
<td>cytosolic gelsolin</td>
</tr>
<tr>
<td>CLD</td>
<td>corneal lattice dystrophy</td>
</tr>
<tr>
<td>DAPI</td>
<td>4,6-diamidino-2-phenylindole</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
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<tr>
<td>FAF</td>
<td>familial amyloidosis of the Finnish type</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>---------</td>
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<tr>
<td>FAP</td>
<td>familial amyloid polyneuropathy</td>
</tr>
<tr>
<td>FBS</td>
<td>fetal bovine serum</td>
</tr>
<tr>
<td>FMF</td>
<td>familial Mediterranean fever</td>
</tr>
<tr>
<td>GI</td>
<td>gastrointestinal</td>
</tr>
<tr>
<td>GSN</td>
<td>gelsolin protein</td>
</tr>
<tr>
<td>GSN</td>
<td>gelsolin gene</td>
</tr>
<tr>
<td>H⁺</td>
<td>hydrogen ion</td>
</tr>
<tr>
<td>HGA</td>
<td>hereditary gelsolin amyloidosis</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>ICTP</td>
<td>carboxyterminal telopeptide of type I collagen</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>La(SSB)</td>
<td>Sjögren’s syndrome antigen B, also called La</td>
</tr>
<tr>
<td>LSG</td>
<td>labial salivary gland</td>
</tr>
<tr>
<td>MMP</td>
<td>matrix metalloproteinase</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PINP</td>
<td>aminoterminal propeptide of type I procollagen</td>
</tr>
<tr>
<td>PIP2</td>
<td>phosphatidylinositol bisphosphate</td>
</tr>
<tr>
<td>PPD</td>
<td>probing pocket depth</td>
</tr>
<tr>
<td>RIA</td>
<td>radioimmunoassay</td>
</tr>
<tr>
<td>Ro(SSA)</td>
<td>Sjögren’s syndrome antigen A, also called Ro</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>SAA</td>
<td>serum amyloid A apolipoprotein</td>
</tr>
<tr>
<td>SAP</td>
<td>serum amyloid P component</td>
</tr>
<tr>
<td>sGSN</td>
<td>secretory gelsolin</td>
</tr>
<tr>
<td>SMA</td>
<td>smooth muscle actin</td>
</tr>
<tr>
<td>SS</td>
<td>Sjögren’s syndrome</td>
</tr>
<tr>
<td>SSA</td>
<td>senile systemic amyloidosis</td>
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</table>
SWS    stimulated whole saliva
TNF    tumor necrosis factor
UWS    unstimulated whole saliva
VPI     visible plaque index
VMGA   viability medium, Goteberg, anaerobically prepared and sterilized
VSMC   vascular smooth muscle cell

In addition, standard three- and one-letter abbreviations of amino acids and one-letter codes of nucleotides are used.
1. INTRODUCTION

In many cases, oral symptoms precede the initiation of disease in target organs, and thus, oral findings may allow early diagnosis and treatment of a systemic disease. On the other hand, in some systemic diseases oral manifestations correlate with disease duration, e.g. dental erosion with gastroesophageal reflux (Meurman et al. 1994). Manifestations in the oral cavity may in certain diseases reflect the severity of the systemic disease, as in inflammatory bowel diseases (Lankarani et al. 2013). Oral infectious diseases and systemic diseases and also general health counteract in several different conditions, e.g. in periodontitis and diabetes mellitus (DM) (Llambes et al. 2015). Thus, the research combining the fields of general and oral health is important.

Amyloidoses are protein misfolding disorders where abnormal fibrillary protein accumulates as amyloid either locally or systemically. Amyloidotic diseases have varying symptoms and prognosis, but they all share the histopathological finding of amyloid deposits. The background can be hereditary, but is more commonly idiopathic or inflammatory. The most common amyloidoses are 1) Alzheimer’s disease, which is a localized amyloidosis and commonly acquired, 2) localized amyloidosis of the pancreas, islet amyloid polypeptide (AIAPP) amyloidosis, related to DM type II and age, and 3) acquired transthyretin wild-type (ATTRwt) amyloidosis, which is commonly related to aging (Tanskanen et al. 2008; Qiu et al. 2009; Su et al. 2012). Systemic acquired amyloidosis, such as immunoglobulin light chain (AL) amyloidosis caused by clonal plasma cells, and amyloid A (AA) amyloidosis secondary to chronic inflammation, are more common than the relatively rare systemic hereditary amyloidoses, e.g. gelsolin (AGel) amyloidosis.

In the 1960s the Finnish ophthalmologist Jouko Meretoja described a disease of hereditary nature affecting the eyes, skin and nerves (Meretoja 1969). According to the discoverer it was then called Meretoja’s disease, later familial amyloidotic polyneuropathy type IV (FAP IV), familial amyloidosis of Finnish type (FAF), and hereditary gelsolin amyloidosis (HGA). Mostly, the name AGel amyloidosis is used according to the amyloid nomenclature (Sipe et al. 2014). It is a late-onset systemic disease, characteristically manifesting with ophthalmological, neurological and
dermatological findings, although other symptoms may also occur (Meretoja 1969).

In the disease, amyloid is found in most tissues and it is especially attached to the blood vessel walls and basement membranes (Meretoja and Teppo 1971). In the 1990s the cause for the disease was found to be a point mutation in the gelsolin-coding gene (GSN) (Levy et al. 1990; Maury et al. 1990; de la Chapelle et al. 1992b). Gelsolin (GSN) is an omnipresent cytosolic (cGSN) and secreted (sGSN) protein with intra- and extracellular functions. The sGSN is considered to be the sole source of amyloid fibrils in AGel amyloidosis (Kangas et al. 1996), and the local production might further increase the amyloid formation in certain tissues and organs (Kivelä et al. 1994; Kiuru 1998). In AGel amyloidosis, amyloid deposits (Haltia et al. 1990a; Maury 1991b) with subsequent cytotoxic effects, are thought to contribute to pathogenesis (Anan et al. 2010). On the other hand, altered cell function has also been observed in patient platelets (Kiuru et al. 2000), but other systemic studies in patient cells have not been presented. Apart from elucidation of AGel amyloidogenesis, the impact of mutant cGSN on cellular function and AGel amyloidosis pathogenesis remains unclear.

Previously, amyloid deposits had been found in an autopsied parotid gland sample of an AGel amyloidosis patient (Meretoja and Teppo 1971). It was also known that some AGel amyloidosis patients have macroglossia (Kiuru et al. 1999a), a relative common finding in AL amyloidosis (Benson 2012b). Oral manifestations have been previously observed in systemic amyloidoses, such as AA (Catalano and Vaughan 1980) and AL (Schima et al. 1994) amyloidosis, in the hereditary amyloidoses such as ALys (Granel et al. 2006) and Aβ2M (Valleix et al. 2012) amyloidosis, and as a separate entity of localized acquired amyloidosis (Paccalin et al. 2005). Apart from systemic hereditary transthyretin (ATTR) amyloidosis, where hyposalivation and altered salivary composition was observed (Johansson et al. 1992), these manifestations have not been extensively studied.

AGel amyloidosis patients had complained to their medical physician of a feeling of dry mouth and loose teeth. This information served as a starting point for this research. In the preliminary study, we encountered two patients with a history of an aggressively progressing periodontitis, which probably had caused or severely contributed to total and/or prominent tooth loss. This further prompted us to investigate dental and periodontal problems of AGel amyloidosis patients.
We hypothesized that AGel amyloidosis could have some adverse effects on the oral health of the patients, and we sought to elucidate these effects by conducting clinical, biochemical, histological, and microbiological studies to verify oral health condition, salivary function and periodontal status of forty heterozygote AGel amyloidosis patients. Further, we speculated that the point mutation could cause alterations in the cGSN function that might have an impact on the pathogenesis of AGel amyloidosis, and therefore, we performed \textit{in vitro} cell studies on oral fibroblasts and vascular smooth muscle cells.
2. REVIEW OF THE LITERATURE

2.1. ORAL HEALTH AND SYSTEMIC DISEASES

2.1.1. Oral tissues

The oral cavity is a unique environment where hard tissue is exposed and surrounded by mucosa. The oral mucosa consists of lamina propria and basement membrane covered with stratified squamous epithelium. This tissue lines the oral cavity. The part of the oral mucosa surrounding the teeth is called gingiva. The teeth are composed of dentin, cementum covering the root of the tooth and enamel overlaying the crown of the tooth. Inside the teeth is the dental pulp tissue containing nerves and blood vessels. The tongue is a muscular structure that is richly supplied with nerves and blood vessels. It is covered with numerous taste buds and papillae (Nanci 2007).

Oral functions, such as eating, digestion and speech, are made possible and supported by the wellbeing of oral tissues.

2.1.2. Periodontium

The periodontium, also called the tooth attachment apparatus, attaches the tooth to the bone tissue of the jaws. It is composed of gingiva, periodontal ligament, root cementum and alveolar bone (Figure 1).
Gingiva comprises epithelial cells lining the underneath connective tissue known as the lamina propria, which has rich blood circulation and innervation. In normal condition, fibroblasts are the most common cells in the lamina propria, but it also contains mast cells, macrophages and inflammatory cells. The gingival extracellular matrix is mostly produced by fibroblasts and its main constituents are collagen and protein-carbohydrate molecules, namely proteoglycans and glycoproteins. Different types of fibers are abundant in the lamina propria and are produced by fibroblasts (Lindhe et al. 2003). The most abundant is collagen type I, which forms gingival fibers bracing the marginal gingiva firmly against the tooth (Romanos et al. 1992; Fiorellini et al. 2012). Other fibers are reticular, oxytalan and elastic fibers (Lindhe et al. 2003).
**Periodontal ligament** attaches teeth to the surrounding alveolar bone and in the cervical tooth part to the *lamina propria* of the gingiva. It transduces the forces generated by masticatory function to the alveolar bone and is essential for the mobility of the tooth. The width of the space of the periodontal ligament varies from 0.2 to 0.4 mm. Cells of the periodontal ligament are mainly fibroblasts, and small amounts of epithelial cells called cell rests of Malassez. The tooth is joined to its surrounding tissues by bundles of collagen fibers. The fiber ends embedded in root cementum and alveolar bone are called Sharpey’s fibers (Figure 1). Also oxytalan fibers, which course parallel to the long axis of the tooth, and a few other elastic system fibers are present in the periodontal ligament, as are also nerves and blood vessels (Lindhe et al. 2003).

**Root cementum** resembles bone and consists of mineralized collagen matrix. Cementum does not undergo physiological remodelling, and thus, it lacks blood vessels, lymph vessels, and innervation, but is characterized by continuing deposition. Different parts of cementum contain varying amounts of cementoblasts, cells producing cementum (Lindhe et al. 2003).

**Alveolar bone** in the tooth socket is covered with a thin layer of bundle bone called alveolar bone proper. In radiographs the bundle bone is opaque (called the lamina dura) because of increased mineral content around fiber bundles, and the alveolar crest is found 1.5-3 mm below the level of cemento-enamel junction (Lindhe et al. 2003).

### 2.1.3. Periodontitis

Periodontitis is an inflammatory response to bacterial deposits (biofilm) on teeth that causes attachment loss. It is measurable as formation of deepened periodontal pockets (≥4 mm probing pocket depth, PPD) and in more advanced stages loosening of teeth and radiographically observed alveolar bone loss. Classification system for periodontal diseases and conditions comprises gingival and periodontal diseases. Periodontitis can be further classified into three major types: chronic periodontitis, aggressive periodontitis, and periodontitis as manifestation of systemic disease (Armitage 1999). These three types of periodontitis are described in more detail in Table 1.
Table 1. Three main types of periodontitis

<table>
<thead>
<tr>
<th>Chronic periodontitis</th>
<th>Aggressive periodontitis</th>
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<tr>
<td>1. Mostly found in adults</td>
<td>1. Disease onset usually under the age of 30 years otherwise medically healthy patients</td>
</tr>
<tr>
<td>2. Slow to moderate rate of progression</td>
<td>2. Rapid rate of progression and familial aggregation</td>
</tr>
<tr>
<td>3. Subgingival calculus frequently found</td>
<td>3. Severity inconsistent with microbial deposits</td>
</tr>
<tr>
<td>4. Associated with a variable microbial pattern</td>
<td>4. <em>A. actinomycetemcomitans</em> commonly found</td>
</tr>
<tr>
<td>Localized: ≤30% of the sites involved</td>
<td>Localized: affecting first molar plus another permanent tooth (incisor) at or near the pubertal age.</td>
</tr>
<tr>
<td>Generalized: &gt;30% of the sites involved</td>
<td>Generalized: affecting at least three teeth other than first molars and incisors.</td>
</tr>
</tbody>
</table>

Periodontitis as manifestation of systemic disease

| 1. Haematologic disorders; acquired neutropenia, leukaemias, or other |
| 2. Genetic disorders; e.g. Down syndrome, Ehlers-Danlos syndrome |
| 3. Not otherwise specified |

The severity of chronic and aggressive periodontitis is evaluated on the basis of the clinical attachment loss (CAL) as slight (CAL 1-2 mm), moderate (CAL 3-4 mm), or severe (CAL ≥ 5mm). *A. actinomycetemcomitans*: *Aggregatibacter actinomycetemcomitans*. Adapted from Armitage 1999, Hinrichs and Novak 2012.

In reports based on national surveys, the proportion of persons with periodontitis (pockets of ≥ 4 mm) varies greatly in different countries from 40% to 70% (Hugoson et al. 1998; Morris et al. 2001; Albandar 2002; Sheiham and Netuveli 2002; Eke et al. 2012). According to the Finnish Health 2000 Survey, where 5255 adults participated in the periodontal study (Suominen-Taipale et al. 2004), the prevalence of periodontitis dentate patients aged over 30 years was 64 % (males 72 % and females 57 %). Thus, periodontitis is a very common infectious disease.
Bacterial biofilm deposits evolve on the tooth surface and cause host cells to secrete proinflammatory cytokines, resulting in destruction of the attachment tissues of the tooth. Mature dental biofilm can contain a large variety of bacterial species; approximately 700 bacterial species or phylotypes can be found in the biofilms of the oral cavity (Paster et al. 2001). Putative periodontal pathogenic species have been identified from the subgingival biofilm, including *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Parvimonas micra*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Streptococcus intermedius* and *Treponema species* (Socransky and Haffajee 2003; Dentino et al. 2013). Three anaerobic bacterial species, namely *P. gingivalis*, *T. forsythia* and *T. denticola* (collectively named “the red complex”), have been associated with severe chronic periodontitis (Socransky et al. 1998). *A. actinomycetemcomitans* has been associated with localized aggressive periodontitis (Zambon 1985). The source of pathogens is usually unknown but transfer from parents is considered to play an important role. However, the onset of periodontitis after the initial colonization of pathogens takes several years or decades depending on host response and behavioural and genetic risk factors (Socransky and Haffajee 2003). Bacteria can be detected by sample cultivation, DNA-based assays, PCR, and electron and confocal laser microscopy (Choi et al. 1994; Socransky et al. 1994; Kroes et al. 1999).

Several physiological factors defend us against the bacteria of the biofilm. Saliva and crevicular fluid flush the gingival sulcus. Turnover of the gingival pocket epithelium removes cells that have been invaded by bacteria. Defense cells, notably neutrophils, release antibacterial substances, such as enzymes, active oxygen species, and defensins, into the gingival sulcus (Socransky and Haffajee 2003). In contrast, persistence of the plaque biofilm causes periodontal tissue destruction directly by compounds released from bacteria (e.g. *P. gingivalis*), but mainly indirectly by inducing an inflammatory immune response. Initially, microbial substances trigger epithelial cells to produce inflammatory mediators, and cells such as polymorphonuclear leucocytes, monocytes, macrophages, and lymphocytes prevail. As the infection continues, antibody-producing plasma cells derived from B cells, and T helper cells appear (Kinane et al. 2003). Periodontal tissue destruction takes place through the action of proteolytic enzymes, most notably matrix metalloproteinases, released from neutrophils, macrophages, osteoclasts, fibroblasts, and epithelial cells (Pöllänen et al. 2012). Destruction of periodontal ligament fibers and alveolar bone results in formation of a space
between the gingiva and the tooth termed as periodontal pocket. These pockets are favourable for
growth of anaerobic pathogenic bacteria (Löe 1981; Carranza and Camargo 2012). They also make
removal of dental biofilm very difficult for the patient. Chronic periodontitis has characterized by
slow site-specific progression with periods of active destruction and quiescent (Socransky et al.
1984). When untreated, periodontal destruction continues and eventually results in tooth loss.

Many factors affecting general health increase the risk for periodontitis. The best known of these
are tobacco smoking (Bergström 1989), poorly controlled and/or long-lasting diabetes mellitus
(DM) (Oliver and Tervonen 1994), and human immunodeficiency virus (HIV) infection (Holmstrup
and Westergaard 1994). Men have more deep pockets and alveolar bone loss than women
(Albandar 2002). Genetic factors, as a form of single-nucleotide polymorphism, are also well-
established risk factors for chronic periodontitis (Mucci et al. 2005; Research, Science and Therapy
Committee of American Academy of Periodontology 2005). Moreover, several studies support the
idea that stress and psychological factors (Peruzzo et al. 2007), obesity (Saito et al. 1998),
metabolic syndrome (Watanabe and Cho 2014), and osteopenia/-porosis, especially in relation to
hormone replacement therapy (Ronderos et al. 2000), increase the risk for periodontitis. In the
Papillon-Lefèvre syndrome, patients have a defect in the gene coding for cathepsin C, an
inflammatory mediator, and early in their lives they suffer from aggressive and easily recurrent
periodontitis (Van Dyke et al. 1984; Nickles et al. 2013).

Vice versa, chronic periodontitis has adverse effect on certain systemic conditions. It increases the
risk for cardiovascular events (Friedewald et al. 2009), may cause low birth weight of newborn
(Heimonen et al. 2009), increases the risk for pneumonia (Paju and Scannapieco 2007), can disturb
glycaemic control in DM (Teeuw et al. 2010), and may promote pancreatic carcinogenesis (Abnet et
al. 2005). Descriptive of the adverse effects of periodontitis to cardio- and cerebrovascular diseases
is that, periodontitis has been shown to cause a 70 % greater risk for heart and vascular diseases,
while vast alveolar bone loss is associated with a five times greater risk for stroke (Buhlin et al.
2011).
2.1.4. Salivary glands and saliva

Saliva is important for the nutrition, protection and lubrication of oral mucosa and for the remineralization of teeth (Mandel 1989).

Major salivary glands are the parotid, submandibular and sublingual glands. Minor salivary glands can be found in the tongue, palate, and labial and buccal mucosa. The functional part of the salivary gland tissue comprises secretory end pieces (acini) and a branched ductal system (Hellquist and Skalova 2014). Secretion from minor salivary glands is primarily mucous and the concentrations of IgA and blood group substances are notably higher than from major salivary glands (Ferguson 1999).

Salivary flow is traditionally divided into resting or unstimulated phase and stimulated phase. The function of minor and submandibular glands is prevalent in the resting phase and that of the other major salivary glands in the stimulated phase. These phases are regulated by the sympathetic, parasympathetic, vascular, acinar and myoepithelial systems (Konttinen et al. 2011). In general, problems in parasympathetic innervation cause impaired salivation and atrophy of salivary glands (Kutchai 1998).

The saliva is an easily reachable body fluid from which several factors affecting oral health or reflecting general health can be measured. IgA is the main immunoglobulin found in saliva under physiological conditions. It is almost totally (95%) produced by salivary gland immunocytes as a host response to an antigenic stimulus (Brandtzaeg 1989). IgA has been implicated as the main specific immune defense mechanism in the oral cavity, participating in the homeostasis of oral microbiota (Smith and Taubman 1992). Elevated levels of salivary IgA have been noted in the elderly (Eliasson et al. 2006), during pregnancy (Bratthall and Widerstrom 1985) and in patients with insulin-dependent DM (Ben-Aryeh et al. 1993). Salivary IgG and IgM are plasma constituents that have usually been found to be related to gingivitis or periodontitis, with inflammation allowing their transudation to gingival crevicular fluid and from there to saliva (Kilian and Bratthall 1999). Salivary albumin is also regarded as a serum ultrafiltrate (Oppenheim 1970). Its concentration varies in relation to different conditions, and it has been suggested to describe the integrity of the
oral mucosa overall (Meurman et al. 2002). Higher salivary albumin, total protein, IgG, and IgM concentrations were found in patients with rheumatic disease than in healthy control subjects, and the salivary IgA, IgG, and IgM concentrations correlated with the severity of focal sialadenitis of the patients. Further, patients with Sjögren’s syndrome (SS) had higher values for salivary IgA and IgM than patients without SS (Helenius et al. 2005). Elevated levels of all three Igs were observed in HIV-infected patients (Mellanen et al. 2001).

The terminology related to diminished salivary flow rate can be somewhat confusing. For clarification, the term xerostomia is commonly used when there is a subjective sense of dry mouth (Tenovuo and Lagerlöf 1999). Hyposalivation is a condition where the salivary flow rate has been measured to be less than the generally accepted critical value. Sicca syndrome is used when, in addition to xerostomia and/or hyposalivation, there is another noted symptom of sicca, usually dry eyes (keratoconjunctivitis sicca); a situation commonly found in SS (Vitali et al. 2002).

Measurement of the saliva secretion is divided into unstimulated and stimulated salivary flow rates. Unstimulated whole saliva is a mixture of saliva secretion in the absence of exogenous stimulus such as chewing. **Table 2** presents the widely accepted reference values of salivary flow rates (Tenovuo and Lagerlöf 1999).

**Table 2.** Secretion rates of unstimulated and stimulated whole saliva (mL/min). Adapted from Textbook of Clinical Cariology (Tenovuo and Lagerlöf 1999).

<table>
<thead>
<tr>
<th></th>
<th>Hyposalivation</th>
<th>Low</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unstimulated saliva (mL/min)</td>
<td>&lt; 0.1</td>
<td>0.1-0.25</td>
<td>0.26-0.35</td>
</tr>
<tr>
<td>Stimulated saliva (mL/min)</td>
<td>&lt; 0.7</td>
<td>0.7-1.0</td>
<td>1.1-3.0</td>
</tr>
</tbody>
</table>
Several factors are known to influence salivary flow rates such as diet, time of day, and body position during measurement (Dawes 1972). On average, females have somewhat lower salivary flow rate than males (Percival et al. 1994). In addition, several medications cause xerostomia and/or hyposalivation as a side effect (Wolff et al. 2008), and in general, the numerous daily drugs of the elderly have been noted to have a negative effect on saliva secretion (Närhi et al. 1993). Surprisingly, xerostomia has little if any relationship with the quantitative salivary flow rate. For example, breathing through the mouth causes evaporation of saliva and can induce xerostomia. A decline in mucous saliva-producing gland outflow (i.e. minor and submandibular/sublingual), commonly age-related, may cause xerostomia (Tenovuo and Lagerlöf 1999), even if the salivary flow rate overall remains adequate (Eliasson et al. 2009). Altogether, mouth dryness may severely affect the quality of life by disturbing speech, eating, use of a prosthesis and even sleep (Nederfors 2000).

2.1.5. Oral manifestations in systemic diseases

Oral symptoms often precede the onset of the systemic disease in main target organs, and thus, oral findings may allow early diagnosis and treatment of the disease.

The oral cavity is the entry point to the gastrointestinal tract and is often involved in conditions affecting the gastrointestinal (GI) system such as inflammatory bowel diseases like Crohn’s disease and ulcerative colitis. Both of these diseases exhibit orofacial symptoms of aphthous ulcers and angular cheilitis (Lankarani et al. 2013). Additionally, Crohn’s disease can manifest in the oral mucosa as diffuse mucosal swelling and cobblestoning, and non-caseation granulomas (Neville 2009a). Generally, oral manifestations of these diseases coincide with the severity of symptoms in other parts of the GI system (Lankarani et al. 2013). Gastroesophageal reflux also affects the GI tract and causes dental erosions that are correlated with the duration of the disease (Meurman et al. 1994). Erosions can be found also in anorexia and bulimia (Chi et al. 2010).

Diseases affecting the skin are often encountered in the oral cavity. Sarcoidosis is an idiopathic disease with prevalent skin and lung involvement, where nearly all organ systems can be involved.
In line with this, oral manifestations, such as periodontitis, enlarged gingiva, and ranulas or nodules of the lips, palate, buccal mucosa, or gingiva, can occur (Blinder et al. 1997). Varying types of oral lesions are found also in lichen planus, lupus erythematosus, pemphigus vulgaris and Behcet’s syndrome (Chi et al. 2010). Oral manifestations of psoriasis include a geographic and/or fissured tongue, which correlates with disease severity (Picciani et al. 2015).

Oral manifestations that can present with DM are gingivitis, periodontitis, bilateral enlargement of parotid glands (sialadenosis), salivary dysfunction, xerostomia, burning mouth syndrome, caries, candidiasis, atrophy of the tongue papillae and delayed wound healing (Neville 2009a; Chi et al. 2010; Leite et al. 2013). Mostly these oral manifestations are related to DM with poor glycaemic control.

HIV infection can manifest with linear gingival erythema and necrotizing ulcerative gingivitis or periodontitis (Schiodt and Pindborg 1987; Chi et al. 2010). Several haematological disorders manifest in the mouth e.g. thrombocytopenia (abnormal hemorrhagic lesions, gingival bleeding), leukemia (mucosal bleeding, gingival enlargement) (Chi et al. 2010), congenital and acquired neutropenia (e.g. severe periodontitis, mucosal ulcerations), Langerhans cell histiocytosis (oral ulcerations, necrotizing gingivitis), and multiple myeloma (myeloma of the jaws, amyloidosis, and macroglossia) (Neville 2009b).

Sjögren’s syndrome (SS) is a systemic autoimmune disease where secretion from exocrine glands, mainly the salivary and lacrimal glands, is diminished (Fox et al. 1984). This causes persistent dryness of the mouth and eyes. It may present as a separate disease entity (primary form) or in relation to chronic inflammatory connective tissue diseases like rheumatoid arthritis or systemic lupus erythematosus (secondary form) (Tenovuo and Lagerlöf 1999; Konttinen et al. 2011). Patients are typically females in their 40s or 50s (Konttinen et al. 2011). Classification criteria for SS are presented in Table 3.

Orofacial manifestations can be observed in several other systemic conditions. These include e.g. vitamin and iron deficiencies, hypo- and hyperthyroidism, hypo- and hyperparathyroidism, and
Addison's disease (Neville 2009a). Sometimes oral symptoms may be the first signs of the disease, as reported in pemphigus vulgaris, thrombocytopenia and Crohn’s disease (Neville 2009a), as well as in APECED, a disease belonging to the Finnish disease heritage (Husebye et al. 2009). In addition, oral manifestations can occur in different forms of systemic amyloidoses, described in more detail in the following section.

**Table 3.** International classification criteria for Sjögren’s syndrome adapted from Vitali et al. 2002.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Subjective xerophthalmia</td>
</tr>
<tr>
<td>2.</td>
<td>Subjective xerostomia</td>
</tr>
<tr>
<td>3.</td>
<td>Ocular involvement. A positive result for at least one of the following:</td>
</tr>
<tr>
<td></td>
<td>a) Schirmer’s I test ≤ 5 mm in 5 min</td>
</tr>
<tr>
<td></td>
<td>b) Rose bengal score or other ocular dye score ≥ 4</td>
</tr>
<tr>
<td>4.</td>
<td>Histopathological findings</td>
</tr>
<tr>
<td></td>
<td>Focus score ≥ 1, i.e. one or more foci of more than 50 lymphocytes per 4 mm² in the minor salivary gland sample</td>
</tr>
<tr>
<td>5.</td>
<td>Salivary gland involvement. A positive result for at least one of the following:</td>
</tr>
<tr>
<td></td>
<td>a) UWS ≤ 1.5 mL in 15 min</td>
</tr>
<tr>
<td></td>
<td>b) Parotid sialography showing the presence of diffuse sialectasias</td>
</tr>
<tr>
<td></td>
<td>c) Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion</td>
</tr>
<tr>
<td>6.</td>
<td>Serological findings</td>
</tr>
<tr>
<td></td>
<td>Presence of antibodies to Ro(SSA) and/or La(SSB) antigens</td>
</tr>
</tbody>
</table>

Primary SS is considered when a) a patient presents any 4 of the 6 items in addition to either histopathological or serological findings being positive or b) three of any four items of numbers 3-6 are observed. Exclusion criteria comprise e.g. past head and neck radiation and other conditions affecting glandular excretion.
The prevalence of primary SS in the general population has been estimated to be 0.09% (Alamanos et al. 2006). However, dry mouth and eyes occasionally occur without any underlying diseases (dry eyes and mouth syndrome) (Price and Venables 2002).

2.2. AMYLOID AND AMYLOIDOTIC DISEASES

2.2.1. History

The term amyloid (starch is amylum in Latin) was first noted in the medical literature in 1854 by German pathologist Rudolf Virchow. He observed staining properties similar to cellulose components of plants in the nervous system (corpora amylacea). Virchow expanded his studies to what was most likely systemic amyloidosis with similar staining characteristics and applied the term amyloid (starch-like) change. The name amyloid was in common use already in 1859. Friedreich and Kekule demonstrated that amyloid is mostly composed of protein, not cellulose (Kyle 2001). The fibrillar nature of amyloid was observed in 1959 (Cohen and Calkins 1959) and the first amyloid protein (immunoglobulin light chain) was identified in 1971 (Glenner et al. 1971).

2.2.2. Amyloid

Amyloid is composed of fibrillar amyloid protein, 31 types of which are currently known (Sipe et al. 2014). In addition to the amyloid fibril protein, amyloid deposits contain proteoglycans, glycosaminoglycans (especially heparan sulfate), apo E, and amyloid P component (Snow et al. 1987; Wisniewski and Frangione 1992; Nuvolone et al. 2012). Definition of an amyloid fibril according to the Amyloid Fibril Protein Nomenclature (2014) is as follows:

"Amyloid fibril is a protein that is deposited as insoluble fibrils, mainly in the extracellular spaces of organs and tissues as a result of sequential changes in protein folding, which results in a
condition known as amyloidosis. An amyloid fibril protein occurs in tissue deposits as rigid, non-branching fibrils approximately 10 nm in diameter. The fibrils bind the dye Congo red and exhibit green, yellow, or orange birefringence when the stained deposits are viewed under polarization microscopy. When isolated from tissues and analyzed with X-ray diffraction, the fibrils exhibit a characteristic cross β diffraction pattern.” (Sipe et al. 2014).

Amyloid can be stained with Congo red (Puchtl et al. 1964), which has a property of enhanced apple-green birefringence of amyloid in tissue sections viewed under polarized light (Divry P. Etude histo-chimique des plaques seniles. J de Neurologie et de Psychiatrie 27:643-57, 1927 cited in: Sipe and Cohen 2000). The fluorochrome dye thioflavin T also stains amyloid by binding to the amyloid fibrils (Biancalana and Koide 2010). This staining method was described by Vassar and Culling in 1959. In the 1960s, a specimen for staining amyloid was obtained mainly from rectal biopsy, and since the 1970s from a subcutaneous fat aspiration biopsy. Still the basic element of amyloid diagnostics is the microscopic view of the biopsy specimen stained with Congo red (Westermark 2012a). In vivo amyloid can be detected by injecting immunolabelled serum amyloid P component (SAP), which is identical to the amyloid P component found in amyloid deposits, and imaging with scintigraphy (Pepys and Dash 1977; Pepys et al. 1979; Hawkins et al. 1990). Mass spectrometry-based proteomics is a new tool that can be used to identify the composition of amyloid fibrils (Dogan 2012).

Although amyloid deposits are considered relatively stable, reduction of amyloid fibril precursor supply can lead to rapid amyloid deposit regression (Hawkins 1997). Generally applicable therapeutic approaches aim at this goal. Prevention of SAP formation prevents amyloid deposit formation in mice (Botto et al. 1997), and the use of anti-SAP antibodies was found to have the same effect (Bodin et al. 2010a). In AGel amyloidosis, the first steps have been taken towards alleviation of amyloidogenesis with chaperone nanobodies targeting pathogenic cleavage of mutant gelsolin (Van Overbeke et al. 2014; Van Overbeke et al. 2015). Recently, knockdown of transthyretin mRNA has been shown to provide targeted treatment for ATTR amyloidosis (Niemietz et al. 2015).
2.2.3. Amyloidoses

Amyloidoses are protein-misfolding disorders of heterogenic origin, prevalence and symptoms. The similarity lies in amyloid tissue deposits, which are found in all amyloidoses. Amyloidotic diseases are named according to the main protein present in the amyloid deposits (Sipe et al. 2014). This still valid recommendation was made at the International Symposium on Amyloidosis held in Helsinki in 1972, at a time when only two amyloid proteins were known (Westermark 2012a). The diseases are designated as A plus suffix of the protein; e.g. A + Gel (gelsolin) = AGel amyloidosis. Only rarely eponyms are used, with the exception of Alzheimer’s disease (Sipe et al. 2014).

Amyloidotic diseases are categorized as local or systemic and acquired or hereditary. The most common and probably also known amyloidosis is Alzheimer’s disease, which is a localized, usually acquired, only rarely inherited form of amyloidosis (Benson 2012a). Some of the localized acquired amyloidoses are fairly common, such as DM type II and age-related amyloidosis affecting the islets of Langerhans, namely AIAPP amyloidosis, while some are rare or have unknown prevalence, like amyloidosis in the injection site of insulin (Westermark 2012b). Systemic acquired amyloidosis, such as AL amyloidosis caused by clonal plasma cells, and AA amyloidosis secondary to chronic inflammation are more common than the relatively rare systemic hereditary amyloidoses, like AGel amyloidosis. In Figure 2, a categorization of the different types of amyloidoses with some examples is illustrated.

AL amyloidosis presents with an estimated incidence of 3-9:100 000 per year in Western countries (Kyle et al. 1992; Pinney et al. 2013). Several different acquired chromosomal alterations are known to cause AL amyloidosis the translocation affecting the immunoglobulin heavy chain being the most common (Fonseca et al. 1998; Hayman et al. 2001). Variant immunoglobulin chains aggregate to amyloid fibrils, which further accumulate to amyloid deposits (Merlini and Stone 2006). Approximately 5% of AL amyloidosis cases occur in association with multiple myeloma (Rajkumar et al. 1998).

AL amyloid deposits are found mainly in kidneys, heart, liver and peripheral nerves. The pathophysiology of this disease is not fully understood. The effect of amyloid deposition is thought
Figure 2. Categorization of the different types of amyloidoses with examples.

to be essential, but it is also known that the non-fibrillar amyloidogenic immunoglobulin light chain has toxic effects on heart tissues. Patients present with distinctive signs of macroglossia, periorbital purpura and shoulder pads, but also with kidney and heart problems, peripheral neuropathy, submandibular swelling, and carpal tunnel syndrome. The leading cause of death in AL amyloidosis patients is chronic or sudden heart failure. The median survival rate reported for 1986-2003 was 3.8 years (Nuvolone et al. 2012).

The treatment of AL amyloidosis is similar to that of multiple myeloma, including stem cell transplantation and different chemotherapies (Wechalekar et al. 2015). Several other treatment modalities are being researched, one of them a pharmacological depletion of the serum amyloid P component (Bodin et al. 2010b).
AA amyloidosis develops in some patients with prevailing chronic inflammatory condition. While AL amyloidosis is the most common in Western countries, AA amyloidosis is found worldwide with an estimated prevalence of 45% of all systemic amyloidoses (Rocken and Shakespeare 2002). The precursor protein of the fibrils in AA amyloidosis is an apolipoprotein, serum amyloid A (SAA). It is a product of the liver in response to inflammatory mediators (Uhlar and Whitehead 1999). For an unknown reason, patients who develop AA amyloidosis fail to degrade the SAA in a normal manner. This leads to smaller fragments of SAA, which eventually deposit as amyloid (van der Hilst 2011).

Formerly the leading causes of AA amyloidosis were infectious diseases such as tuberculosis, malaria, leprosy, and chronic osteomyelitis. Rheumatic diseases, including rheumatoid arthritis, ankylosing spondylitis and juvenile idiopathic arthritis, have since commonly caused AA amyloidosis. Lately, however, with therapeutic developments their prevalence as the cause of AA amyloidosis has decreased significantly. Other diseases associated with AA amyloidosis are granulomatous diseases, such as sarcoidosis and Crohn’s disease, and malignancies such as mesothelioma and Hodgkin’s lymphoma (Ombrello and Aksentijevich 2012). AA amyloidosis can accompany also hereditary autoinflammatory diseases, like familial Mediterranean fever (FMF), in which 11% of the patients show AA amyloidosis (Touitou et al. 2007). Approximately 6% of all AA amyloidosis cases have no identified disease association (Lachmann et al. 2007).

The organ involvement in AA amyloidosis varies, but most often it affects the kidneys, causing proteinuria, and the gastrointestinal tract, causing diarrhoea and malabsorption (Gertz and Kyle 1991). Less frequently, also hepatomegaly and splenomegaly are present, and rarely also the heart, tongue and skin are affected (Ombrello and Aksentijevich 2012). The median survival after diagnosis is 11.1 years, and high SAA levels have an adverse effect on prognosis (Lachmann et al. 2007).

Biologic medications such as anti-TNF medications, IL-1 inhibitors, and IL-6 inhibitors have been used to reduce the inflammatory state (Ombrello and Aksentijevich 2012). Novel therapeutic strategies targeting the formation of amyloid fibrils and amyloid deposition may suppress amyloidogenesis and hence preserve also renal function (Real de Asua et al. 2014).
Systemic hereditary amyloidoses, in contrast to systemic acquired amyloidoses, are fairly rare diseases and have varying prevalence. A retrospective study of 284 patients diagnosed with ATTR or non-ATTR amyloidosis at the Mayo Clinic in Rochester, Minnesota, between 1970 and 2013 roughly describes the prevalence of different systemic hereditary amyloidoses; ATTR amyloidosis was proven in 93% of patients, but AFib (N=9), AApoI (N=6), AGel (N=3), ALys (N=1), and AApoII (N=1) amyloidoses presented in the rest of the patients (Zhen et al. 2015). Ethnic origin greatly affects the prevalence of systemic hereditary amyloidoses; in Finland, for example, a similar study would have produced a very different kind of result. Table 4 presents the precursor protein, mutations, symptoms, severity, prognosis and available treatment of systemic hereditary amyloidoses.

AGel amyloidosis is one of the ten systemic hereditary amyloidoses known today (Benson 2012a; Rowczenio et al. 2014), and it is discussed in more detail later in this thesis.
Fibril proteins listed according to the Amyloid Nomenclature. The origin and normal function of the precursor protein and number of known mutations are included. Main target organs listed and the severity of the disease at a scale of slowly progressing-progressive-lethal are listed. Treatment guidelines are included, if available. CNS: central nervous system, PNS: peripheral nervous system, ANS: autonomic nervous system.

*) In advanced cases, amyloid deposits are found in many organs.

**) Evidence of hereditary nature is based on family history.

See Appendix 1 for references.

### Table 4. Systemic hereditary amyloidoses in humans.

<table>
<thead>
<tr>
<th>Fibril protein</th>
<th>Precursor protein</th>
<th>Origin of protein</th>
<th>Normal function</th>
<th>Number of known mutations</th>
<th>Main target organ/-s</th>
<th>Severity of the disease</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL</td>
<td>Immunoglobulin light chain</td>
<td>Clonal plasma cells</td>
<td>Role in humoral immune response</td>
<td>1</td>
<td>All organs except CNS</td>
<td>Lethal</td>
<td>Stem cell transplantation, chemotherapy</td>
</tr>
<tr>
<td>ATTR-Transferrin, variants</td>
<td>Liver (hepatic)</td>
<td>Metabolism of iron</td>
<td>Transferrin receptor</td>
<td>&gt;100</td>
<td>PNS, ANS, heart, eye, leptomen</td>
<td>Lethal</td>
<td>Liver transplantation</td>
</tr>
<tr>
<td>Ab2M</td>
<td>β2-Microglobulin, variant</td>
<td>All nucleated cells</td>
<td>Role in fibrillogenesis</td>
<td>1</td>
<td>GI symptoms, ANS</td>
<td>Slowly progressing</td>
<td>Not available</td>
</tr>
<tr>
<td>AApoloaprotein A I</td>
<td>Apolipoprotein A I, variants</td>
<td>Liver, small intestine</td>
<td>Constituent of HDL</td>
<td>5</td>
<td>Kidney*</td>
<td>Slowly progressing</td>
<td>Kidney transplantation</td>
</tr>
<tr>
<td>AApoloaprotein A II</td>
<td>Apolipoprotein A II, variants</td>
<td>Liver, small intestine</td>
<td>Constituent of HDL</td>
<td>15</td>
<td>Heart, liver, kidney, PNS, testis</td>
<td>Lethal</td>
<td>Liver transplantation</td>
</tr>
<tr>
<td>AGelsolin</td>
<td>Gelsolin, variants</td>
<td>All nucleated cells</td>
<td>Actin modulation and clearance</td>
<td>2</td>
<td>PNS, cornea, skin</td>
<td>Slowly progressing</td>
<td>Only symptom alleviating</td>
</tr>
<tr>
<td>ALys</td>
<td>Lysozyme, variants</td>
<td>Macrophages and PMNs</td>
<td>Bacteriolytic enzyme</td>
<td>7</td>
<td>Mainly kidney</td>
<td>Slowly progressing</td>
<td>Kidney transplantation</td>
</tr>
<tr>
<td>ALECT2</td>
<td>Leukocyte Chemotactic Factor-2</td>
<td>Mainly liver</td>
<td>No specific function verified</td>
<td>not known</td>
<td>Kidney, primarily</td>
<td>Progressive</td>
<td>Not available</td>
</tr>
<tr>
<td>AFib</td>
<td>Fibrinogen α, variants</td>
<td>Liver</td>
<td>Major role in blood coagulation</td>
<td>1</td>
<td>Kidney, primarily</td>
<td>Lethal</td>
<td>Liver (and kidney) transplantation</td>
</tr>
<tr>
<td>ACys Cystatin C</td>
<td>Cystatin C, variants</td>
<td>Hematopoietic cells</td>
<td>Cysteine protease inhibitor</td>
<td>1</td>
<td>PNS, skin</td>
<td>Lethal</td>
<td>Not available</td>
</tr>
<tr>
<td>ABriP</td>
<td>BriPP, variants</td>
<td>Coded from chromosome 13</td>
<td>Protein not normally found</td>
<td>1</td>
<td>CNS, primarily</td>
<td>Lethal</td>
<td>Not available</td>
</tr>
</tbody>
</table>

See Appendix 1 for references.
2.2.4. Oral manifestations in systemic amyloidoses

The first report of oral findings caused by systemic amyloidosis dates back to 1971, when Kuczynski et al. presented a “70-year-old patient who suffered from sicca syndrome and renal, hepatic and cardiac diseases”. Autopsy studies revealed that amyloid deposits had accumulated in several different organs, including the parotid glands. No sign of inflammation was observed so it was concluded that the amyloidosis was of primary type (AL amyloidosis) and not secondary to an inflammatory disease (Kuczynski et al. 1971). Reports of sicca symptoms and salivary gland biopsy findings in systemic amyloidoses are presented in Table 5.

Table 5. Sicca symptoms and salivary gland biopsy findings in systemic amyloidoses.

<table>
<thead>
<tr>
<th></th>
<th>Amyloidosis</th>
<th>Sicca symptoms</th>
<th>Amyloid</th>
<th>Inflammation</th>
<th>Atrophy</th>
<th>Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acquired</strong></td>
<td>AL</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>yes*</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>yes</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Hereditary</strong></td>
<td>ATTR</td>
<td>yes</td>
<td>yes</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>ALys</td>
<td>yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Aβ2M</td>
<td>yes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>AGel</td>
<td>ND</td>
<td>yes**</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Sjögren’s syndrome (SS) has sometimes been diagnosed concomitantly with systemic amyloidosis, as in AL amyloidosis (Delevaux et al. 2001; Perlat et al. 2009). In the case of Delevaux et al. (2001), the lymphocytic infiltration finding met the histopathological criteria of SS (focus score ≥ 1), but also amyloid was found in the labial salivary gland specimen.

Saliva secretion and its protein composition have been studied only in ATTR amyloidosis. In addition to hyposalivation, elevated protein glycosylation and concentrations of salivary protein, amylase, lysozyme, salivary peroxidase, IgA, hexosamines, sialic acid, fucose, phosphate and potassium were observed in the patient group (Johansson et al. 1992). The researchers concluded that patients with ATTR amyloidosis have a greater risk for caries due to their amyloidotic disease.

The first publications of amyloid-related macroglossia date back to the 1940s (Weber et al. 1947). Ever since amyloid deposits of tongue has been reported in different types of systemic amyloidoses, namely in haemodialysis associated Aβ2M (Fuchs et al. 1987), AL (Gertz and Kyle 1996), AGel (Kiuru et al. 1999a) and AA (Koloktronis et al. 2003) amyloidoses. In AL amyloidosis 10-20% of the patients present with tongue enlargement (Benson 2012b). Macroglossia may induce altered speech, excessive salivation, and difficulties in eating (Angiero et al. 2010). Prominent enlargement of the tongue can be treated surgically (Cobb et al. 2013; Pau et al. 2013).

Only a small number of studies have addressed the relationship of amyloidosis and periodontal health. Pathology of periodontal tissues is a rare finding in systemic amyloidosis and seems to present rarely as localized severe periodontitis (Khoury et al. 2004). One case report describes advanced periodontitis in a Turkish man with AA amyloidosis (Cengiz et al. 2010). Interestingly, patients who have familial Mediterranean fever (FMF) with amyloidosis have been reported to have more often moderate to severe generalized periodontitis than FMF patients without amyloidosis (Cengiz et al. 2009). According to that report, periodontal therapy reduced the levels of acute-phase reactants and thus could also alleviate the disease burden of FMF patients. Serum amyloid A protein levels have been noted to be higher in patients with periodontitis (Ardila and Guzman 2015).

Localized amyloidosis has been described in nearly all organ systems, also in oral cavity, but they do not evolve into systemic amyloidosis (Paccalin et al. 2005). Localized amyloidosis in the oral
cavity can present in the soft and hard palate, salivary glands, tongue, buccal and labial mucosa, and floor of the mouth as soft nodules with yellow, red, blue or purple colouring (Kurokawa et al. 1998; Nandapalan et al. 1998; Pentenero et al. 2006; O'Reilly et al. 2013). Surgical removal seems to be the treatment guideline. Especially, if amyloid is found in the tongue, systemic AL amyloidosis should be suspected.

2.3. HEREDITARY GELSONIN (AGel) AMYLOIDOSIS

2.3.1. A member of the Finnish disease heritage

In 1960’s the Finnish ophthalmologist Jouko Meretoja described a previously unknown disease with hereditary nature affecting eyes, skin and nerves (Meretoja 1969). According to the discoverer it was then called Meretoja’s disease, later familial amyloidotic polyneuropathy type IV (FAP IV), familial amyloidosis of the Finnish type (FAF), and hereditary gelsolin amyloidosis (HGA). Mostly, the name AGel amyloidosis is used according to the amyloid nomenclature (Sipe et al. 2014). It characteristically manifests with ophthalmological, neurological and dermatological findings, although other symptoms may also occur (Meretoja 1969). Irrespective of the ethnic origin these symptoms are similar in the AGel amyloidosis patients (Kiuru 1998). Almost all patients are heterozygotes. Few reported homozygotes exhibit more severe clinical findings and do not survive over their thirties without dialysis or renal transplantation (Maury et al. 1992; Ardalan et al. 2007).

The Finnish disease heritage comprises diseases, which are markedly overrepresented in Finland (Norio 2003a). The prevalence of them varies from 1:176 000 of Northern epilepsy to the prevalence of AGel amyloidosis, which is 1:6 000 (Norio 2003b). The cause for this overrepresentation of certain diseases in Finland lies in the national and regional isolation (Norio 2003a). There are altogether 36 described diseases in the Finnish disease heritage. Most of them (32) are transmitted in an autosomal recessive manner, unlike AGel amyloidosis and three others, which are autosomal dominantly inherited (Norio 2003b). The ancestral mutation carriers of AGel amyloidosis have been traced to two areas in Finland, Southern-Häme and Kymenlaakso (Meretoja 1973). It has been suggested that this disorder originates in Finland from one point mutation.
several centuries ago (Meretoja 1973; Meretoja 1976). This is supported also by the genetic homogeneity of the AGel amyloidosis patients in Finland (Kiuru 1998), and the uniform haplotypes in some families (de la Chapelle et al. 1992b; Paunio et al. 1995).

2.3.2. Epidemiology

At present, there are two gelsolin gene mutations identified to cause AGel amyloidosis (de la Chapelle et al. 1992a). The nucleotide guanine (G) is substituted with either adenine (A) or thymine (T) in the gelsolin gene, and the mutation is informed as c.640G>A/T, while previously it was reported as c.654G>A/T. The estimated number of Finnish AGel amyloidosis patients, affected with c.640G>A varies from 400 patients (Kiuru 1992) to 1000 gene carriers (Meretoja et al. 1976), while in other countries the reported number of patients is approximately 140. These patients have been reported from several European countries, North and South America, Iran, Japan and India (Figure 3) (Kiuru-Enari and Haltia 2013; Maramattom and Chickabasaviah 2013; Gonzalez-Rodriguez et al. 2014; Alabdali et al. 2015). The lack of a common founder behind the cases outside Finland favours the idea that AGel amyloidosis in these cases is caused by sporadic mutations (Kiuru-Enari and Haltia 2013), and that the affected nucleotide may represent a mutational hot spot in the gelsolin gene (de la Chapelle et al. 1992b; Paunio et al. 1995).

2.3.3. Clinical findings

AGel amyloidosis in heterozygote patients is a late-onset disease, with slowly progressing systemic symptoms that often present with phenotype variations. Corneal lattice dystrophy (CLD) is usually the first sign in patients manifesting between their 3rd and 4th decades and often causing visual impairment at advanced age (Meretoja 1969). Corneal nerve damage has been reported to correlate with clinical symptoms (Rothstein et al. 2002). Patients also complain of eye dryness, photophobia and general eye irritability (Meretoja 1969; Kiuru 1992; Chastan et al. 2006).
Figure 3. Worldwide distribution of AGel amyloidosis. The black dots indicate countries where AGel amyloidosis has been reported up to year 2015. The big dot on Finland represents the larger accumulation of cases.

Characteristic facial palsy is commonly the initial symptom of cranial neuropathy, presenting in the 4th or 5th decade of life (Meretoja 1969; Kiuru-Enari and Haltia 2013). Other evident signs of cranial neuropathy are myokymias, corneal hypesthesia and reflex loss, and weakness of the masseter and tongue muscles (Kiuru 1992; Kiuru 1998). Peripheral, mostly sensory somatic nerves are affected, and the symptoms become evident often at the age of fifty (Kiuru 1992; Kiuru and Seppäläinen 1994). Symptoms are typically distal numbness and paresthesia, predominant loss of vibration sense, and absent or decreased tendon reflexes. In aged patients also touch and position senses can be impaired. With age difficulties in handling and identifying small objects may appear (Kiuru 1992) as well as severe ataxia causing ambulation loss (Tanskanen et al. 2007). Autonomic
neuropathy in AGel amyloidosis is often minor, if noted at all (Kiuru and Seppäläinen 1994). Orthostatic hypotension, postural dizziness and fainting, gastrointestinal disturbances, dysuria, impotence, and/or decreased perspiration may occur (Kiuru 1992; Kiuru and Seppäläinen 1994).

The most typical dermatological finding in AGel amyloidosis patients is cutis laxa, which means abnormally loose skin (Kiuru-Enari et al. 2005). Dermatological symptoms progress with age too, and appear as skin atrophy, increased vulnerability, abnormal scarring and thinning, and/or loss of body and scalp hair (Meretoja 1972; Kiuru 1992). Cutis laxa also markedly alters the facial appearance, restricts mimics, decreases visual fields, and impairs speech and other oral functioning, causing a significant handicap to the patient. Together these two main organ manifestations, neurological and dermatological, produce a typical facial appearance, which is described to be mask like or sad (Figure 4). This facial appearance has been observed in AGel patients, irrespective of familial or ethnic origin (Kiuru-Enari and Haltia 2013).

Renal involvement in AGel amyloidosis varies greatly from intermittent proteinuria in heterozygous gelsolin gene carriers to nephrotic syndrome in young homozygous gene carriers (Meretoja 1973; Kiuru 1992; Maury et al. 1992). Amyloid angiopathy affecting most blood vessels is a pathological characteristic of AGel amyloidosis (Kiuru et al. 1999c). Consequently, symptoms of cardio- and cerebrovascular involvement have been reported in AGel amyloidosis patients (Meretoja 1969; Kiuru 1992; Kiuru and Seppäläinen 1994; Kiuru 1995), but their prevalences remain unknown. Patients also use more prescribed medicines for blood pressure and cholesterol than their non-affected relatives (Laine et al. 2010). Patients report more bruises than healthy control persons, probably due to amyloidotic vessel wall involvement together with altered platelet shape change (Kiuru et al. 2000; Laine et al. 2010).

Quite commonly hypoglossal neuropathy causes furrowing and fasciculations of the tongue in older patients, while macroglossia is rather rarely observed (Kiuru et al. 1999b). The combination of tissue laxity and neurological problems induce dysarthria, dysphagia and drooling, which is in some aged patients further excavated by prominent lower lip protrusion (Kiuru 1998). Sleep apnea can also occur in AGel amyloidosis (Kiuru et al. 1999a). The recent FIN-GAR (Finnish Gelsolin Amyloidosis Patient Registry) study in 227 patients showed cardiac and severe renal problems to be
more prevalent than earlier considered (Nikoskinen et al. 2015). AGel amyloidosis does not reduce fertility (Meretoja 1973). Many patients reach advanced age, although average life span is somewhat shortened (Meretoja 1973).

**Figure 4.** a) Overall facial appearance of a 79-year-old patient with severe AGel amyloidosis, specifically manifested as b) furrowed and macroglossic tongue and c-f) cutis laxa affecting the scalp, thumb, back and ears. d,f) AGel amyloidosis causes the tissues to retain the deformed state after pressure of a few minutes. Figures published with the permission of the British Journal of Dermatology (Kiuru-Enari et al. 2005).
2.3.4. Histopathological findings

Amyloid deposits in AGel amyloidosis stain with Congo red, which is typical of all amyloidoses (Meretoja 1969). Further characterization of amyloid deposits can be made immunohistochemically with antibodies raised against gelsolin amyloid precursor protein (Haltia et al. 1990a; Haltia et al. 1990b; Maury and Baumann 1990; Maury 1991a) and with antisera against e.g. amyloid P component and apo-E (Haltia et al. 1990a). Amyloid fibrils can be detected with immunoelectron microscopy (Verga et al. 2012). Mass spectrometry-based proteomics can be used in diagnostics, also in gelsolin-related amyloidoses (Sethi et al. 2013; Efebera et al. 2014), and is especially useful if the sample size is very small (Lavatelli et al. 2011; Dogan 2012).

AGel amyloid is found in most tissues, particularly attached to the blood vessel walls and basement membranes (Meretoja and Teppo 1971). AGel amyloid deposits in the eyes are a common feature in different kindreds and found especially in the corneal lattice lines, under Bowman’s membrane, and in the scleral drainage area (Kivelä et al. 1994; Kiuru 1998). In the peripheral nerves, the perineurial and endoneurial layers are affected with amyloid deposits, and they are also found in arteries (intimal and medial layers) of nearly every organ and in capillaries (Meretoja and Teppo 1971). Later, a widespread spinal, less cerebral, and meningeal angiopathy has been observed in patients (Kiuru et al. 1999c). In the skin, amyloid deposits have been noted in basement membranes of the epidermis, sweat and sebaceous glands and in layers of the dermis (Meretoja 1969; Meretoja and Teppo 1971) and encircling elastic fibers co-localizing with their amyloid P component (Kiuru-Enari et al. 2005).

2.3.5. Molecular genetics and pathogenesis

A point mutation of c.640G>A/T causes AGel amyloidosis with systemic amyloid deposits (Levy et al. 1990; Maury et al. 1990; de la Chapelle et al. 1992b). All Finnish and most European, North and South American, and Asian kindreds carry the c.640G>A mutation (Kiuru-Enari and Haltia 2013; Gonzalez-Rodriguez et al. 2014), while c.640G>T carriers have been reported from Denmark, France, former Czechoslovakia, and Brazil (Kiuru-Enari and Haltia 2013).
The point mutation c.640G>A/T in gelsolin protein (GSN) coding gene (GSN) for gelsolin causes substitution of single amino acid (p.D214N or p.D214T) (Levy et al. 1990; Maury et al. 1990; de la Chapelle et al. 1992b; Maury et al. 2000), and prevents normal binding of Ca\(^{2+}\) ion (Isaacson et al. 1999). Consequently, the GSN molecule undergoes pathological cleavage intracellularly by furin (Huff et al. 2003). This abnormally fragmented 68 kDa GSN is secreted and further cleaved by the MT1 matrix metalloproteinase (MMP 14) to 8 and 5 kDa amyloidogenic fragments (Page et al. 2005). These fragments undergo abnormal protein folding and form amyloid fibrils. The variant secretory gelsolin (sGSN) is considered to be the sole source of AGel amyloid (Kangas et al. 1996), and the local production might further increase the amyloid formation in certain tissues and organs, like the peripheral nervous systems, and ocular, dermal, renal, and striated and smooth muscular tissues (Kivelä et al. 1994; Kiuru 1998).

In AGel amyloidosis, amyloid depositions (Haltia et al. 1990a; Maury 1991b) with subsequent cytotoxic effects are thought to contribute to pathogenesis (Anan et al. 2010). It has been proposed that formation of oligomeric aggregates during amyloidogenesis with proteotoxic and cytotoxic effects may have pathogenetic significance in AGel amyloidosis, similar to other amyloidoses (Solomon et al. 2012). On the other hand, systematic cell studies of platelets in AGel amyloidosis patients showed altered platelet shape change, suggesting cytosolic gelsolin (cGSN) dysfunction (Kiuru et al. 2000). In homozygote AGel amyloidosis patients, plasma gelsolin lacks both actin severing and nucleating activities, and in heterozygote patients the activity was about half of the control values (Weeds et al. 1993). Thus, possible malfunctions of cGSN and/or sGSN due to the c.640G>A mutation cannot be excluded as contributing factors in the pathogenesis.

2.3.6. Gelsolin

Gelsolin protein (GSN) has multiple roles in health and disease. To date, only five disease-causing gelsolin gene (GSN) mutations are known: c.640G>A/T causing systemic hereditary AGel amyloidosis (Levy et al. 1990; Maury et al. 1990; de la Chapelle et al. 1992b; Maury et al. 2000), c.580G>A and c.633C>A causing localized renal amyloidosis (Sethi et al. 2013; Efebera et al. 2014), and quite recently a GSN mutation causing hereditary thrombocytosis (Pianta et al. 2013).
Figure 5 presents an overview of the diverse functions of GSN. This thesis concentrates on the roles of GSN exposed in the in vitro cell studies that we performed with AGel amyloidosis cells.

Figure 5. Multiple roles of gelsolin. Aβ: amyloid β protein, the major component of amyloid plaques in the brains of individuals with Alzheimer’s disease. sGSN: secretory gelsolin, cGSN: cytosolic gelsolin, CNS: central nervous system, PIP2: phosphatidylinositol biphosphates. Adapted from Li et al. 2012.
The gelsolin gene (GSN) is located on chromosome 9 at q33.2 (Kwiatkowski et al. 1986). The gene codes for all three forms of gelsolin: secretory gelsolin (sGSN) and cytosolic (cGSN), with differing initiation of the transcription site (Yin et al. 1984), and gelsolin-3, which is expressed mainly in cerebral oligodendrocytes, lungs, and testis (Vouyiouklis and Brophy 1997). sGSN is expressed mainly by smooth and skeletal muscle cells (Kwiatkowski et al. 1988), and it scavenges and severs actin filaments released from cells during inflammation and injury (Lind et al. 1986; Vasconcellos et al. 1994).

cGSN is present in most types of cells (Paunio et al. 1997), especially in smooth muscle cells, where its level can reach up to 0.1% of the total protein amount (Fock et al. 2005). The major function of cGSN is to modulate cytoskeletal actin by severing, capping, and uncapping actin filaments and nucleating the growth of actin monomers to filaments. The actin cytoskeleton is crucial in generation of force and facilitation of cell movement (Sun et al. 1999). Gelsolin is activated with calcium (Ca^{2+}) or pH < 7, leading to the severing of actin (Yin and Stossel 1979; Yin et al. 1980; Lamb et al. 1993). After severing, gelsolin caps the barbed end of actin and prevents filament elongation, which results in disassembly of the actin network (Yin et al. 1981; Sun et al. 1999). Binding of phosphatidylinositol biphosphates (PIP2) to cGSN induces uncapping of the acting filament, allowing it to grow again (Jannmey and Stossel 1987). Active cGSN can also nucleate actin filament growth from actin monomers (Yin et al. 1981). The Ca^{2+}-dependent actin-severing ability of cGSN is considered to be the major factor of cell migration, also in fibroblasts (Arora and McCulloch 1996). Figure 6 presents the actin-modulating function of gelsolin. cGSN has the capacity to both enhance and inhibit apoptosis (Li et al. 2012), and it has several roles in signal transduction, e.g. in membrane ruffling and cell protrusion (Azuma et al. 1998; De Corte et al. 2002) and in matrix protein adhesion complexes (Li et al. 2012).

Tissue remodelling by collagen phagocytosis performed by fibroblasts is a crucial phenomenon and cGSN contributes to several stages of this process. It participates in both integrin and Fc receptor mediated phagocytosis (Arora et al. 2004). The cGSN/actin- and cGSN/non-muscle myosin IIA-processes are essential in collagen binding and internalization (Arora et al. 2005; Arora et al. 2013), and cGSN has been observed abundantly around nascent phagosomes (Yin et al. 1981). In cyclosporine-induced gingival overgrowth, the phagocytosis of collagen by cultured fibroblasts is
decreased (Kataoka et al. 2000), leading to accumulation of collagen in gingival tissue. This is mediated by cGSN (Chan et al. 2007). Cyclosporine blocks the release of Ca\(^{2+}\) from endoplasmic reticulum disrupting cGSN’s actin-severing activity (Arora et al. 2001), and thus, the fibroblasts are unable to form actin fibers for lamellipodia to engulf collagen.

Concerning cGSN in AGel amyloidosis, *in vitro* studies favouring both normal (Kangas et al. 1999) and abnormal actin modulation (Westberg et al. 1999) have been presented. Murine GSN knockout studies demonstrated reduced migration capacity of neutrophils and fibroblasts (Witke et al. 1995), and delayed filopodia retraction (Lu et al. 1997). In summary, gelsolin is an omnipresent protein with many roles in cell structure and function.

**Figure 6.** Gelsolin is activated by Ca\(^{2+}\) or H\(^+\) (pH<7) or by proteases (e.g. caspase 3, chymotrypsin). The proteases produce gelsolin, which is capable of interacting with both monomeric and polymeric actin. Phosphatidylinositol biphosphates (PIP2) expose the barbed end of actin by inactivating gelsolin, thus enabling actin to grow again. Adapted from Janmey et al. 1998.
2.3.7. Diagnosis and treatment

The diagnosis of AGel amyloidosis can be set by demonstrating corneal lattice dystrophy (CLD) combined with other typical signs, especially facial nerve palsy and cutis laxa (Kiuru 1998), and/or molecular genetically by showing the c.640G>A mutation (Paunio et al. 1992). Demonstration of CLD with family history of AGel amyloidosis is also sufficient for diagnosis. In unclear cases and families without previous molecular genetic diagnosis, verification of the fully penetrant c.640G>A GSN mutation is recommended.

Treatment at present is merely symptom-alleviating, but can notably improve the quality of life of AGel patients. Eye symptoms are treated with lubricants and topical antibiotics (Carrwik and Stenevi 2009) or surgically (Mattila et al. 2015). Facial appearance due to hanging skin can create severe social disability and plastic surgery is often needed, sometimes repeatedly (Pihlamaa et al. 2011). The first steps have been taken towards alleviation of gelsolin amyloidogenesis with chaperone nanobodies targeting pathogenic cleavage of mutant gelsolin in a mouse model (Van Overbeke et al. 2014; Van Overbeke et al. 2015). Antibody to serum amyloid P protein (SAP) has also been presented as a successful treatment of any amyloidosis based on a mouse study, where clearance of massive amyloid deposits was observed (Bodin et al. 2010a). In anticipation of future treatment options, the first studies depicting the natural disease history (Nikoskinen et al. 2015) and biomarkers enabling follow-up of such treatments have recently been published (Pihlamaa et al. 2015).
3. AIMS OF THE STUDY

The aims of the present study were as follows:

1) to assess oral health problems and manifestations in AGel amyloidosis patients. (I-II).

2) to analyse salivary secretion/flow rates, salivary protein composition and labial salivary gland (LSG) histology in AGel amyloidosis patients (I,II).

3) to examine periodontal health condition and subgingival microbial profile in AGel amyloidosis patients (III).

4) to investigate oral fibroblasts and vascular smooth muscle cells (VSMCs) of AGel amyloidosis patients in vitro, with a focus on cytoskeletal actin and gelsolin distribution, cell migration, and collagen type I metabolism (IV).

The hypothesis was that AGel amyloidosis has some adverse effects on saliva or periodontal health of the patients.
4. SUBJECTS, MATERIALS AND METHODS

More detailed descriptions of the materials and methods used are given in the original publications (I-IV).

4.1. STUDY SUBJECTS

Patients were invited to participate in the study through their patient organization with open invitation (Finnish Amyloidosis Association, SAMY, www.suomenamyloidoosiyhdistys.fi). Forty patients volunteered, and according to inclusion criteria (adult, non-smoker during the last year, not pregnant, no antibiotics during past 3 months) applicable number of them participated to the different study settings. The diagnosis of AGel amyloidosis was set by demonstrating the typical clinical features by an experienced neurologist (SK-E), and in most cases confirmed with family history. Molecular genetic confirmation of the diagnosis was available in 23 patients (Paunio et al. 1992). Age- and sex-matched controls were recruited for the different studies, but age-matching was not possible for the vascular smooth muscle cell (VSMC) samples because of the scarce control material. Table 6 presents the different study settings, numbers of the participating patients and controls and their demographic characteristics. The case report (I) patient is included in study settings 1 and 2.

The Ethics Committee of Helsinki University Hospital approved the research protocol. Patients were informed of the research in both a spoken and written form, and they gave their written consent. The clinical part of the research was performed at the Department of Oral and Maxillofacial Diseases, Faculty of Medicine, University of Helsinki, Finland. The histopathological studies were performed at the Department of Pathology, Faculty of Medicine, University of Helsinki. In vitro cell studies were conducted at the Scientific Research Laboratory of the Institute of Dentistry and at Meilahti Clinical Proteomics Core Facility, both in Biomedicum, University of Helsinki.
Table 6. Number and demographic characteristics of the AGel amyloidosis patient and control subjects in different study settings.

<table>
<thead>
<tr>
<th>Study setting</th>
<th>N</th>
<th>Female</th>
<th>Male</th>
<th>Age ± SD</th>
</tr>
</thead>
<tbody>
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<td><strong>1. Questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>40</td>
<td>29</td>
<td>11</td>
<td>60.4 ± 9.6</td>
</tr>
<tr>
<td><strong>2a. Salivary flow rate; UWS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.2 ± 9.7</td>
</tr>
<tr>
<td>Controls</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.0 ± 9.8</td>
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<tr>
<td><strong>2a. Salivary flow rate; SWS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.2 ± 9.7</td>
</tr>
<tr>
<td>Controls</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.0 ± 9.8</td>
</tr>
<tr>
<td><strong>2b. Salivary composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.2 ± 9.7</td>
</tr>
<tr>
<td>Controls</td>
<td>27</td>
<td>21</td>
<td>6</td>
<td>60.0 ± 9.8</td>
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<td><strong>2c. LSG biopsy</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>7</td>
<td>4</td>
<td>3</td>
<td>57.3 ± 12.2</td>
</tr>
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<td>-</td>
<td>49.0</td>
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<td><strong>3a. Periodontal study</strong></td>
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<td>Patients</td>
<td>36</td>
<td>26</td>
<td>10</td>
<td>60.9 ± 9.3</td>
</tr>
<tr>
<td>Controls*</td>
<td>5255</td>
<td>2782</td>
<td>2473</td>
<td>49.5 ± 13.2</td>
</tr>
<tr>
<td><strong>3b. Oral yeast sample</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>36</td>
<td>26</td>
<td>10</td>
<td>60.9 ± 9.3</td>
</tr>
<tr>
<td>Controls</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>55.2 ± 4.6</td>
</tr>
<tr>
<td><strong>4a. Oral fibroblast study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>62.5 ± 10.4</td>
</tr>
<tr>
<td>Controls</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>62.3 ± 10.9</td>
</tr>
<tr>
<td><strong>4b. VSMC study</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>63.1 ± 9.2</td>
</tr>
<tr>
<td>Controls</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>46.8 ± 19.2</td>
</tr>
</tbody>
</table>

4.2. QUESTIONNAIRE

Patients filled in a questionnaire that assessed their general diseases, medications, onset of A Gel amyloidosis, smoking habits and possible oral problems. In addition, questions about oral hygiene habits were posed, and patients were asked whether they felt that A Gel amyloidosis was complicating their oral hygiene procedures (I-III). The questionnaire is attached as Appendix 2.

4.3. CLINICAL AND RADIOLOGICAL ORAL EXAMINATION

Oral mucous membranes and the tongue of patients were examined macroscopically, and the number of teeth counted. Patients were informed of any dental caries lesions observed.

A thorough periodontal examination was performed by a dentist (PJ) with supervision by a periodontist (AN). Probing pocket depth (PPD) and clinical attachment level (CAL) were measured with a WHO probe at six sites per tooth. PPD and CAL measurements ≥ 4 mm were registered. Furcation involvement was evaluated with a furcation probe. Visible plaque index (VPI) and bleeding on probing (BOP) were checked at four sites of each tooth.

Panoramic tomographies were taken and analysed by a specialist. Radiographs and clinical findings were compared to ones taken previously, if available (III).

4.4. SPECIMEN COLLECTIONS AND ANALYSES

4.4.1. Saliva samples and biochemical analyses

Patients were informed not to eat or drink for one hour before sample collection. Salivary flow rate measurements were performed before the clinical oral examination. For the measurement of unstimulated whole saliva (UWS) flow rate (mL/min), saliva was collected for 15 minutes by
passive expectoration. To measure stimulated whole saliva (SWS) flow rate (mL/min), a piece of paraffin (1 g) was chewed and saliva was collected for 5 min. The cut-off values of hyposalivation for UWS and SWS flow rates were set according to the literature to < 0.1 mL/min and < 0.7 mL/min, respectively (Ericsson and Hardwick 1978; Tenovuo and Lagerlöf 1999; Nederfors 2000) (I-II).

The SWS samples were centrifuged (10 000 rpm, 4 min) and stored at -70°C until analysed. All analyses were carried out immediately after thawing. Standards and controls were included in all analyses. The concentrations (mg/mL or µg/mL) of total protein, albumin, and immunoglobulins A, G and M were analysed. The secretion rates (mg/min or µg/min) of each protein were calculated by multiplying the SWS flow rate values with concentrations (Dimitriou et al. 2002) (II).

4.4.2. Labial salivary gland biopsies and histological analyses

Labial salivary gland (LSG) samples were taken from patients who volunteered for the procedure and were considered suitable by the dentist. For example, patients with AGel-related drooping of the lower lip were excluded to prevent infection of the operation area. Under local anaesthesia, minor salivary glands were obtained from the lower labial lip through an incision.

Paraffin sections from the samples were stained with haematoxylin and eosin and Herovici stains, revealing collagen fibers. Amyloid was stained with modified Puchtler’s alkaline Congo red (Puchtler and Sweat 1962), and the amyloid protein was characterized by performing immunohistochemistry with rabbit antiserum raised against a low molecular weight AGel subunit (Haltia et al. 1990b). The tissue lymphocytes were identified with leucocyte common antigen, CD45, to evaluate the presence of inflammation (I-II). The deposition of gelsolin amyloid, the degree of inflammation and glandular atrophy were classified with a semi-quantitative five-step grading system (0-4) to improve comparability between the variables (Table 7).
Table 7. Grading system for amyloid, inflammation, and atrophy in labial salivary gland specimen.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Amyloid</th>
<th>Inflammation</th>
<th>Atrophy</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no amyloid</td>
<td>no inflammation</td>
<td>no atrophy or fibrosis</td>
</tr>
<tr>
<td>1</td>
<td>thin strikes of amyloid affecting &lt; 10% of GT</td>
<td>slight infiltrate</td>
<td>minimal, patchy areas of fibrosis affecting &lt; 10% of GT and with normal intervening tissue</td>
</tr>
<tr>
<td>2</td>
<td>distinct amyloid deposits affecting 10-40% of GT</td>
<td>moderate infiltrate</td>
<td>diffuse fibrotic areas with acinar atrophy affecting 10-40% of GT</td>
</tr>
<tr>
<td>3</td>
<td>distinct amyloid deposits affecting &gt;40-70% of GT</td>
<td>1 focus of at least 50 lymphocytes/4 mm²</td>
<td>diffuse fibrotic areas with acinar atrophy affecting &gt;40-70% of GT</td>
</tr>
<tr>
<td>4</td>
<td>extensive amyloid deposits affecting &gt;70% of GT</td>
<td>&gt;1 focus of at least 50 lymphocytes/4 mm²</td>
<td>diffuse fibrotic change with wide spread replacement of acini affecting &gt;70% of GT</td>
</tr>
</tbody>
</table>

A five-step grading system (0–4) to classify the degree of amyloid, inflammation and atrophy in labial salivary gland specimens of AGel amyloidosis patients. GT: glandular tissue.
4.4.3. Oral microbiological samples and analyses

Oral yeasts were studied because we expected to find hyposalivation, a known risk for oral candidiasis (Parvinen and Larmas 1981). Yeast samples were taken with a sterile cotton stick from the dorsal surface of the tongue from study patients and controls. Cultivation was done on special solid growth medium and fungi identified according to their morphological features on different growth medias and metabolic tests (HUSLAB, Helsinki). Yeast growth was graded from 0 to 4 (0 = no growth, 1 = minor growth, 2 = growth on that half of the petri plate where the sample was spreaded, 3 = growth also on the other half of the plate, 4 = growth on the whole plate).

Subgingival plaque samples for bacterial analyses were collected from periodontal pockets with PPD ≥ 4 mm using sterile periodontal curettes. The pooled samples from five study patients were transferred to small vials containing 2 mL of VMGA III medium and transported to the HUSLAB, Helsinki for anaerobic culture of six periodontal pathogens. In addition, the pooled samples of 24 patients were sent frozen to the Oral Microbiology Laboratory at the University of Bern for bacterial analysis. The samples were processed by the checkerboard DNA-DNA hybridization method, as previously described (Socransky et al. 1994; Socransky et al. 2004; Katsoulis et al. 2005). A total of 39 bacterial species were assessed with this method. The results were compared with the microbiological findings obtained from several studies done in the same laboratory and comprising people with various periodontal conditions (Persson et al. 2009; Brito et al. 2013) (III).

4.5. IN VITRO STUDIES ON ORAL FIBROBLASTS AND VASCULAR SMOOTH MUSCLE CELLS

Oral fibroblasts were obtained from palatal tissue specimens. Each sample was divided into six pieces and dried onto petri dishes for 10 min before adding culture medium. The cells were cultured at 37°C in 5% CO₂ with Dulbecco’s modified eagle medium (high glucose, GlutaMAX™, and pyruvate) supplemented with 10% foetal bovine serum (FBS), penicillin 50 units/mL, streptomycin 50 µg/mL, and amphotericin B 2.5 µg/mL. All of these were purchased from Gibco®, as was also
0.25% Trypsin EDTA. Cell lines of passage 2-3 were used in all experiments, and the cells were confirmed to be negative for mycoplasma with DAPI staining.

Vascular smooth muscle cell (VSMC) lines were established from facial blood vessels. Tissue samples were collected from plastic surgery operations at the Department of Plastic Surgery, Töölö Hospital, Helsinki University Hospital. The VSMCs were isolated by surgical preparation from the blood vessel wall. Small pieces of the vessel wall were dried onto petri dishes for 20 min before addition of culture medium. The cells were cultured in Dulbecco’s modified eagle medium containing low glucose, 10% FBS, L-glutamine and penicillin/streptomycin at 37°C in 5% CO₂. All experiments on primary VSMCs were carried out on cells in passage 2-3, and the cells were confirmed to be negative for mycoplasma with VenorGeM® mycoplasma detection kit (Minerva Biolabs GmbH, Berlin, Germany) and DAPI staining.

VSMCs were identified by Western blotting the cell lysates. Antibodies used for characterization were mouse anti-α-SMA (Sigma-Aldrich, St. Louis, MO, USA) and goat anti-mouse IRDye® 680LT (LI-COR, Lincoln, NE, USA). Fibroblasts served as negative controls for VSMCs in this experiment. RNA isolation and sequencing were performed to confirm transcription of GSN in the patient cells. Fluorescent cell staining methods were used to evaluate possible differences in actin cytoskeleton and cGSN distribution in patient cells relative to controls. Cell migration was studied with QCM™ Colorimetric Cell Migration Assay (Chemicon International, Billerica, MA, USA). Migration of fibroblasts was evaluated by measuring the absorbance values (AV) at the time points of 11 and 22 hours and migration of VSMCs at the time point of 22 hours. Radioimmunoassay (RIA) analyses were performed to assess collagen type I synthesis and degradation (PINP RIA -kit and ICTP RIA -kit, Orion Diagnostica, Oulu, Finland). Staurosporine is a protein kinase C inhibitor with the known effect of rapid disruption of actin microfilaments by inhibition of actin and cGSN induction (Miyamoto and Wu 1990; Yu and Gotlieb 1992). Both cell types were incubated with 50 nM staurosporine (Yu and Gotlieb 1992; Koivisto et al. 2003), and the ratio of rounded cells to cuboidal, stellate, or spindle-shaped cells was determined by counting cells from an area of 850 μm x 640 μm (IV).
4.6. STATISTICAL METHODS

The following tests were used for the statistical analyses. Median values were used when the distribution of the values was skewed.

Non-parametric Spearman rank correlation coefficients were calculated to assess the relationships between hyposalivation (UWS or SWS) and age (as a continuous variable), gender, and number of daily drugs (<3 versus more). The same analysis was performed to evaluate the correlation between histopathological findings and xerostomia and hyposalivation (UWS).

Chi-square tests were used to analyse the possible association between the number of daily drugs with xerostomia as a side effect and xerostomia and hyposalivation (UWS and SWS), as well as hyposalivation (SWS) between patients and controls. The same test was used to compare the mean values of the periodontal parameters between the two age groups, and also to compare the mean values of migration rate and parameters from RIA analyses between the patient and control cell lines.

Wilcoxon signed-rank tests were used to compare the median values of SWS and concentrations and secretion rates of salivary proteins between patients and controls.

Mann-Whitney U-test was used to assess differences in the quantity of each bacterial species by defined group.

All statistics were performed with PASW® Statistics 18.0 software (SPSS Inc., Chicago, IL, USA) or SPSS® Statistics 22 software (IBM® Corp., New York, NY, USA) (II-IV).
5. RESULTS

5.1. QUESTIONNAIRE

According to the questionnaire, 88.9% of patients reported a sense of dry mouth (xerostomia) and 81.5% dry eyes (xerophthalmia). Concomitant xerostomia and xerophthalmia (sicca symptoms) were reported by 74.1% of patients. A progressive tendency of increased symptoms with age was noted (II).

Approximately 25% of patients did not use any daily drugs. Prescribed drugs were mainly for the heart and circulatory diseases. The number of daily drugs ($\geq 3$) and drugs with xerostomia as a side effect were not related to xerostomia reported by patients (I, II).

Four patients were excluded from the periodontal study because of current smoking, which is a known risk factor for periodontitis (Bergström 1989), and thus, obscures the findings. Most patients (68%) brushed their teeth twice a day and 44% performed daily interdental hygiene. Only four patients felt that AGel amyloidosis hindered their oral hygiene procedures due to sensomotor impairment of their hands, typical in AGel amyloidosis (III).

5.2. SALIVARY SECRETION, COMPOSITION AND LABIAL SALIVARY GLAND HISTOLOGY

5.2.1. Salivary flow rates

The patients had significantly lower median flow rates of SWS than controls (mean 0.64 mL/min versus mean 1.60 mL/min, $p<0.001$). Hyposalivation was present in approximately 2/3 patients according to both UWS (66.7 %) and SWS (63 %) flow rates. Only four patients had UWS or SWS
flow rates at a normal level, $\geq 0.1$ mL/min and $\geq 0.7$ mL/min, respectively.

The hyposalivation (UWS or SWS) observed in AGel amyloidosis patients did not correlate with age or gender. No differences were found in prevalence of hyposalivation when the effect of medications and drugs with xerostomia as a side effect was evaluated.

5.2.2. Salivary protein composition

Patients’ mean salivary albumin and IgG concentrations were significantly higher and their secretion rates of salivary total protein and IgA were significantly lower than those of controls. These data are presented in Table 8.

5.2.3. Labial salivary gland histopathology

Patient LSG samples showed varying grades of amyloid in Congo red staining. These amyloid deposits were immunoreactive with the antibody against the AGel subunit, verifying the presence of amyloid composed of variant GSN (Figure 7). In addition, varying amounts of gland atrophy and inflammation were present. Most of the AGel amyloid deposits were seen around the small salivary gland acini attached to the basement membranes and in the blood vessel walls. The amounts of amyloid, inflammation and atrophy were graded semi quantitatively from 0 to 4 (Table 7, p. 55). In these specimens, the amount of amyloid roughly paralleled the amount of salivary gland atrophy (Figure 8). No correlation was found between the grade of histopathological findings and the presence of xerostomia or hyposalivation (UWS) (II).
Table 8. Results of biochemical salivary protein analyses in AGel amyloidosis patients and controls.

<table>
<thead>
<tr>
<th></th>
<th>Patients (N=27)</th>
<th>Controls (N=27)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein concentration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (mg/mL)</td>
<td>1.49 (0.79)</td>
<td>1.55 (0.69)</td>
<td>0.169</td>
</tr>
<tr>
<td>Albumin (μg/mL)</td>
<td>47.60 (39.50)</td>
<td>26.70 (19.30)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IgA (μg/mL)</td>
<td>48.1 (70.00)</td>
<td>59.00 (30.50)</td>
<td>0.436</td>
</tr>
<tr>
<td>IgG (μg/mL)</td>
<td>21.30 (18.60)</td>
<td>8.90 (6.20)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IgM (μg/mL)</td>
<td>2.54 (2.86)</td>
<td>1.70 (1.23)</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Protein secretion rate</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein (mg/min)</td>
<td>0.83 (0.65)</td>
<td>2.60 (1.95)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Albumin (μg/min)</td>
<td>33.00 (28.01)</td>
<td>40.01 (35.54)</td>
<td>0.064</td>
</tr>
<tr>
<td>IgA (μg/min)</td>
<td>32.55 (26.37)</td>
<td>98.77 (96.42)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>IgG (μg/min)</td>
<td>11.07 (10.96)</td>
<td>15.71 (12.16)</td>
<td>0.078</td>
</tr>
<tr>
<td>IgM (μg/min)</td>
<td>1.54 (1.38)</td>
<td>2.81 (3.23)</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Values are shown as medians (interquartile range). Highly significant p-values (p<0.001) are marked with an asterisk (*).

Previously, one female study patient with xerostomia, xerophthalmia, focus score 2 (i.e. more than one focus of ≥ 50 lymphocytes in 4 mm²) in LSG biopsy sample, and slightly elevated rheumatoid factor had been diagnosed with SS at the age of 41 years. She was later diagnosed with AGel amyloidosis, and in re-evaluation of the original LSG biopsy sample AGel amyloid deposits in addition to mild inflammation were observed. Despite persisting sicca symptoms according to the questionnaire, we did not observe hyposalivation in the salivary flow rate measurements. Thus, Congo red staining of LSG specimen is essential for excluding amyloidosis as a cause of sicca symptoms, even if the more common SS is otherwise suspected (1).
Figure 7. Atrophy, inflammation and deposition of amyloid in labial small salivary glands of a patient with AGel amyloidosis. Stainings are as follows: haematoxylin eosin (a), Herovici (b), Congo red (c) with polarization (d), immunohistochemical staining against AGel subunit (e), and against leucocyte common antigen, CD45 (f). Asterisk: partially atrophied gland; triangle: fibrotic tissue; arrowhead: lymphocytic infiltrate; arrow: amyloid deposits (c, d) and precursor protein (e). Original magnification x 100.
Figure 8. Grade (0 – 4) of amyloid deposition, inflammation, and atrophy of the labial salivary glands in the biopsy specimens from seven AGel amyloidosis patients in increasing age from 40 years (1) to 72 years (7). Grading system presented in Table 7, p.55.

5.3. ORAL MUCOSA, PERIODONTAL CONDITION AND MICROBIOLOGICAL FINDINGS

All patients had macroscopically healthy oral mucous membranes, except two who had an atrophic tongue. We did not observe macroglossia in our study patients.

Thirty-six patients were involved in the periodontal study. Visible plaque index (VPI) and bleeding on probing (BOP) values reflected good oral hygiene (Table 9). However, nearly all patients (89%) had at least one probing pocket depth (PPD) ≥ 4 mm and many (38%) had PPD ≥ 6 mm. Typically, deepened pockets were found in molar teeth. For comparison, in the Health 2000 Survey, 64% of subjects had one or more PPD ≥ 4 mm (Suominen-Taipale et al. 2004). CAL measurements
converged with PPDs, indicating that the study patients had not had a prominent history of previous gingival recession or gingival overgrowth. Observations from panoramic tomographies were consistent with PPD and CAL measurements.

The patients were divided into two age groups to determine whether progression of their systemic disease, AGel amyloidosis, correlated with periodontal findings (Table 9). The older age group had significantly less teeth, especially molars; they also more frequently had less than twenty teeth left. In general, most lost teeth were molars and comparably fewer incisors. Altogether 21% and 45% of the molars were lost in the younger and older age groups, respectively. The number of teeth left was approximately the same as in the Health 2000 Survey (mean: all periodontal study patients 21.5, national survey: 22.8) (Suominen-Taipale et al. 2004) (III).

The most common oral yeast detected in cultivation was *Candida albicans*. Growth of values 2-4 was detected in 56% of patients, but in only 10% (1/10) of healthy controls. One patient had clinically detectable candidiasis under a removable prosthesis.

Periodontal microbiological samples from five first patients studied at HUSLAB by bacterial culturing method showed that *Tannerella forsythia* and *Prevotella intermedia* -group bacteria were present in all samples and *Porphyromonas gingivalis* and *Peptostreptococcus micros* in four samples. All except one sample (8x10^5) had bacteria ≥ 1x10^7. Further, 51 samples from 24 patients studied with the DNA-DNA checkerboard method showed that, when using ≥ 1 x 10^5 bacterial cells as the unit of observation, bacteria known to be associated with chronic periodontitis were found in many samples. This data is presented in Table 10. When the subject was used as a unit of observation and with PPDs ≥ 6 mm to define severe chronic periodontitis (N=10), statistical analysis failed to identify any species differing by periodontal site-based diagnosis at the p<0.001 level.
Table 9. Results of the periodontal study in two age categories of AGel amyloidosis patients.

<table>
<thead>
<tr>
<th></th>
<th>Age group 43-59 years (N=17)</th>
<th>Age group 60-79 years (N=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean number of teeth</strong></td>
<td>24.4</td>
<td>18.5*</td>
</tr>
<tr>
<td><strong>Mean number of molars</strong></td>
<td>6.4</td>
<td>4.4*</td>
</tr>
<tr>
<td><strong>Number (%) of patients with &lt;20 teeth left</strong></td>
<td>2/17 (11.8 %)</td>
<td>9/19 (47.4 %)*</td>
</tr>
<tr>
<td><strong>Mean % of VPI</strong></td>
<td>11.4</td>
<td>19.2</td>
</tr>
<tr>
<td><strong>Mean % of BOP</strong></td>
<td>11.1</td>
<td>11.2</td>
</tr>
<tr>
<td><strong>Mean number (range) of PPD ≥ 4 mm</strong></td>
<td>5.7 (0 - 15)</td>
<td>8.8 (0 – 20)</td>
</tr>
<tr>
<td><strong>Mean number (range) of PPD ≥ 6 mm</strong></td>
<td>0.9 (0 – 10)</td>
<td>1.0 (0 – 5)</td>
</tr>
<tr>
<td><strong>Mean number (range) of CAL ≥ 4 mm</strong></td>
<td>6.2 (0 - 20)</td>
<td>8.6 (0 – 20)</td>
</tr>
<tr>
<td><strong>Mean number (range) of CAL ≥ 6 mm</strong></td>
<td>1.6 (0 – 18)</td>
<td>1.5 (0 – 6)</td>
</tr>
</tbody>
</table>

VPI: visible plaque index, BOP: bleeding on probing, PPD: probing pocket depth, CAL: clinical attachment level. Significant differences (p<0.05) are indicated with an asterisk (*).
Table 10. Distribution of 39 bacteria strains used in the DNA-DNA -checkerboard analysis and the proportions (%) of sites with bacteria cells ≥1 x 10^5.

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Reference Strain</th>
<th>≥1 x 10^5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces israelii</td>
<td>ATCC 12102</td>
<td>80.87</td>
</tr>
<tr>
<td>Actinomyces naeslundii</td>
<td>ATTC 43146</td>
<td>73.1</td>
</tr>
<tr>
<td>Actinomyces odontolyticus</td>
<td>ATTC 17929</td>
<td>62.3</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans (Y4)</td>
<td>ATCC 29523</td>
<td>75.0</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans (a)</td>
<td>ATCC 43718</td>
<td>53.8</td>
</tr>
<tr>
<td>Campylobacter gracilis</td>
<td>ATCC 33236</td>
<td>57.8</td>
</tr>
<tr>
<td>Campylobacter rectus</td>
<td>ATCC 33238</td>
<td>78.8</td>
</tr>
<tr>
<td>Campylobacter showae</td>
<td>ATCC 51146</td>
<td>28.8</td>
</tr>
<tr>
<td>Capnocytophaga gingivalis</td>
<td>ATCC 33612</td>
<td>36.5</td>
</tr>
<tr>
<td>Capnocytophaga ochracea</td>
<td>ATCC 33596</td>
<td>73.1</td>
</tr>
<tr>
<td>Capnocytophaga putitigena</td>
<td>ATCC 33612</td>
<td>86.5</td>
</tr>
<tr>
<td>Eikenella corrodens</td>
<td>ATCC 23834</td>
<td>50.0</td>
</tr>
<tr>
<td>Eubacterium saburreum</td>
<td>ATCC 33271</td>
<td>65.4</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp.nucleatum</td>
<td>ATCC 25586</td>
<td>96.2</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp.polymorphum</td>
<td>ATCC 10953</td>
<td>78.8</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp.vincentii</td>
<td>ATCC 49256</td>
<td>88.8</td>
</tr>
<tr>
<td>Fusobacterium periodonticum</td>
<td>ATCC 33693</td>
<td>96.2</td>
</tr>
<tr>
<td>Lactobacillus acidophilus</td>
<td>ATCC 11975</td>
<td>69.2</td>
</tr>
<tr>
<td>Neisseria mucosa</td>
<td>ATCC 19696</td>
<td>92.3</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>ATCC 33277</td>
<td>44.2*</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>ATCC 25611</td>
<td>64.4</td>
</tr>
<tr>
<td>Prevotella melaninogenica</td>
<td>ATCC 25845</td>
<td>88.5</td>
</tr>
<tr>
<td>Prevotella nigrescens</td>
<td>ATCC 33563</td>
<td>73.1</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>ATCC 11827/11728</td>
<td>40.4</td>
</tr>
<tr>
<td>Selenomonas noxia</td>
<td>ATCC 43541</td>
<td>61.5</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>ATCC 25923</td>
<td>61.5</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>ATCC 33397</td>
<td>67.3</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>ATCC 27823(M32b)</td>
<td>17.3</td>
</tr>
<tr>
<td>Streptococcus gordonii</td>
<td>ATCC 10558</td>
<td>76.9</td>
</tr>
<tr>
<td>Streptococcus intermedius</td>
<td>ATCC 27335</td>
<td>62.3</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>ATCC 49456</td>
<td>40.4</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>ATCC 35037</td>
<td>46.2</td>
</tr>
<tr>
<td>Streptococcus oralis</td>
<td>ATCC 10556</td>
<td>32.7</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>ATCC 25175</td>
<td>34.6</td>
</tr>
<tr>
<td>Tannerella forsythia</td>
<td>ATCC 43037</td>
<td>88.5*</td>
</tr>
<tr>
<td>Treponema denticola</td>
<td>Boston B1</td>
<td>50.0*</td>
</tr>
<tr>
<td>Treponema socranskii</td>
<td>ATCC D40DR2</td>
<td>86.5</td>
</tr>
<tr>
<td>Veillonella parvula</td>
<td>ATCC10790</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The proportions of sites with red complex bacteria are marked with asterisk (*).
5.4 Case Reports

These two cases contributed significantly to instigating the studies of the thesis and are therefore presented here. The data are derived from oral examinations, dental history records and interviews of patients, and panoramic tomographies compared with previously taken ones (unpublished observations). The cases represent rapidly progressing severe periodontitis in AGel amyloidosis patients.

Case 1

A male dental technician of 58 years age. He was diagnosed with AGel amyloidosis at the age of thirty. The disease had caused impairment of vision and polyneuropathy of legs and hands. Additionally, the patient had high blood pressure due to insufficient kidney functions of unknown origin, and severe hearing impairment. He was a former smoker.

Previous dental history: The patient had visited a dentist regularly between the ages of 26 and 35. His dental charts, serial intra-oral bitewing radiographs taken at the ages of 26, 35 and 41 years, and panoramic tomographies taken at the ages of 35, 41 and 42 years were available. At the age of 26, mild horizontal alveolar bone loss and dental calculus were seen in the molar areas. Nine years later (at the age of 35), marginal bone support was almost at the same level (Figure 9a). Six years later, at the age of 41 years, alveolar bone loss had proceeded to 5-6 mm (on average 1 mm per year). According to the panoramic tomography at the age of 41, alveolar bone loss had reached the apical third of some molar teeth and suspected furcation lesions are seen. Additionally, vertical intrabony lesions were identified at several teeth (Figure 9b). At this time, intensive periodontal therapy had been started by a periodontist. Required surgical procedures were performed, and after one year prosthetic constructions were made to establish a functional occlusion in the upper jaw. When the patient attended the present study at the age of 58 years, he had full upper and lower dentures.
Figure 9. Case 1. a) Panoramic tomography at the age of 35 years. b) Panoramic tomography six years later, at the age of 41 years.
Case 2

A female 55 years of age. AGel amyloidosis was diagnosed at the age of 25. Blood pressure was medicated, and the patient had quit smoking at the age of 53.

Previous dental history: The patient reported that she had mainly lived abroad the adult age and that she had visited dentist regularly. Previous dental charts were not available, but the dental history was evident from panoramic tomographies taken at the ages of 44 and 55 years. When the patient came to this study, she had already lost five molars and one premolar, but no incisors. The remaining molars had deep pockets and little or no alveolar bone support. The panoramic tomographies (Figure 10a, b) showed that the bone loss in the molar regions had been unusually extensive. Furthermore, the rate of periodontal attachment loss had been very rapid, on average 1.2 mm of alveolar bone loss per year, as calculated from the worst molar sites. Periodontal treatment consisted of extraction of teeth 18, 25, 37 and 46 and conventional scaling and root planing with oral hygiene instructions in three sessions. The patient responded well to treatment.
Figure 10. Case 2. **a)** Panoramic tomography at the age of 44 years. **b)** Panoramic tomography 11 years later, at the age of 55 years.
5.5. ORAL FIBROBLASTS AND VASCULAR SMOOTH MUSCLE CELLS

Patient cells expressed normal and variant gelsolin in an approximately 1:1 ratio, whereas in healthy control cells there was no evidence of variant GSN, as expected. Both fibroblasts and VSMCs grew normally in an elongated way and formed orientated parallel bundles of cells in culture. The growth rate of the cells was similar in the patient and control cell groups. No systematic difference was observed in actin cytoskeleton morphology or cGSN distribution between AGel amyloidosis fibroblasts or VSMCs versus healthy control cells. Figure 11a-d shows fluorescent stainings of the control and patient fibroblasts and VSMCs.

We found no difference in the migration ability of fibroblasts and VSMCs between patients and controls. In line with this, the synthesis (PINP) and degradation (ICTP) of collagen type I measured with RIA remained similar between patient and control cells. After staurosporine incubation, the patient fibroblasts obtained a rounded shape more rapidly than control cells, whereas the VSMCs showed no marked difference in cell shape after incubation. Data from these experiments are presented in Table 11 (IV).
Figure 11a-d. Fluorescent stainings of fibroblast control (a) and patient (b) and VSMC control (c) and patient (d) cells. Cells were double-stained for phalloidin staining actin (green) and GSN (red). Nuclei were visualized with DAPI (blue). Magnification x 400. Scale bars: 20 µm.
Table 11. Values from cell study experiments performed on patient and control cell lines.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Patient cells</th>
<th>Controls cells</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration, AV-value</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) fibroblasts, 11 h</td>
<td>0.286 ± 0.952</td>
<td>0.353 ± 0.134</td>
<td>0.239</td>
</tr>
<tr>
<td>b) fibroblasts, 22 h</td>
<td>0.368 ± 0.144</td>
<td>0.317 ± 0.152</td>
<td>0.452</td>
</tr>
<tr>
<td>c) VSMCs, 22 h</td>
<td>0.292 ± 0.201</td>
<td>0.299 ± 0.195</td>
<td>0.930</td>
</tr>
<tr>
<td>Collagen type I metabolism, RIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) fibroblasts, PINP (ng/mL)</td>
<td>53.6 ± 13.5</td>
<td>61.0 ± 25.3</td>
<td>0.484</td>
</tr>
<tr>
<td>b) fibroblasts, ICTP (μg/mL)</td>
<td>8.5 ± 3.7</td>
<td>10.5 ± 5.4</td>
<td>0.398</td>
</tr>
<tr>
<td>c) VSMCs, PINP (ng/mL)</td>
<td>57.3 ± 15.0</td>
<td>63.9 ± 18.0</td>
<td>0.489</td>
</tr>
<tr>
<td>d) VSMCs, ICTP (μg/mL)</td>
<td>21.8 ± 5.8</td>
<td>20.7 ± 3.2</td>
<td>0.678</td>
</tr>
<tr>
<td>Staurosporine incubation, shape change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) fibroblasts, 30 min</td>
<td>0.501 ± 0.274</td>
<td>0.204 ± 0.112</td>
<td>0.003*</td>
</tr>
<tr>
<td>b) VSMCs, 90 min</td>
<td>0.403 ± 0.156</td>
<td>0.311 ± 0.083</td>
<td>0.208</td>
</tr>
</tbody>
</table>

Values presented as mean ± SD. AV: absorbance value, RIA: radioimmunoassay, PINP: aminoterminal propeptide of type I procollagen (measuring synthesis), ICTP: carboxyterminal telopeptide of type I collagen (measuring degradation). A significant difference (p<0.05) is indicated with an asterisk (*).
6. DISCUSSION

6.1. GENERAL AND METHODOLOGICAL ASPECTS

Patients were invited to the study through their patient organization SAMY. This may have caused bias in that patients in fairly good condition, i.e. able to move independently, participated in the study, while those with more severe manifestations of AGel amyloidosis were not reached. Females were probably overrepresented because of this outreach channel. This could have affected the average plaque index, and thus, also the periodontal condition since females in general perform more daily oral hygiene procedures and have fewer deepened periodontal pockets than males (Suominen-Taipale et al. 2004). Twenty-seven patients (21 women, 6 men) participated in the saliva study. Women generally have lower saliva secretion than men (Percival et al. 1994). This could have exaggerated the results from the salivary secretion measurements.

Although the total number of patients in the different study settings is not very high, it represents 5-10% of estimated number of AGel amyloidosis patients in Finland. Smoking has been shown to increase the severity and extent of periodontal disease. Four patients were current smokers and were excluded from the periodontal study. The rest reported not smoking during the past year, but smoking history was not systematically explored. Smoking is a known risk factor for periodontitis (Bergström 1989; Bergström 2004; Zeng et al. 2014), and this aspect should had been elucidated more carefully, if a consistent increase was found in the prevalence of periodontitis in AGel amyloidosis patients.

We used results from the Health 2000 Survey as controls for the periodontal study. We rationalized that comparing AGel amyloidosis patients with a very large control group of the average Finnish population is more reliable than comparing these patients with any other similarly sized control group, gathered from e.g. volunteers or unaffected relatives. Volunteers might present differing socioeconomic level and other confounding factors (e.g. smoking, diabetes, age) that could have hindered comparability.
6.2. SALIVARY FINDINGS: XEROSTOMIA, HYPOSALIVATION, SALIVA COMPOSITION AND LABIAL SALIVARY GLAND HISTOPATHOLOGY

Our major oral findings among AGel amyloidosis patients were prevalent xerostomia and reduced salivary flow rates together with diminished secretion rates of salivary IgA. Histological observations of gelsolin amyloid deposition, atrophy and inflammation in the minor LSGs may partly explain these findings.

6.2.1. Xerostomia and hyposalivation

The study patients frequently experienced concomitant xerostomia and xerophthalmia, i.e. sicca symptoms (74%). This is in line with the Finnish questionnaire study, where a high prevalence of xerostomia was reported by AGel amyloidosis patients (48.8%, N = 160; mean age (SD) = 58.7 (25.8) years) compared with healthy relatives (1.8%, N = 111) (Laine et al. 2010). The higher prevalence of xerostomia in our series (88.9%) could to some extent be related to the higher mean age (60.2 (SD 9.7) years) of our patients, but it is also possible that patients suffering from xerostomia were more likely to volunteer for this study. Altogether, xerostomia (with or without hyposalivation) may severely affect the quality of life by disturbing speech, eating, use of a prosthesis, and even sleep (Nederfors 2000). Xerophthalmia, prevalent also in our study (81.5%), was reported in the largest AGel patient series by over 95% of the 227 patients, often as the first sign of disease (Nikoskinen et al. 2015). Sicca symptoms and amyloid in salivary glands have been observed in other types of amyloidosis as well, namely in AL (Gogel et al. 1983), AA (Catalano and Vaughan 1980), and ATTR amyloidoses (Johansson et al. 1992). Besides ATTR amyloidosis (Johansson et al. 1992), AGel amyloidosis is the only other amyloidotic disease where saliva secretion and composition have been reported.

Hyposalivation (UWS or SWS) was observed in approximately 2/3 patients, although xerostomia was reported by nearly 90%. This may be due to the observation that xerostomia is related to hyosecretion of minor salivary glands, while the whole salivary flow rate can remain at an adequate level (Eliasson et al. 2009). Several factors, particularly female gender and polypharmacy,
may diminish salivary flow rate (Percival et al. 1994; Wolff et al. 2008). Although most of the study patients were females and several used daily drugs, we did not observe correlations between these factors and hyposalivation. It is possible that AGel amyloidosis-related hyposalivation disguised their impact.

6.2.2. Salivary protein composition

We observed elevated concentrations of serum-born factors albumin and IgG, which represent leakage through oral mucosa, as has been demonstrated for salivary IgG in burning mouth syndrome, taste disturbances, and xerostomia (Nagler and Hershkovich 2005). Increased serum albumin concentration could possibly explain high salivary albumin, but in general AGel amyloidosis patients have shown low serum albumin values (Kiuru 1992). Low serum albumin was observed also in ATTR amyloidosis (Johansson et al. 1992). Interestingly, in AGel amyloidosis leakage of blood vessel walls (Kiuru et al. 1999c; Kiuru-Enari et al. 2005) and renal glomeruli has been previously demonstrated (Maury et al. 1992; Ardalan et al. 2007; Yamanaka et al. 2013). Pathological studies are at present not available, but such mucosal leakage could via AGel deposition and/or connective tissue derangement, analogous to other AGel tissues, contribute to high albumin and IgG values. Elevated salivary albumin concentrations have been observed in HIV-infected patients and in the elderly and suggested to be due to mucosal leakage with generalized immune deficiency or age (Mellanen et al. 2001; Meurman et al. 2002). Similarly, higher concentrations of salivary albumin, IgG, and IgM have been noted in patients with rheumatic diseases, some even with SS, than in controls and have been speculated to be caused by autoimmune inflammation of these patients’ salivary glands (Helenius et al. 2005).

Secretion rate of salivary IgA, the main specific immune defence mechanism in the oral cavity (Smith and Taubman 1992), was severely reduced in our patient group, probably reflecting salivary gland atrophy. As a parallel finding, diminished salivary IgA concentration has been previously observed in ATTR amyloidosis (Johansson et al. 1992). Reduction in salivary IgA could be expected to result in incomplete antimicrobial function of the saliva, further aggravating the effects of salivary hypossecretion such as caries activity and mucosal changes (Tenovuo and Lagerlöf 1999).
6.2.3. Labial salivary gland histopathology

AGel amyloid was deposited around salivary acini, mainly associated to basement membranes, one of the predominant sites of AGel deposition in general (Meretoja 1969; Meretoja and Teppo 1971). Amyloid deposits are commonly found in the arteries of nearly every organ and in capillaries (Meretoja and Teppo 1971; Kiuru et al. 1999c). This previous finding was confirmed in our study, when AGel amyloid deposits were seen relatively extensively in the vascular tissue. The reason for AGel amyloid depositing in certain tissues is not yet known. In the skin, AGel amyloid has been found encircling elastic fibers, which are degenerated (Kiuru-Enari et al. 2005), and also in blood vessel walls (Koskelainen et al., submitted). In all biopsies amyloid deposits co-occurred with atrophy and chronic inflammation. Often lymphocytic infiltration in salivary glands points to SS, since it is one of its diagnostic criteria (Vitali et al. 2002). Without a known family history of AGel amyloidosis or Congo red staining of LSG sample, this kind of histopathological finding, together with AGel-related sicca symptoms, can mislead to a diagnosis of SS (I). In line with our histopathological findings were observations in ATTR amyloidosis, where amyloid deposits were found around blood vessels in the LSG sample and in the parenchyma of all salivary glands (Johansson et al. 1992).

AGel amyloid deposits as a mechanical obstacle, and atrophy in the minor salivary glands – possibly related to amyloid toxicity – could be reasons for the diminished UWS flow rate. While amyloid deposits have also been found in the parotid gland (Meretoja and Teppo 1971), similar findings could be associated with reduced SWS flow rate. In addition, problems in parasympathetic innervation cause impaired saliva secretion and atrophy of salivary glands in other conditions (Kutchai 1998). The submandibular and sublingual salivary glands receive their parasympathetic input from the facial nerve. This nerve is the predilection site of AGel neuropathy, which is known to cause autonomic dysfunction too (Kiuru-Enari and Haltia 2013). In AGel amyloidosis, trigeminal nerve involvement with subsequent weak masseter function (Kiuru-Enari and Haltia 2013) might also diminish biting-stimulated saliva secretion. Thus, the pathogenesis of hyposalivation in AGel amyloidosis could be also multifactorial. Interestingly, in ATTR amyloidosis, autonomic dysfunction, ATTR-related malnutrition, and/or amyloid deposits in the salivary glands have been suggested as mechanisms behind hyposalivation (Johansson et al. 1992). Overall, patients with
AGel amyloidosis often had quantitative and qualitative salivary abnormalities, which may alter the physiological role of saliva in the maintenance of a healthy oral environment.

6.3. ORAL AND PERIODONTAL HEALTH WITH MICROBIOLOGICAL FINDINGS

6.3.1. Oral tissue manifestations

No notable changes in the oral mucosa that could have been related to systemic amyloidosis were observed. Macroglossia was not evident here, although previously reported in some AGel amyloidosis patients (Kiuru et al. 1999a). However, the patients in the study of Kiuru et al. were older, had a history of snoring, and showed obstructive sleep apnea and/or laxity of oro-and hypopharyngeal tissues, factors that are likely to co-occur with macroglossia.

6.3.2. Periodontal condition

Because several systemic diseases have been found to increase the risk of oral diseases, including periodontal problems, we studied oral soft tissues and periodontal condition of AGel amyloidosis patients.

Concerning the periodontal condition, our main finding was that overall periodontal health of the study patients did not markedly differ from that of the general Finnish population, as reported in the Finnish Health 2000 Survey (Suominen-Taipale et al. 2004). The microbiological findings supported the periodontal findings. The moderate mean VPI and BOP indices, both < 15%, reflected good oral hygiene of the patients, but still, 83% of them had one or more PPD ≥ 4 mm and 36% had one or more PPD ≥ 6 mm. The corresponding values according to the Health 2000 Survey are 64% and 21% (Suominen-Taipale et al. 2004). Furthermore, when the study patients had advanced periodontitis the type of disease appeared somewhat unusual. Deep periodontal pockets were primarily found around molar teeth, and most of the lost teeth were molars. However, the
molar teeth are often affected by periodontitis and their prognosis is worse than that of other teeth due to their root morphology, which poses challenges for periodontal treatment (Novak and Takei 2012). In general, maintaining the anterior teeth is more important for patients for aesthetic reasons, and also hygiene procedures are easier to perform on the anterior area. Similarly, in the Finnish population, according to the Health 2000 Survey, mandibulary molars were the most commonly lost, followed by maxillary molars and premolars (Suominen-Taipale et al. 2004). Significant differences in the number of teeth (mean 24.4 versus 18.5) and molars (mean 6.4 versus 4.4) in the older patient group (60-79 years) relative to the younger patient group (40-59 years) could be partly due to the progressive nature of the symptoms in AGel amyloidosis with time. Even in individuals without systemic diseases, age alters periodontal tissue consistency and possibly also oral microflora. Untreated periodontitis progresses with age, but age alone does not present a risk for periodontitis (Needleman 2012). Taken together, diseases progressing with age may cause measurable changes in the periodontium, as was found in this study.

Unpublished reports of the two study patients (Case reports 1 and 2) suggest that AGel amyloidosis might, in some cases, be associated with aggressively progressing periodontitis combined with unusually extensive alveolar bone loss. The female patient’s periodontitis was rapidly progressive, leading to loss of molars. The male patient had not responded to earlier periodontal treatment and the periodontitis had progressed, resulting in loss of all teeth before the age of 58 years. Confounding factors, such as smoking history, had undoubtedly played a role in their disease progression. The aggressive nature of periodontitis of these patients could also be incidental and not directly related to their systemic disease.

Epidemiological data of the prevalence of aggressive periodontitis is concentrated on local aggressive periodontitis typically diagnosed at puberty or before the age of 30 years (Hinrichs and Novak 2012). The estimated prevalence of aggressive periodontitis varies from 0.1% to 15% in different populations. No data are available concerning the prevalence of aggressive periodontitis in adult populations (Stabholz et al. 2010). Although only two patients had aggressively progressive periodontitis, AGel amyloidosis is not excluded as a confounding factor since certain AGel amyloidosis-related manifestations present only in a small fraction of patients. Because both AGel amyloidosis and periodontitis progress with age, this association is probably more common in older
patient populations. The number of patients in our study was too small for definitive associations, but as periodontitis is a multifactorial disease and AGel amyloidosis presents with phenotypic variation, excluding confounding factors even from larger study populations is very difficult.

6.3.3 Microbiological findings

*Candida albicans*, the most common specie of the *Saccharomycetaceae* family, is found in 30-35% of the oral cavities of healthy individuals. In our study group over half of the patients were positive for oral *Candida albicans*. This fairly prevalent finding is in line with the observation of diminished salivary flow rates, known to impair the local mucosal defense towards microbes (Cheng et al. 2015), which in AGel amyloidosis might be further promoted by the reduced salivary IgA secretion rates.

The subgingival bacterial samples were examined with a DNA-DNA checkerboard method. Bacteria commonly associated with adult chronic periodontitis were found, as assessed by comparison with the microbiological findings of other studies conducted in the same laboratory. Subgingival bacterial flora analyses and periodontal clinical findings taken together suggest that there is probably no specific AGel amyloidosis-related periodontal health condition. According to the prevailing theory, the ecological plaque hypothesis, pathogenic bacteria alone do not cause periodontitis (Hinrichs and Novak 2012). It leaves open the possibility that the development of periodontitis in some AGel amyloidosis patients may be partly due to amyloid deposition in the periodontal tissues, possibly combined with connective tissue derangement, defective function of some cell types, or an inadequate antimicrobial defence system of the periodontium.
In this study setting, we set out to elucidate whether oral fibroblasts and VSMCs exhibit changes implying altered cGSN function, which has been noted earlier in other cell types (Westberg et al. 1999; Kiuru et al. 2000). The main finding was that there were no consistent differences in actin cytoskeleton morphology and intracellular gelsolin distribution, cell migration, and collagen type I metabolism between patient and control cell lines.

Both GSN alleles, healthy and mutated, were equally expressed in AGel amyloidosis patients. Despite this, the patient cell lines did not differ in their actin cytoskeleton morphology or intracellular gelsolin distribution from healthy control cell lines. A similar finding was earlier observed in fixed mice gelsolin-null fibroblasts transduced with either wild-type or mutant GSN (Kangas et al. 1999); this finding is now confirmed in patient fibroblasts and VSMCs. Surprisingly, migration ability and collagen type I metabolism were not significantly different between our patient and control cell lines, although gelsolin plays an important role especially in cell migration and collagen metabolism (Sun et al. 1999; Arora et al. 2013). Probably oral fibroblasts and VSMCs can maintain normal function through 1) cGSN coded by the wild-type allele, 2) function of other members of the gelsolin superfamily, or 3) action of other proteins with partial functional similarity to GSN overtaking the role of variant GSN. Interestingly, the leaky scanning mechanism of translation initiation in gelsolin knockout fibroblasts transduced with sGSN mutation produced also cGSN (Kangas et al. 1999).

Staurosporine is a protein kinase C inhibitor with a wide spectrum of fairly non-specific effects on cells (Tamaoki and Nakano 1990). Of these, best known is inhibition of actin-cGSN induction, and thus, rapid disruption of the actin microfilaments (Miyamoto and Wu 1990; Yu and Gotlieb 1992). On the other hand, staurosporine induces alterations in a concentration-dependent and cell type-specific manner, and thus, in cultured keratinocytes staurosporine has been found to induce rapid lamellipodia and filopodia formation (Koivisto et al. 2003). In our study, incubation with staurosporine showed that patient fibroblasts transformed to a rounded shape faster than controls, but we did not observe a similar transformation difference in the VSMCs. Platelets of Finnish AGel amyloidosis patients have also shown slower and more profound shape transformation than control
platelets (Kiuru et al. 2000). These could reflect aberrant cGSN function during cell activation when only the wild-type GSN allele in the patient’s cells is coding for the normal cGSN. Interestingly, the mutation site, c.640G>A, is responsible for the GSN actin binding feature (Sun et al. 1999). On the other hand, abnormal GSN fragmentation itself, as demonstrated in multiple different cells of non-amyloidotic mice after caspase 3 cleavage, caused the cells to round up (Kothakota et al. 1997). Patients’ VSMCs did not show a difference similar to fibroblasts, potentially illustrating that cells of different lineages contribute individually to AGel amyloidosis, as suggested by experimental \textit{in vitro} studies (Paunio et al. 1998).

These results suggest that in oral fibroblasts and VSMCs the GSN defect does not grossly impair the physiological actin cytoskeleton-associated function of cGSN. The significance of minor shape changes noted in staurosporine-treated oral fibroblasts requires further clarification.
Hyposalivation with altered salivary composition constitutes a risk for oral health in AGel amyloidosis patients. It may therefore present an increased risk for oral diseases, such as caries and oral candidiasis, in these patients. In this study, unusual caries activity was not noted and oral candidiasis was observed in only one patient, but this may be explained by better-than-average oral hygiene practices (Suominen-Taipale et al. 2004). However, in general, these patients need to follow enhanced strategies of preventive procedures such as diet advice, extra fluoride, xylitol, probiotics, lubricants and optimized periodic dental check-ups.

In addition to xerophthalmia, a well-recognized symptom in AGel amyloidosis patients, common xerostomia, should be included in the main symptoms of this disease. These sicca symptoms not only greatly affect the quality of life, but can also lead to incorrect diagnosis. Therefore, salivary gland biopsy and Congo red staining analysis are good tools for identifying AGel amyloidosis patients, as has been suggested also in other forms of amyloidoses (Do Amaral et al. 2009).

According to our study, AGel amyloidosis patients do not have generalized increased risk for periodontitis. Still, there is a possibility that in some AGel amyloidosis patients periodontitis may proceed in an aggressive and treatment-resistant manner. Possible underlying mechanisms could be at the tissue level, with amyloid deposits, especially in basement membranes, hindering defence mechanisms and normal tissue turnover. At the cellular level, altered function of cGSN could affect some important cell functions such as cell migration or phagocytic ability. However, we did not observe such changes in the cellular study of oral fibroblasts. AGel amyloid is known to affect elastic fibres (Kiuru-Enari et al. 2005) and possibly also oxytalan fibres (Hintner and Breathnach 1988) Therefore, exploring the periodontal ligament from extracted teeth would be beneficial in assessing possible alterations in the tooth attachment apparatus of AGel amyloidosis patients. As mentioned, periodontitis is a multifactorial chronic infectious disease. AGel amyloidosis manifestations can also vary greatly between individuals. Thus, it is challenging to evaluate the input of solely AGel amyloidosis on the risk of periodontitis progression. The good oral hygiene and regular dental check-ups needed to treat dry mouth are also prerequisites for periodontal health. The relationship between systemic diseases and oral health is being increasingly recognized and
investigated. AGel amyloidosis is a novel systemic disease in this field. More studies are needed to assess the pathomechanism of AGel amyloidosis and to develop new treatment strategies for the disease. Until that, knowledge of the manifestations, prevention of further tissue damages, and relieving of the symptoms are primary targets.
This study was carried out at the Department of Oral and Maxillofacial Diseases and in the Scientific Research Laboratory in Biomedicum at the University of Helsinki, Finland. I thank the former Dean of the Institute of Dentistry, Professor Jarkko Hietanen, and the Head of the Department of Oral and Maxillofacial Diseases, Professor Christian Lindqvist, for the opportunity to perform research at these excellent facilities.

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Vaasa, February 2016

Pirjo Juusela
REFERENCES


with gastritis and inflammatory bowel disease as prevailing symptoms. BMC Gastroenterol 14:159-230X-14-159.


APPENDICES

Appendix 1: references to Table 3.

AL amyloidosis (Benson et al. 2015).

ATTR amyloidosis (Andrade 1952; Plante-Bordeneuve and Said 2011; Ueda and Ando 2014; Sekijima 2015).

Ab2M amyloidosis (Valleix et al. 2012).


AApoAII amyloidosis (Brewer and Rader 1991; Benson et al. 2001; Magy et al. 2003; Yazaki et al. 2003b).


ALect amyloidosis (Benson et al. 2008; Larsen et al. 2010; Khalighi et al. 2013; Larsen et al. 2014).

AFIb amyloidosis (Benson et al. 1993; Uemichi et al. 1994; Gillmore et al. 2009; Stangou et al. 2010).


ABri amyloidosis (Worster-Drought et al. 1933; Ghiso et al. 2001; Vidal et al. 1999;).
Appendix 2: Questionnaire

Hampaiston ja suun alueen ongelmat perinnöllisessä gelsoliiniamyloidiosissa

Helsingin yliopisto, hammaslääketieteen laitos, suubilogian osasto
HLL Pirjo Juusela, EHL Anja Nieminen, EL Sari Kiuru-Enari

Nimi: ________________________________________________________________

Sosiaaliturvatusun: _________________________________________________

Sairaudet: ___________________________________________________________
______________________________________________________________

Lääkitykset: _________________________________________________________
______________________________________________________________

Rengastakaa oikea vaihtoehto:

Oletteko käyttäneet antibiootteja viimeisen 3 kk aikana?

Kyllä  En

Tupakoitteko?   Kyllä  En

Paljonko?  < 10 savuketta/pv  10-20 savuketta/pv  > 1 aski/pv

Onko geenivirhe todettu geenitestillä?  Kyllä  Ei
Milloin Teillä oli ensimmäiset oireet sairaudestanne ja minkälaisia ne olivat? Jos vastaustila ei riitä, niin voitte jatkaa kääntöpuolelle.

Onko Teillä kuivat silmät?  Kyllä  Ei

Kuvaikaa minkälaisia hampaiston tai suun alueen ongelmia (esim. hampaiden liikkumista, suun kuivuutta, suun sieni-infektio tms.) Teillä on ollut ja milloin.

Miten hoidatte hampaitanne?

Hampaiden harjaus:  kerran/pv  2 kertaa/pv  >2 kertaa/pv
Hammavälien puhdistus: ei lainkaan  pari krt/vko  joka päivä
hammastikulla  hammashangalla  väliharjoilla

Vaikeuttaako sairautenne mielestänne hampaiden puhdistusta?

Kyllä  Ei

Kiitos vaivannäöstä! Ottakaa tämä paperi mukaanne kun tulette tutkimukseen.