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ORAL HEALTH IN HEREDITARY GELSOLIN AMYLOIDOSIS

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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 ccontributing 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 nimitykseltään 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. Tyypillisiä 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-amyloidoosin aiheuttaa gelsoliinigeenin pistemutaatio c.640G>A/T, joka ilmoitettiin aiemmin c.654G>A/T. Kyseinen geeni ohjaa sekä solunsisäisen että eritettävän eli sekretorisen gelsoliiniproteinin muodostumista. Solunsisäisellä gelsoliinilla on monia tehtäviä. Se säätelee solun aktiinitukirangan muodostusta ja osallistuu useisiin solutoimintoihin, kuten solun muodonmuutoksiin, liikkumiseen ja fagosytoosiin. Geenivirheestä johtuen gelsoliini pilkkoutuu epänormaalisti, jonka seurauksena sekretorisesta gelsoliinista on ajateltu muodostuvan säikeisiä amyloidikertymiä liki kaikkiin kudoksiin ja elimiin. Taudin patogeneesiä ei vielä täysin tunneta, mutta oletetaan, että amyloidikertymät ja niiden muodostumisprosessi aiheuttavat taudissa ilmenevät oireet.

AGel-amyloidoosipotilaat olivat kertoneet lääkärilleen suun alueen ongelmista, kuten kuivan suun tunteesta sekä heiluvista ja lohkeilevista hampaista. Tämä tieto toimi lähtökohtana tälle tutkimukselle, jonka tarkoituksena oli kliinisin, biokemiallisin, histologisin ja mikrobiologisin menetelmin selvittää tämän systeemisairauden vaikutusta potilaiden suun terveyden tilaan. Vastaavanlaista tutkimusta ei ole aiemmin tehty. Syljenerityksen mittaaminen, syljen proteiinikonsentraation määrittäminen sekä hampaiden kiinnityskudosten eli parodontiumin tilan kartoittaminen, kliinisesti ja mikrobiologisesti, olivat päätutkimuskohteita. Lisäksi tutkimme suun alueen sidekudossoluja ja verisuonten sileitä lihassoluja *in vitro* arvioidaksemme geenimutaation

vaikutusta solunsisäisen gelsoliinin toimintaan ja siten taudin patogeneesiin yleensä ja mahdollisten parodontaaliongelmien ilmenemiseen. Potilaat kutsuttiin tutkimukseen potilasjärjestön, Suomen Amyloidoosiyhdistys ry., kautta. Neljäkymmentä, ikäjakaumaltaan 39-79-vuotiasta, kyselykaavakkeen täyttänyttä vapaaehtoista osallistui tutkimukseen.

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 hiivatulehdukselle.

Pienten sylkirauhasten histopatologisissa tutkimuksissa havaittiin gelsoliiniamyloidikertymien lisäksi rauhasatrofiaa ja lieväasteista tulehdusta. Tämä uusi löydös voi osin selittää muutokset syljenerityksessä ja sen koostumuksessa. Aiemmin amyloidia on todettu myös potilaan parotis-rauhasessa. Yhden tutkimuspotilaan kohdalla sicca-oireet (kuivat silmät ja kuiva suu) sekä tulehdusmuutos pienten sylkirauhasten kudosnäytteessä olivat harhaanjohtaneet Sjögrenin syndrooma diagnoosiin. Myöhemmin potilaalla todettiin kuitenkin olevan ainoastaan AGel-amyloidoosi.

Tutkimustemme mukaan AGel-amyloidoosipotilailla ei keskimäärin ole kohonnutta riskiä parodontiitille saman ikäiseen suomalaiseen populaatioon verrattuna. Kahdella tutkimuspotilaalla todettiin kuitenkin varsin aggressiivisesti edennyt parodontiitti, johon heidän yleissairautensa on saattanut myötävaikuttaa. Sekä AGel-amyloidoosi että synnyltään ja etenemiseltään monitekijäinen parodontiitti kumuloituvat iän myötä. Myös tässä tutkimuksessa vanhemmalla potilasryhmällä oli enemmän syventyneitä ientaskuja ja menetettyjä hampaita, etenkin poskihampaita, kuin nuoremmalla ikäryhmällä. Potilaiden ientaskuissa oli lisäksi samoja bakteereja kuin kroonisessa parodontiitissa yleisesti.

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

jakauma, migraationopeus ja tyypin 1 kollageenin metabolia. Ainoastaan potilaiden sidekudossolujen reaktio proteiinikinaasi-inhibiittori staurosporiinille erosi verrokkisoluista, ja tulos vaatii jatkotutkimuksia. Saadut tulokset viittaavat siihen, että potilaiden suun sidekudossolut ja verisuonten sileälihassolut kykenevät suoriutumaan pääosin normaalisti edellä mainituista toiminnoista *in vitro* olosuhteissa. Kokeet eivät kuitenkaan poissulje mahdollisuutta, että geenimutaatio myötävaikuttaisi solunsisäisen gelsoliinin kautta taudin patogeneesiin.

Periytyvästä systeemisairaudestaan johtuen AGel-amyloidoosipotilailla vaikuttaa olevan hypolivaation ja alentuneen syljen IgA pitoisuuden myötä suurentunut riski suun alueen sairauksiin. Näin ollen potilaat hyötyvät erityisesti ehkäisevästä hammashoidosta kuten ruokavalioneuvonnasta, suuhygieniaohjeista, lisäfluorista, suuta kosteuttavista tuotteista, säännöllisistä hammastarkastuksista ja ennen kaikkea tehokkaasta suun omahoidosta. Suomenkielinen vuonna 2011 ilmestynyt potilasopas Meretojan taudista antaa tietoa sairauden kulusta ja sen hoidosta sekä potilaille että hoitohenkilökunnalle (www.suomenamyloidoosiyhdistys.fi).

AVAINSANAT: AGel amyloidoosi, suun terveys, hyposalivaatio, syljen IgA, pienet sylkirauhaset, parodontiiitti, sidekudossolut, sileälihassolut.

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ORIGINAL PUBLICATIONS I-IV

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I Juusela P, Tanskanen M, Nieminen A, Uitto VJ, Blåfield H, Kiuru-Enari S. Hereditary gelsolin amyloidosis mimicking Sjögren's syndrome.
Clinical Rheumatology 2009; 28(11): 1351-4.

II Juusela P, Tanskanen M, Nieminen A, Kari K, Suominen L, Uitto VJ, Kiuru-Enari S. Xerostomia in hereditary gelsolin amyloidosis.
Amyloid. 2013; 20(1): 39-44.

III Juusela P, Persson R, Nieminen A, Kiuru-Enari S, Uitto V-J.
Relation of gelsolin amyloidosis and periodontal health.
Clinical Oral Investigations 2015; 19(2): 229-35.

IV Juusela P*, Koskelainen S*, Nieminen A, Baumann M, Salo T, Risteli J, Uitto V-J, Kiuru-Enari S.
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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ABBREVIATIONS

AA	amyloid A
AApoAI	amyloid apolipoprotein AI
AApoAII	amyloid apolipoprotein AII
Aβ2M	amyloid β2 microglobulin
ABri	amyloid ABriPP
ACys	amyloid cystatin C
AFib	amyloid fibrinogen α
AGel	amyloid gelsolin
AIAPP	amyloid islet amyloid polypeptide
AL	amyloid light chain
ALECT	amyloid leukocyte chemotactic factor-2
ALys	amyloid lysozyme
APECED	autoimmune polyendocrinopathy candidiasis ectodermal dystrophy
Apo E	apolipoprotein E
ATTR	amyloid transthyretin
ATTRwt	amyloid transthyretin, wild type
AV	absorbance value
BOP	bleeding on probing
Ca^{2+}	calcium ion
CAL	clinical attachment level
cGSN	cytosolic gelsolin
CLD	corneal lattice dystrophy
DAPI	4,6-diamidino-2-phenylindole
DNA	deoxyribonucleic acid
DM	diabetes mellitus
EDTA	ethylenediaminetetraacetic acid
FAF	familial amyloidosis of the Finnish type

FAP	familial amyloid polyneuropathy
FBS	fetal bovine serum
FMF	familial Mediterranean fever
GI	gastrointestinal
GSN	gelsolin protein
GSN	gelsolin gene
H^+	hydrogen ion
HGA	hereditary gelsolin amyloidosis
HIV	human immunodeficiency virus
ICTP	carboxyterminal telopeptide of type I collagen
Ig	immunoglobulin
IL	interleukin
kDa	kilodalton
La(SSB)	Sjögren's syndrome antigen B, also called La
LSG	labial salivary gland
MMP	matrix metalloproteinase
mRNA	messenger ribonucleic acid
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PINP	aminoterminal propeptide of type I procollagen
PIP2	phosphatidylinositol bisphosphate
PPD	probing pocket depth
RIA	radioimmunoassay
Ro(SSA)	Sjögren's syndrome antigen A, also called Ro
RNA	ribonucleic acid
SAA	serum amyloid A apolipoprotein
SAP	serum amyloid P component
sGSN	secretory gelsolin
SMA	smooth muscle actin
SS	Sjögren's syndrome
SSA	senile systemic amyloidosis

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 promted us to investigate dental and periodontal problems of AGel amyloidosis patients.

We hypothetized 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 *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**).

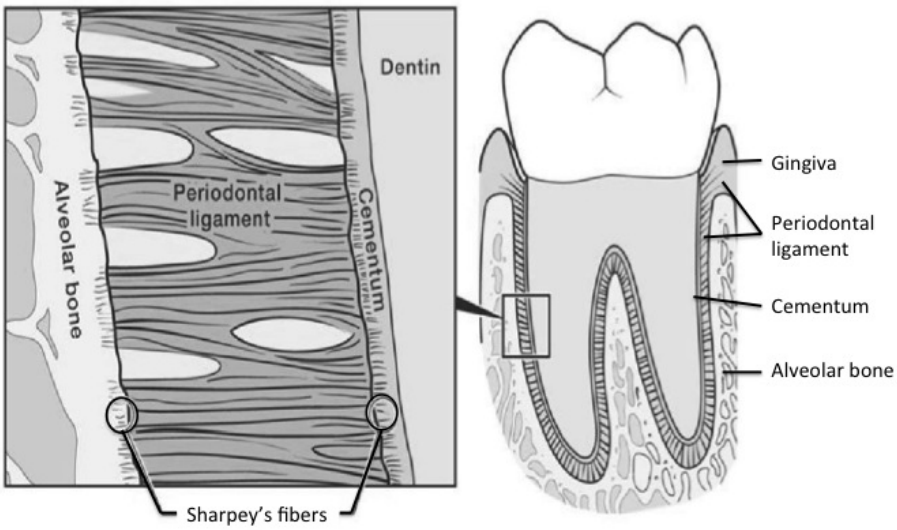

Figure 1. Structure of healthy periodontium.

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

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

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 attachement 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 wihich 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).

	Hyposalivation	Low	Normal
Unstimulated saliva (mL/min)	< 0.1	0.1-0.25	0.26-0.35
Stimulated saliva (mL/min)	< 0.7	0.7-1.0	1.1-3.0

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.

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

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 fibrillary 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 fibrillary 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 (Puchtler 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

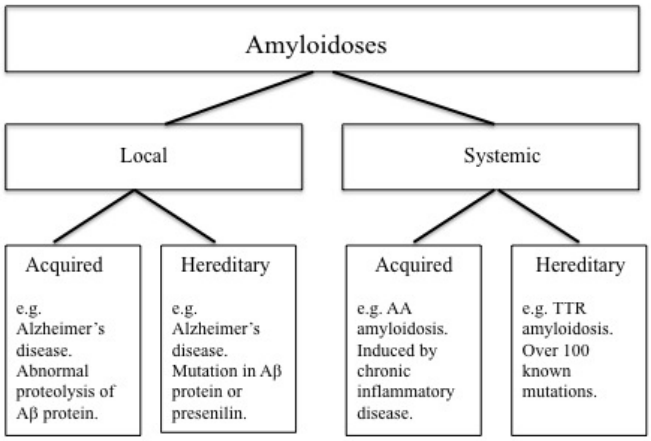

Figure 2. Categorization of the different types of amyloidoses with examples.

to be essential, but it is also known that the non-fibrillary 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.

Table 4. Systemic hereditary amyloidoses in humans.

Sarake1	Sarake2	Sarake3	Sarake4	Sarake5	Sarake6	Sarake8	Sarake9
Fibril protein	**Precursor protein**	**Origin of protein**	**Normal function**	**Number of known mutations**	**Main target organ/-s**	**Severity of the disease**	**Therapy**
AL	Immunoglobulin light chain	Clonal plasma cells	Role in humoral immuno response	1	All organs except CNS	Lethal	Stem cell transplantation, chemotherapy
ATTR	Transthyretin, variants	Liver	Transporter protein	>100	PNS, ANS, heart, eye, leptomen	Lethal	Liver transplantation
Ab2M	β2-Microglobulin, variant	All nucleated cells	Role in fibrillogenesis	1	GI symptoms, ANS	Slowly progressing	Not available
AApoAI	Apolipoprotein A I, variants	Liver, small intestine	Major constituent of HDL	15	Heart, liver, kidney, PNS, testis	Lethal	Liver transplantation
AApoAII	Apolipoprotein A II, variants	Liver, small intestine	Constituent of HDL	5	Kidney*	Slowly progressing	Kidney transplantation
AGel	Gelsolin, variants	All nucleated cells	Actin modulation and clearance	2	PNS, cornea, skin	Slowly progressing	Only symptom alleviating
ALys	Lysozyme, variants	Macrophages and PMNs	Bacteriolytic enzyme	7	Mainly kidney	Slowly progressing	Kidney transplantation
ALECT2	Leukocyte Chemotactic Factor-2	Mainly liver	No spesific function verified	not known**	Kidney, primarily	Progressive	Not available
AFib	Fibrinogen α, variants	Liver	Major role in blood coagulation	1	Kidney, primarily	Lethal	Liver (and kidney) transplantation
ACys	Cystatin C, variants	Hematopoietic cells	Cysteine protease inhibitor	1	PNS, skin	Lethal	Not available
ABri	ABriPP, variants	Coded from chromosome 13	Protein not normally found	1	CNS, primarily	Lethal	Not available

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.

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.

			Salivary gland biopsy findings			
	Amyloidosis	Sicca symptoms	Amyloid	Inflammation	Atrophy	Fibrosis
Acquired	AL	yes	yes	no	yes	yes*
	AA	yes	yes	no	yes	ND
Hereditary	ATTR	yes	yes	ND	ND	ND
	ALys	yes	-	-	-	-
	Aβ2M	yes	-	-	-	-
	AGel	ND	yes**	ND	ND	ND

*) only in one out of eight cases, **) an autopsy sample. ND: not determined. References:
AL amyloidosis (Kuczynski et al. 1971; Gogel et al. 1983; Yokota et al. 1984; Itoh et al. 1991; Myssiorek et al. 1992; Schima et al. 1994; Richey and Bennion 1996; Jardinet et al. 1998).
AA amyloidosis (Catalano and Vaughan 1980).
ATTR amyloidosis (Johansson et al. 1992).
ALys amyloidosis (Valleix et al. 2002; Granel et al. 2006; Girnius et al. 2012).
Aβ2M amyloidosis (Valleix et al. 2012).
AGel amyloidosis (Meretoja and Teppo 1971).

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 GELSOLIN (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 medicins 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.

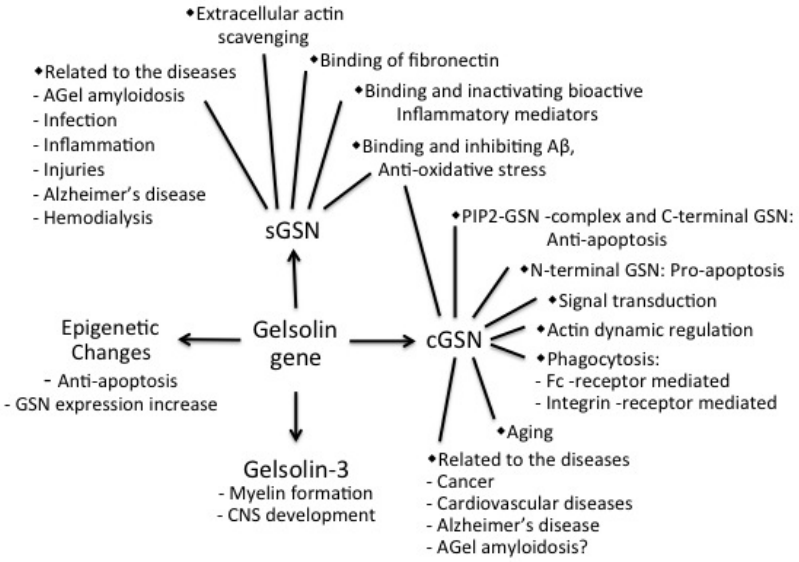

Figure 5. Multiple roles of gelsolin. Aβ: amyloid β protein, the major component of amyloid plaques in the brains of individuals with Alzheimer's disease. sGSN: secretory gelsolin, cGSN: cytosolic gelsolin, CNS: central nervous system, PIP2: phosphatidylinositol biphosphates. Adapted from Li et al. 2012.

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 (Janmey 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 endoplastic 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.

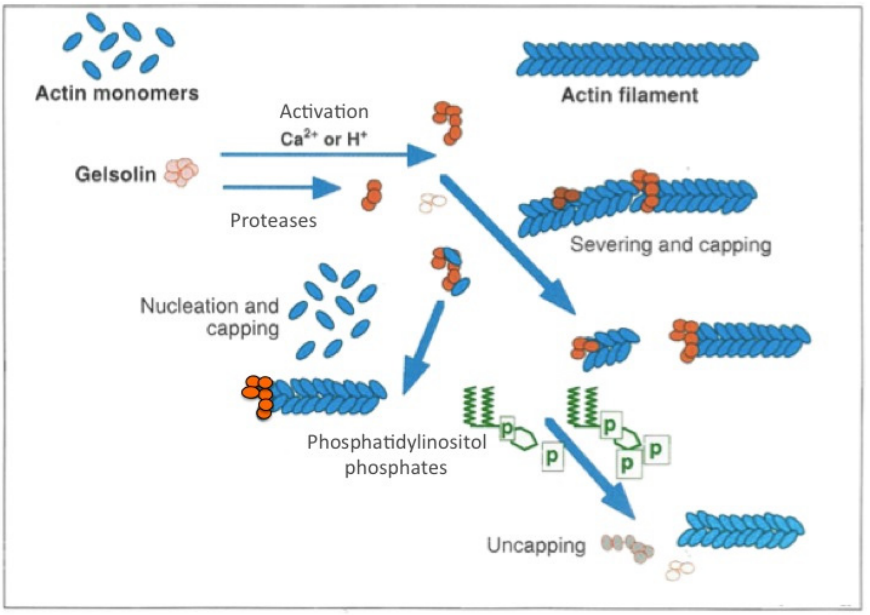

Figure 6. Gelsolin is activated by Ca^{2+} or H^{+} (pH<7) or by proteases (e.g. caspase 3, chymotrypsin). The proteases produce gelsolin, which is capable of interacting with both monomeric and polymeric actin. Phosphatidylinositol biphosphates (PIP2) expose the barbed end of actin by inactivating gelsolin, 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.

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

N: number of patients and controls, Age ± SD: mean age ± standard deviation in years, UWS: unstimulated whole saliva, SWS: stimulated whole saliva, LSG: labial salivary gland, VSMC: vascular smooth muscle cell. *) Controls from the Finnish Health 2000 Survey (Suominen-Taipale et al. 2004).

4.2. QUESTIONNAIRE

Patients filled in a questionnaire that assessed their general diseases, medications, onset of AGel amyloidosis, smoking habits and possible oral problems. In addition, questions about oral hygiene habits were posed, and patients were asked whether they felt that AGel 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 $\geq$ 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^{o}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.

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

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% CO_2 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% CO_2. 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 (≥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, ≥ 0.1 mL/min and ≥ 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.

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

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 mm^2) 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 (**I**).

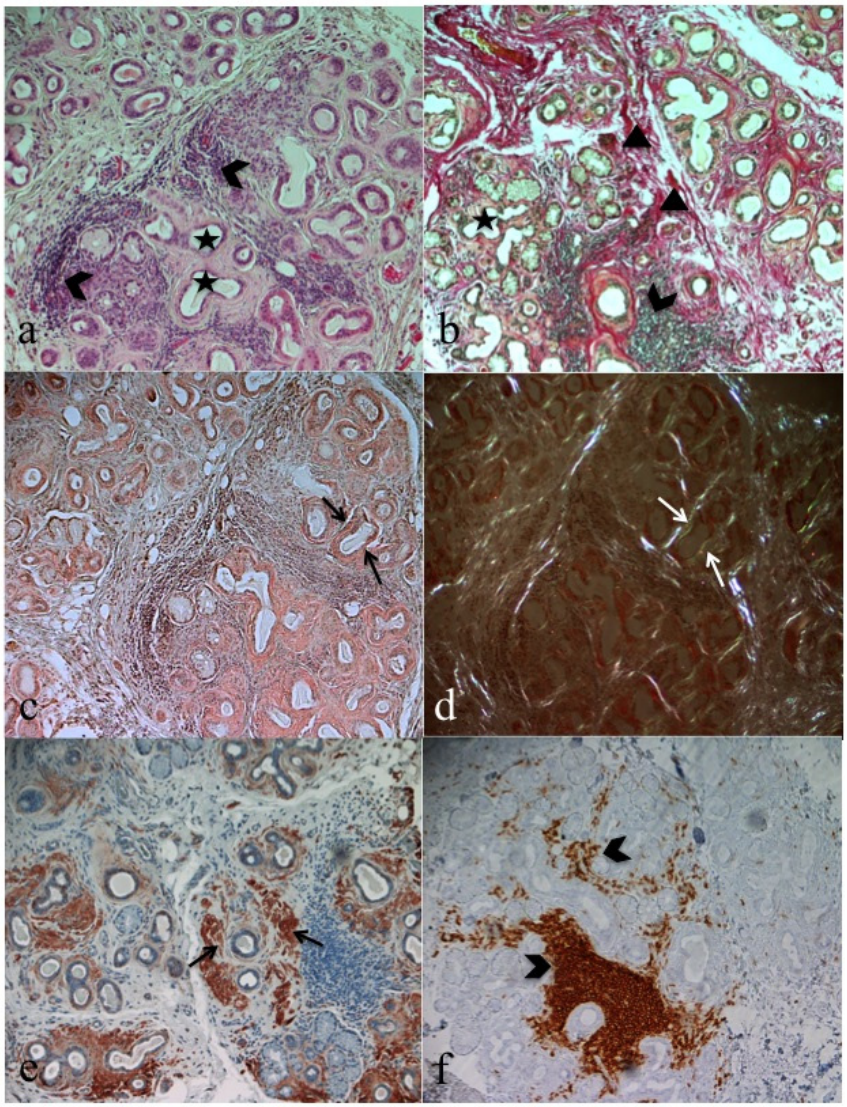

Figure 7. Atrophy, inflammation and deposition of amyloid in labial small salivary glands of a patient with AGel amyloidosis. Stainings are as follows: haematoxylin eosin (a), Herovici (b), Congo red (c) with polarization (d), immunohistochemical staining against AGel subunit (e), and against leucocyte 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.

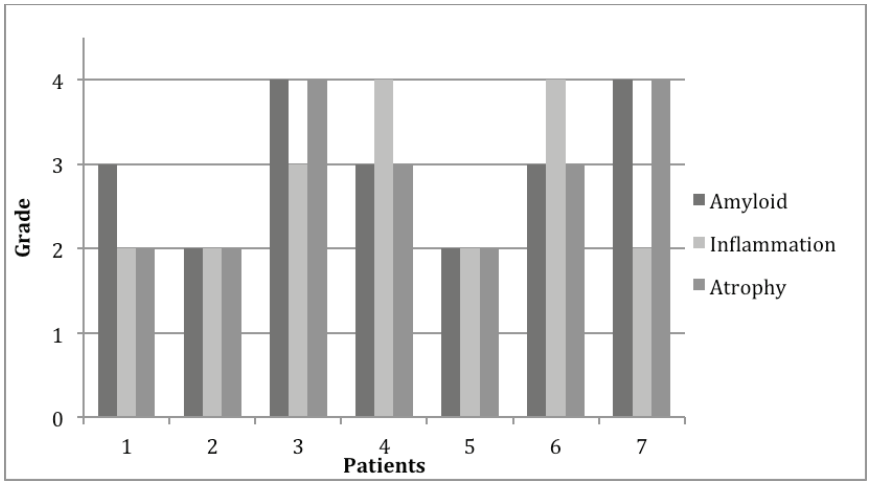

Figure 8. Grade (0 – 4) of amyloid deposition, inflammation, and atrophy of the labial salivary glands in the biopsy specimens from seven AGel amyloidosis patients in increasing age from 40 years (1) to 72 years (7). Grading system presented in **Table 7**, p.55.

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 $\geq 1x10^7$. Further, 51 samples from 24 patients studied with the DNA-DNA checkerboard method showed that, when using $\geq 1 \times 10^5$ 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 $\geq$ 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.

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

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.

Bacterial species	**Reference Strain**	**≥1 x 10^5**
Actinomyces israelii	ATCC 12102	80.87
Actinomyces naeslundii	ATTC 43146	73.1
Actinomyces odontolyticus	ATTC 17929	62.3
Aggregatibacter actinomycetemcomitans (Y4)	ATCC 29523	75.0
Aggregatibacter actinomycetemcomitans (a)	ATCC 43718	53.8
Campylobacter gracilis	ATTC 33236	57.8
Campylobacter rectus	ATTC 33238	78.8
Campylobacter showae	ATTC 51146	28.8
Capnocytophaga gingivalis	ATTC 33612	36.5
Capnocytophaga ochracea	ATTC 33596	73.1
Capnocytophaga sputigena	ATTC 33612	86.5
Eikenella corrodens	ATTC 23834	50.0
Eubacterium saburreum	ATTC 33271	65.4
Fusobacterium nucleatum sp.nucleatum	ATTC 25586	96.2
Fusobacterium nucleatum sp.polymorphum	ATTC 10953	78.8
Fusobacterium nucleatum sp.vincentii	ATTC 49256	88.8
Fusobacterium periodonticum	ATTC 33693	96.2
Lactobacillus acidophilus	ATTC 11975	69.2
Neisseria mucosa	ATTC 19696	92.3
Porphyromonas gingivalis	ATTC 33277	44.2*
Prevotella intermedia	ATTC 25611	64.4
Prevotella melaninogenica	ATTC 25845	88.5
Prevotella nigrescens	ATTC 33563	73.1
Propionybacterium acnes	ATTC 11827/11728	40.4
Selenomonas noxia	ATTC 43541	61.5
Staphylococcus aureus	ATTC 25923	61.5
Streptococcus anginosus	ATTC 33397	67.3
Streptococcus anginosus	ATTC 27823(M32b)	17.3
Streptococcus gordonii	ATTC 10558	76.9
Streptococcus intermedius	ATTC 27335	62.3
Streptococcus mitis	ATCC 49456	40.4
Streptococcus mutans	ATCC 35037	46.2
Streptococcus oralis	ATTC 10556	32.7
Streptococcus sanguinis	ATTC 25175	34.6
Tannerella forsythia	ATTC 43037	88.5*
Treponena denticola	Boston B1	50.0*
Treponema socranskii	ATTC D40DR2	86.5
Veilionella parvula	ATTC10790	100.0

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 attachement 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.

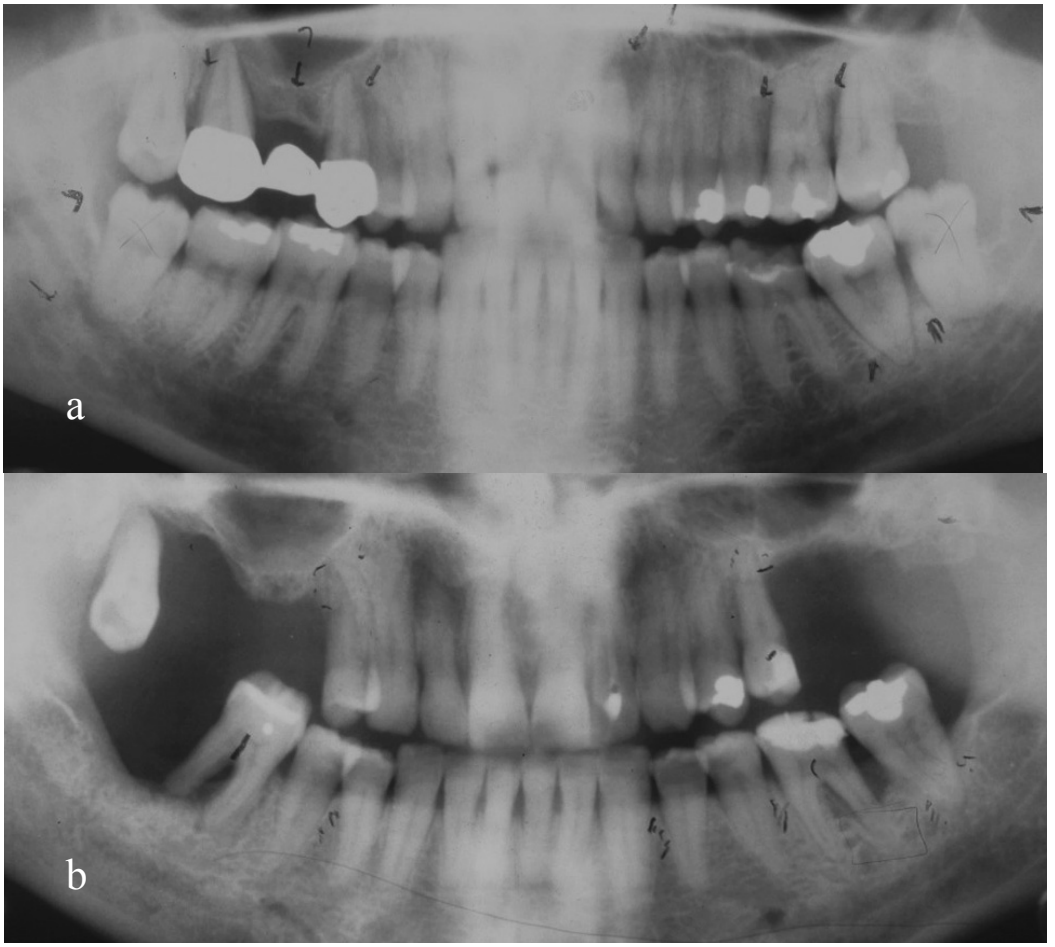

Figure 10. Case 2. a) Panoramic tomography at the age of 44 years. **b)** Panoramic tomography 11 years later, at the age of 55 years.

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**).

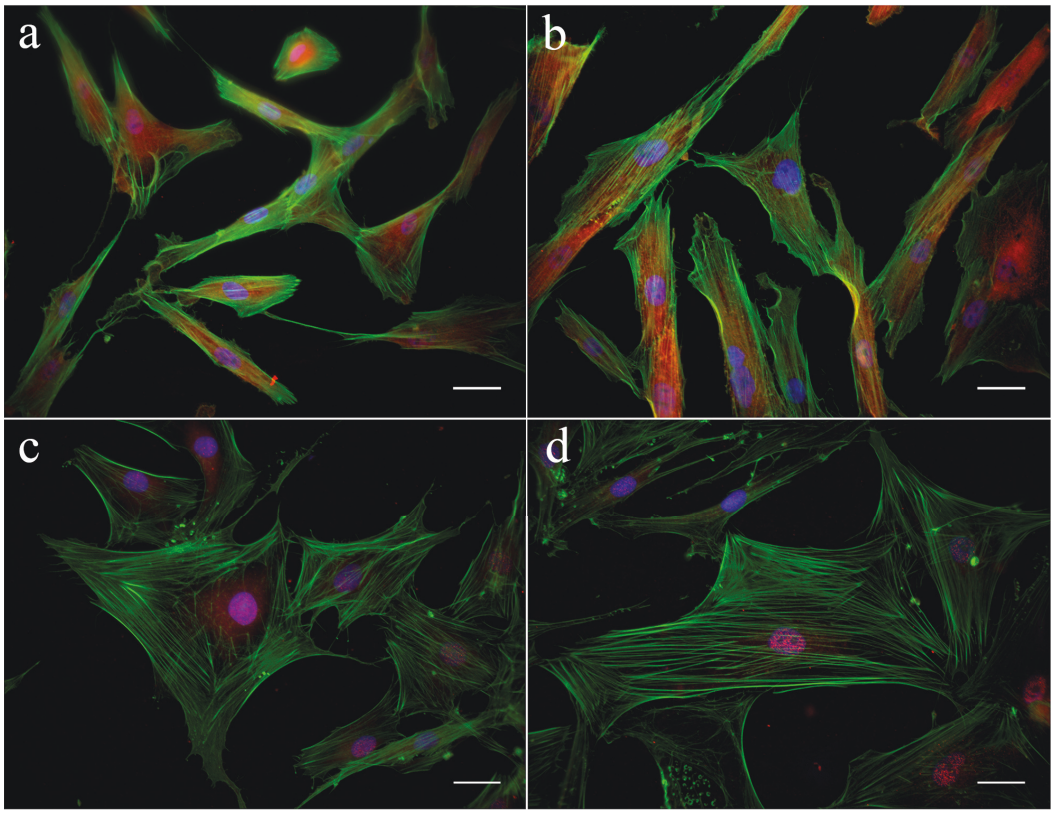

Figure 11a-d. Fluorescent stainings of fibroblast control (a) and patient (b) and VSMC control (c) and patient (d) cells. Cells were double-stained for phalloidin staining actin (green) and GSN (red). Nuclei were visualized with DAPI (blue). Magnification x 400. Scale bars: 20 µm.

Table 11. Values from cell study experiments performed on patient and control cell lines.

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

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 hyposecretion 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 hyposecretion 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.

6.4. ORAL FIBROBLASTS AND VASCULAR SMOOTH MUSCLE CELLS *IN VITRO*

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 *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.

7. CONCLUSIONS

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.

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REFERENCES

Abnet CC, Kamangar F, Dawsey SM, Stolzenberg-Solomon RZ, Albanes D, Pietinen P, Virtamo J, Taylor PR (2005). Tooth loss is associated with increased risk of gastric non-cardia adenocarcinoma in a cohort of Finnish smokers. Scand J Gastroenterol 40:681-687.

Abrahamson M, Grubb A, Olafsson I, Lundwall A (1987). Molecular cloning and sequence analysis of cDNA coding for the precursor of the human cysteine proteinase inhibitor cystatin C. FEBS Lett 216:229-233.

Alabdali M, Barnett C, Abraham A, Jaafari DA, Bril V (2015). Gelsolin Familial Amyloidosis Peripheral Neuropathy in Canada: A Case Report. Can J Neurol Sci 42:1-3.

Alamanos Y, Tsifetaki N, Voulgari PV, Venetsanopoulou AI, Siozos C, Drosos AA (2006). Epidemiology of primary Sjogren's syndrome in north-west Greece, 1982-2003. Rheumatology 45:187-191.

Albandar JM (2002). Global risk factors and risk indicators for periodontal diseases. Periodontol 2000 29:177-206.

Anan I, Kiuru-Enari S, Obayashi K, Ranlov PJ, Ando Y (2010). Investigation of AGE, their receptor and NF-kappaB activation and apoptosis in patients with ATTR and Gelsolin amyloidosis. Histol Histopathol 25:691-699.

Andrade C (1952). A peculiar form of peripheral neuropathy; familiar atypical generalized amyloidosis with special involvement of the peripheral nerves. Brain 75:408-427.

Angiero F, Seramondi R, Magistro S, Crippa R, Benedicenti S, Rizzardi C, Cattoretti G (2010). Amyloid deposition in the tongue: clinical and histopathological profile. Anticancer Res 30:3009-3014.

Ardalan MR, Shoja MM, Kiuru-Enari S (2007). Amyloidosis-related nephrotic syndrome due to a G654A gelsolin mutation: the first report from the Middle East. Nephrol Dial Transplant 22:272-275.

Ardila CM, Guzman IC (2015). Comparison of serum amyloid A protein and C-reactive protein levels as inflammatory markers in periodontitis. J Periodontal Implant Sci 45:14-22.

Armitage GC (1999). Development of a classification system for periodontal diseases and conditions. Ann Periodontol 4:1-6.

Arora PD, McCulloch CA (1996). Dependence of fibroblast migration on actin severing activity of gelsolin. J Biol Chem 271:20516-20523.

Arora PD, Silvestri L, Ganss B, Sodek J, McCulloch CA (2001). Mechanism of cyclosporin-induced inhibition of intracellular collagen degradation. J Biol Chem 276:14100-14109.

Arora PD, Glogauer M, Kapus A, Kwiatkowski DJ, McCulloch CA (2004). Gelsolin mediates collagen phagocytosis through a rac-dependent step. Mol Biol Cell 15:588-599.

Arora PD, Chan MW, Anderson RA, Janmey PA, McCulloch CA (2005). Separate functions of gelsolin mediate sequential steps of collagen phagocytosis. Mol Biol Cell 16:5175-5190.

Arora PD, Wang Y, Bresnick A, Dawson J, Janmey PA, McCulloch CA (2013). Collagen remodeling by phagocytosis is determined by collagen substrate topology and calcium-dependent interactions of gelsolin with nonmuscle myosin IIA in cell adhesions. Mol Biol Cell 24:734-747.

Azuma T, Witke W, Stossel TP, Hartwig JH, Kwiatkowski DJ (1998). Gelsolin is a downstream effector of rac for fibroblast motility. EMBO J 17:1362-1370.

Ben-Aryeh H, Serouya R, Kanter Y, Szargel R, Laufer D (1993). Oral health and salivary composition in diabetic patients. J Diabetes Complications 7:57-62.

Benson MD, Liepnieks J, Uemichi T, Wheeler G, Correa R (1993). Hereditary renal amyloidosis associated with a mutant fibrinogen alpha-chain. Nat Genet 3:252-255.

Benson MD, Liepnieks JJ, Yazaki M, Yamashita T, Hamidi Asl K, Guenther B, Kluve-Beckerman B (2001). A new human hereditary amyloidosis: the result of a stop-codon mutation in the apolipoprotein AII gene. Genomics 72:272-277.

Benson MD, James S, Scott K, Liepnieks JJ, Kluve-Beckerman B (2008). Leukocyte chemotactic factor 2: A novel renal amyloid protein. Kidney Int 74:218-222.

Benson MD (2012a). The Hereditary Amyloidoses. In: Picken MM, Dogan A, Herrera GA (eds) Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 53-68.

Benson MD (2012b). Amyloidosis of the Gastrointestinal Tract and Liver. In: Picken MM, Dogan A, Herrera GA (eds) Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 339-360.

Benson MD, Liepnieks JJ, Kluve-Beckerman B (2015). Hereditary systemic immunoglobulin light-chain amyloidosis. Blood 21:3281-6.

Bergström J (1989). Cigarette smoking as risk factor in chronic periodontal disease. Community Dent Oral Epidemiol 17:245-247.

Bergström J (2004). Tobacco smoking and chronic destructive periodontal disease. Odontology 92:1-8.

Biancalana M, Koide S (2010). Molecular mechanism of Thioflavin-T binding to amyloid fibrils. Biochim Biophys Acta 1804:1405-1412.

Blinder D, Yahatom R, Taicher S (1997). Oral manifestations of sarcoidosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 83:458-461.

Bodin K, Ellmerich S, Kahan MC, Tennent GA, Loesch A, Gilbertson JA, Hutchinson WL, Mangione PP, Gallimore JR, Millar DJ, Minogue S, Dhillon AP, Taylor GW, Bradwell AR, Petrie A, Gillmore JD, Bellotti V, Botto M, Hawkins PN, Pepys MB (2010). Antibodies to human serum amyloid P component eliminate visceral amyloid deposits. Nature 468:93-97.

Botto M, Hawkins PN, Bickerstaff MC, Herbert J, Bygrave AE, McBride A, Hutchinson WL, Tennent GA, Walport MJ, Pepys MB (1997). Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene. Nat Med 3:855-859.

Bowman SJ, Ibrahim GH, Holmes G, Hamburger J, Ainsworth JR (2004). Estimating the prevalence among Caucasian women of primary Sjogren's syndrome in two general practices in Birmingham, UK. Scand J Rheumatol 33:39-43.

Brandtzaeg P (1989). Salivary immunoglobulins. In: Tenovuo JO (ed) Human Saliva: clinical chemistry and microbiology, 2th edn. CRC Press, pp. 2-54.

Bratthall D, Widerstrom L (1985). Ups and downs for salivary IgA. Scand J Dent Res 93:128-134.

Brewer HB,Jr, Rader DJ (1991). HDL: structure, function and metabolism. Prog Lipid Res 30:139-144.

Brito F, Zaltman C, Carvalho AT, Fischer RG, Persson R, Gustafsson A, Figueredo CM (2013). Subgingival microflora in inflammatory bowel disease patients with untreated periodontitis. Eur J Gastroenterol Hepatol 25:239-245.

Buhlin K, Mantyla P, Paju S, Peltola JS, Nieminen MS, Sinisalo J, Pussinen PJ (2011). Periodontitis is associated with angiographically verified coronary artery disease. J Clin Periodontol 38:1007-1014.

Carranza FA, Camargo PM (2012). The Periodontal Pocket. In: Newman MG, Takei HH, Carranza FA (eds) Carranza's clinical periodontology, 11th ed. Elsevier Saunders, pp. 127-139.

Carrwik C, Stenevi U (2009). Lattice corneal dystrophy, gelsolin type (Meretoja's syndrome). Acta Ophthalmol 87:813-819.

Catalano MA, Vaughan JH (1980). Secondary amyloidosis and sicca syndrome. Arthritis Rheum 23:1067.

Cengiz MI, Bagci H, Cengiz S, Yigit S, Cengiz K (2009). Periodontal disease in patients with familial Mediterranean fever: from inflammation to amyloidosis. J Periodontal Res 44:354-361.

Cengiz MI, Wang HL, Yildiz L (2010). Oral involvement in a case of AA amyloidosis: a case report. J Med Case Rep 4:200.

Chan MW, Arora PD, McCulloch CA (2007). Cyclosporin inhibition of collagen remodeling is mediated by gelsolin. Am J Physiol Cell Physiol 293:C1049-58.

Chastan N, Baert-Desurmont S, Saugier-Veber P, Derumeaux G, Cabot A, Frebourg T, Hannequin D (2006). Cardiac conduction alterations in a French family with amyloidosis of the Finnish type with the p.Asp187Tyr mutation in the GSN gene. Muscle Nerve 33:113-119.

Cheng L, Cheng X, Deng M, Deng X, Du Q, Ge Y, Guo Q, He J, Jia W, Kang D, Kuang Y, Li C, Li M, Li Y, Li Y, Liu C, Liu X, Lu C, Peng X, Qiu W, Sun Y, Wang H, Wang S, Wang Y, Wei Y, Xiao L, Xiao X, Xu X, Yang K, Zeng J, Zhang K, Zhang W, Zheng X, Zhou H, Zhou S, Zhou X, Zhou Y, Zhu Z (2015). Oral mucosal microbes. In: Zhou X, Li Y (eds) Atlas of Oral Microbiology; from health microflora to diseases, Academic Press, Oxford, pp. 95-107.

Chi AC, Neville BW, Krayer JW, Gonsalves WC (2010). Oral manifestations of systemic disease. Am Fam Physician 82:1381-1388.

Choi BK, Paster BJ, Dewhirst FE, Gobel UB (1994). Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis. Infect Immun 62:1889-1895.

Cobb AR, Boyapati R, Walker DM, Dunaway DJ, Lloyd TW (2013). The surgical management of severe macroglossia in systemic AL amyloidosis. Br J Oral Maxillofac Surg 51:e72-4.

Cohen AS, Calkins E (1959). Electron microscopic observations on a fibrous component in amyloid of diverse origins. Nature 183:1202-1203.

Dawes C (1972). Circadian rhythms in human salivary flow rate and composition. J Physiol 220:529-545

Dawes C (2008). Salivary flow patterns and the health of hard and soft oral tissues. J Am Dent Assoc 139 Suppl:18S-24S.

De Corte V, Bruyneel E, Boucherie C, Mareel M, Vandekerckhove J, Gettemans J (2002). Gelsolin-induced epithelial cell invasion is dependent on Ras-Rac signaling. EMBO J 21:6781-6790.

de la Chapelle A, Kere J, Sack GH Jr, Tolvanen R, Maury CP (1992a). Familial amyloidosis, Finnish type: G654----a mutation of the gelsolin gene in Finnish families and an unrelated American family. Genomics 13:898-901.

de la Chapelle A, Tolvanen R, Boysen G, Santavy J, Bleeker-Wagemakers L, Maury CP, Kere J (1992b). Gelsolin-derived familial amyloidosis caused by asparagine or tyrosine substitution for aspartic acid at residue 187. Nat Genet 2:157-160.

Delevaux I, Andre M, Amoura Z, Kemeny JL, Piette JC, Aumaitre O (2001) Concomitant diagnosis of primary Sjogren's syndrome and systemic AL amyloidosis. Ann Rheum Dis 60:694-695.

Dentino A, Lee S, Mailhot J, Hefti AF (2013). Principles of periodontology. Periodontol 2000 61:16-53.

Dimitriou L, Sharp NC, Doherty M (2002). Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers. Br J Sports Med 36:260-264.

Divry P (1927). Etude histo-chimique des plaques seniles. J de Neurologie et de Psychiatrie 27:643-57.

Do Amaral B, Coelho T, Sousa A, Guimaraes A (2009). Usefulness of labial salivary gland biopsy in familial amyloid polyneuropathy Portuguese type. Amyloid 16:232-238.

Dogan A (2012). Classification of Amyloidosis by Mass Spectrometry-Based Proteomics. In: Picken MM, Dogan A, Herrera GA (eds) Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 261-272.

Efebera YA, Sturm A, Baack EC, Hofmeister CC, Satoskar A, Nadasdy T, Nadasdy G, Benson DM, Gillmore JD, Hawkins PN, Rowczenio D (2014). Novel gelsolin variant as the cause of nephrotic syndrome and renal amyloidosis in a large kindred. Amyloid 21:110-112.

Eke PI, Dye BA, Wei L, Thornton-Evans GO, Genco RJ, CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina, Chapel Hill, USA), Gordon Douglass (Past President, American Academy of Periodontology), Roy Page (University of Washin (2012). Prevalence of periodontitis in adults in the United States: 2009 and 2010. J Dent Res 91:914-920.

Eliasson L, Birkhed D, Osterberg T, Carlen A (2006). Minor salivary gland secretion rates and immunoglobulin A in adults and the elderly. Eur J Oral Sci 114:494-499.

Eliasson L, Birkhed D, Carlen A (2009). Feeling of dry mouth in relation to whole and minor gland saliva secretion rate. Arch Oral Biol 54:263-267.

Ericsson Y, Hardwick L (1978). Individual diagnosis, prognosis and counselling for caries prevention. Caries Res 12 Suppl 1:94-102.

Ferguson DB (1999). The flow rate and composition of human labial gland saliva. Arch Oral Biol 44 Suppl 1:S11-4.

Fiorellini JP, Kao DWK, Kim DM, Uzel NG (2012). The Normal Periodontium. In: Newman MG, Takei HH, Carranza FA (eds) Carranza's clinical periodontology, 11th ed. edn. Elsevier Saunders, pp. 11-32

Fock U, Jockusch BM, Schubert WD, Hinssen H (2005). Topological assignment of the N-terminal extension of plasma gelsolin to the gelsolin surface. Biochem J 385:659-665.

Fonseca R, Ahmann GJ, Jalal SM, Dewald GW, Larson DR, Therneau TM, Gertz MA, Kyle RA, Greipp PR (1998). Chromosomal abnormalities in systemic amyloidosis. Br J Haematol 103:704-710.

Fox RI, Howell FV, Bone RC, Michelson P (1984). Primary Sjogren syndrome: clinical and immunopathologic features. Semin Arthritis Rheum 14:77-105.

Friedewald VE, Kornman KS, Beck JD, Genco R, Goldfine A, Libby P, Offenbacher S, Ridker PM, Van Dyke TE, Roberts WC, American Journal of Cardiology, Journal of Periodontology (2009). The American Journal of Cardiology and Journal of Periodontology editors' consensus: periodontitis and atherosclerotic cardiovascular disease. J Periodontol 80:1021-1032.

Fuchs A, Jagirdar J, Schwartz IS (1987). Beta 2-microglobulin amyloidosis (AB2M) in patients undergoing long-term hemodialysis. A new type of amyloid. Am J Clin Pathol 88:302-307.

Gertz MA, Kyle RA (1991) Secondary systemic amyloidosis: response and survival in 64 patients. Medicine (Baltimore) 70:246-256.

Gertz MA, Kyle RA (1996). Myopathy in primary systemic amyloidosis. J Neurol Neurosurg Psychiatry 60:655-660.

Ghiso J, Pons-Estel B, Frangione B (1986). Hereditary cerebral amyloid angiopathy: the amyloid fibrils contain a protein which is a variant of cystatin C, an inhibitor of lysosomal cysteine proteases. Biochem Biophys Res Commun 136:548-554.

Ghiso JA, Holton J, Miravalle L, Calero M, Lashley T, Vidal R, Houlden H, Wood N, Neubert TA, Rostagno A, Plant G, Revesz T, Frangione B (2001). Systemic amyloid deposits in familial British dementia. J Biol Chem 276:43909-43914.

Gillmore JD, Stangou AJ, Lachmann HJ, Goodman HJ, Wechalekar AD, Acheson J, Tennent GA, Bybee A, Gilbertson J, Rowczenio D, O'Grady J, Heaton ND, Pepys MB, Hawkins PN (2006). Organ transplantation in hereditary apolipoprotein AI amyloidosis. Am J Transplant 6:2342-2347.

Gillmore JD, Lachmann HJ, Rowczenio D, Gilbertson JA, Zeng CH, Liu ZH, Li LS, Wechalekar A, Hawkins PN (2009). Diagnosis, pathogenesis, treatment, and prognosis of hereditary fibrinogen A alpha-chain amyloidosis. J Am Soc Nephrol 20:444-451.

Girnius S, Skinner M, Spencer B, Prokaeva T, Bartholomew C, O'Hara C, Seldin DC, Connors LH (2012). A new lysozyme tyr54asn mutation causing amyloidosis in a family of Swedish ancestry with gastrointestinal symptoms. Amyloid 19:182-185.

Glenner GG, Terry W, Harada M, Isersky C, Page D (1971). Amyloid fibril proteins: proof of homology with immunoglobulin light chains by sequence analyses. Science 172:1150-1151.

Gogel HK, Searles RP, Volpicelli NA, Cornwell GG 3rd (1983). Primary amyloidosis presenting as Sjogren's syndrome. Arch Intern Med 143:2325-2326.

Gonzalez-Rodriguez J, Ramirez-Miranda A, Hernandez-Da Mota SE, Zenteno JC (2014). TGFBI, CHST6, and GSN gene analysis in Mexican patients with stromal corneal dystrophies. Graefes Arch Clin Exp Ophthalmol 252:1267-1272.

Granel B, Valleix S, Serratrice J, Cherin P, Texeira A, Disdier P, Weiller PJ, Grateau G (2006). Lysozyme amyloidosis: report of 4 cases and a review of the literature. Medicine (Baltimore) 85:66-73.

Gudmundsson G, Hallgrimsson J, Jonasson TA, Bjarnason O (1972). Hereditary cerebral haemorrhage with amyloidosis. Brain 95:387-404.

Haltia M, Ghiso J, Prelli F, Gallo G, Kiuru S, Somer H, Palo J, Frangione B (1990a). Amyloid in familial amyloidosis, Finnish type, is antigenically and structurally related to gelsolin. Am J Pathol 136:1223-1228.

Haltia M, Prelli F, Ghiso J, Kiuru S, Somer H, Palo J, Frangione B (1990b). Amyloid protein in familial amyloidosis (Finnish type) is homologous to gelsolin, an actin-binding protein. Biochem Biophys Res Commun 167:927-932.

Hamidi Asl K, Liepnieks JJ, Nakamura M, Parker F, Benson MD (1999). A novel apolipoprotein A-1 variant, Arg173Pro, associated with cardiac and cutaneous amyloidosis. Biochem Biophys Res Commun 257:584-588.

Hawkins PN, Lavender JP, Pepys MB (1990). Evaluation of systemic amyloidosis by scintigraphy with 123I-labeled serum amyloid P component. N Engl J Med 323:508-513.

Hawkins PN (1997). Diagnosis and treatment of amyloidosis. Ann Rheum Dis 56:631-633.

Hayman SR, Bailey RJ, Jalal SM, Ahmann GJ, Dispenzieri A, Gertz MA, Greipp PR, Kyle RA, Lacy MQ, Rajkumar SV, Witzig TE, Lust JA, Fonseca R (2001). Translocations involving the immunoglobulin heavy-chain locus are possible early genetic events in patients with primary systemic amyloidosis. Blood 98:2266-2268.

Heimonen A, Janket SJ, Kaaja R, Ackerson LK, Muthukrishnan P, Meurman JH (2009). Oral inflammatory burden and preterm birth. J Periodontol 80:884-891.

Helenius LM, Meurman JH, Helenius I, Kari K, Hietanen J, Suuronen R, Hallikainen D, Kautiainen H, Leirisalo-Repo M, Lindqvist C (2005). Oral and salivary parameters in patients with rheumatic diseases. Acta Odontol Scand 63:284-293.

Hellquist HB, Skalova A (2014). Histology. In: Hellquist HB, Skalova A (eds). Histopathology of the salivary glands, Springer, pp. 1-20.

Hinrichs JE, Novak MJ (2012). Classification and epidemiology of periodontal diseases. In: Newman MG, Takei HH, Carranza FA (eds). Carranza's clinical periodontology, 11th ed. edn. Elsevier Saunders, pp. 33-65.

Hintner H, Breathnach SM (1988). Amyloid P components in normal human skin and skin with lesions. Hautarzt 39:712-716.

Holmstrup P, Westergaard J (1994). Periodontal diseases in HIV-infected patients. J Clin Periodontol 21:270-280.

Huff ME, Page LJ, Balch WE, Kelly JW (2003). Gelsolin domain 2 Ca^{2+} affinity determines susceptibility to furin proteolysis and familial amyloidosis of finnish type. J Mol Biol 334:119-127.

Hugoson A, Norderyd O, Slotte C, Thorstensson H (1998). Distribution of periodontal disease in a Swedish adult population 1973, 1983 and 1993. J Clin Periodontol 25:542-548.

Husebye ES, Perheentupa J, Rautemaa R, Kampe O (2009). Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med 265:514-529.

Isaacson RL, Weeds AG, Fersht AR (1999). Equilibria and kinetics of folding of gelsolin domain 2 and mutants involved in familial amyloidosis-Finnish type. Proc Natl Acad Sci USA 96:11247-11252.

Itoh T, Ohashi S, Tsujino T, Takenaka M, Kodama H, Kishihara M, Inatome T, Inoh T (1991). Primary amyloidosis with dry eyes and dry mouth--a case report. Jpn J Med 30:597-599.

Janmey PA, Stossel TP (1987). Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. Nature 325:362-364.

Janmey PA, Stossel TP, Allen PG (1998). Deconstructing gelsolin: identifying sites that mimic or alter binding to actin and phosphoinositides. Chem Biol 5:R81-5.

Jardinet D, Westhovens R, Peeters J (1998). Sicca syndrome as an initial symptom of amyloidosis. Clin Rheumatol 17:546-548.

Jean E, Ebbo M, Valleix S, Benarous L, Heyries L, Grados A, Bernit E, Grateau G, Papo T, Granel B, Daniel L, Harle JR, Schleinitz N (2014). A new family with hereditary lysozyme amyloidosis

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

Jensson O, Gudmundsson G, Arnason A, Blondal H, Petursdottir I, Thorsteinsson L, Grubb A, Lofberg H, Cohen D, Frangione B (1987). Hereditary cystatin C (gamma-trace) amyloid angiopathy of the CNS causing cerebral hemorrhage. Acta Neurol Scand 76:102-114.

Johansson I, Ryberg M, Steen L, Wigren L (1992). Salivary hypofunction in patients with familial amyloidotic polyneuropathy. Oral Surg Oral Med Oral Pathol 74:742-748.

Kangas H, Paunio T, Kalkkinen N, Jalanko A, Peltonen L (1996). In vitro expression analysis shows that the secretory form of gelsolin is the sole source of amyloid in gelsolin-related amyloidosis. Hum Mol Genet 5:1237-1243.

Kangas H, Ulmanen I, Paunio T, Kwiatkowski DJ, Lehtovirta M, Jalanko A, Peltonen L (1999). Functional consequences of amyloidosis mutation for gelsolin polypeptide -- analysis of gelsolin-actin interaction and gelsolin processing in gelsolin knock-out fibroblasts. FEBS Lett 454:233-239.

Kataoka M, Shimizu Y, Kunikiyo K, Asahara Y, Yamashita K, Ninomiya M, Morisaki I, Ohsaki Y, Kido JI, Nagata T (2000). Cyclosporin A decreases the degradation of type I collagen in rat gingival overgrowth. J Cell Physiol 182:351-358.

Katsoulis J, Heitz-Mayfield LJ, Weibel M, Hirschi R, Lang NP, Persson GR (2005). Impact of sample storage on detection of periodontal bacteria. Oral Microbiol Immunol 20:128-130.

Khalighi MA, Yue A, Hwang MT, Wallace WD (2013). Leukocyte chemotactic factor 2 (LECT2) amyloidosis presenting as pulmonary-renal syndrome: a case report and review of the literature. Clin Kidney J 6:618-621.

Khoury S, Dusek JJ, Anderson GB, Vigneswaran N (2004). Systemic amyloidosis manifesting as localized, severe periodontitis. J Am Dent Assoc 135:617-623.

Kilian M, Bratthall D (1999). Caries Immunology. In: Thylstrup A, Fejerskov O (eds). Textbook of Clinical Cariology, 2th edn, Munksgaard, pp. 355-366.

Kinane DF, Berglundh T, Lindhe J (2003). Host-Parasite Interactions in Periodontal Diseases. In: Lindhe J, Karring T, Lang NP (eds). Clinical Periodontology and Implant Dentistry, 4th edn, Blackwell Munksgaard, pp. 150-178.

Kiuru S (1992). Familial amyloidosis of the Finnish type (FAF). A clinical study of 30 patients. Acta Neurol Scand 86:346-353.

Kiuru S, Seppäläinen AM (1994). Neuropathy in familial amyloidosis, Finnish type (FAF): electrophysiological studies. Muscle Nerve 17:299-304.

Kiuru S (1995). Familial amyloidosis, Finnish type (FAF). Clinical, histological and amyloid protein studies. Dissertation, University of Helsinki, Department of Neurology.

Kiuru S (1998). Gelsolin-related familial amyloidosis, Finnish type (FAF), and its variants found worldwide. Amyloid 5:55-66.

Kiuru S, Nieminen T, Partinen M (1999a). Obstructive sleep apnoea syndrome in hereditary gelsolin-related amyloidosis. J Sleep Res 8:143-149.

Kiuru S, Salonen O, Haltia M (1999b). Gelsolin-related spinal and cerebral amyloid angiopathy. Ann Neurol 45:305-311.

Kiuru S, Javela K, Somer H, Kekomäki R (2000). Altered platelet shape change in hereditary gelsolin Asp187Asn-related amyloidosis. Thromb Haemost 83:491-495.

Kiuru-Enari S, Haltia M (2013). Hereditary gelsolin amyloidosis. In: Said G, Krarup C (eds). Handbook of Clinical Neurology: Peripheral Nerve Disorders, 3rd series, Elsevier, pp. 659-681.

Kiuru-Enari S, Keski-Oja J, Haltia M (2005) Cutis laxa in hereditary gelsolin amyloidosis. Br J Dermatol 152:250-257.

Kivelä T, Tarkkanen A, Frangione B, Ghiso J, Haltia M (1994). Ocular amyloid deposition in familial amyloidosis, Finnish: an analysis of native and variant gelsolin in Meretoja's syndrome. Invest Ophthalmol Vis Sci 35:3759-3769.

Koivisto L, Alavian K, Häkkinen L, Pelech S, McCulloch CA, Larjava H (2003). Glycogen synthase kinase-3 regulates formation of long lamellipodia in human keratinocytes. J Cell Sci 116:3749-3760.

Koloktronis A, Chatzigiannis I, Paloukidou N (2003). Oral involvement in a case of AA amyloidosis. Oral Dis 9:269-272.

Konttinen YT, Porcar A, Porola P, Koskenpato K, Rodriguez ML, Pöllänen R, Stegaev V, Virkki L, Spaan M, Przybyla BD (2011). Neurobiology and Hormonal Control of Lacrimal and Salivary Gland Function. In: Fox RI, Fox CM (eds). Sjögren's Syndrome, Springer, pp. 151-175.

Kothakota S, Azuma T, Reinhard C, Klippel A, Tang J, Chu K, McGarry TJ, Kirschner MW, Koths K, Kwiatkowski DJ, Williams LT (1997). Caspase-3-generated fragment of gelsolin: effector of morphological change in apoptosis. Science 278:294-298.

Koskelainen S, Pihlamaa T, Suominen S, Zhao F, Salo T, Risteli J, Baumann M, Kalimo H, Kiuru-Enari S (submitted). Gelsolin amyloid angiopathy. Acta Neuropathologica.

Kroes I, Lepp PW, Relman DA (1999). Bacterial diversity within the human subgingival crevice. Proc Natl Acad Sci U S A 96:14547-14552.

Kuczynski A, Evans RJ, Mitchinson MJ (1971). Sicca syndrome due to primary amyloidosis. Br Med J 2:506.

Kurokawa H, Takuma C, Tokudome S, Yamashita Y, Kajiyama M (1998). Primary localization amyloidosis of the sublingual gland. Fukuoka Igaku Zasshi 89:216-220.

Kutchai HC (1998). The gastrointestinal system. In: Berne RM, Levy MN (eds) Physiology, 4th edn, Mosby, USA, pp. 617-622.

Kwiatkowski DJ, Mehl R, Izumo S, Nadal-Ginard B, Yin HL (1988). Muscle is the major source of plasma gelsolin. J Biol Chem 263:8239-8243.

Kwiatkowski DJ, Stossel TP, Orkin SH, Mole JE, Colten HR, Yin HL (1986). Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. Nature 323:455-458.

Kyle RA, Linos A, Beard CM, Linke RP, Gertz MA, O'Fallon WM, Kurland LT (1992). Incidence and natural history of primary systemic amyloidosis in Olmsted County, Minnesota, 1950 through 1989. Blood 79:1817-1822.

Kyle RA (2001). Amyloidosis: a convoluted story. Br J Haematol 114:529-538.

Lachmann HJ, Goodman HJ, Gilbertson JA, Gallimore JR, Sabin CA, Gillmore JD, Hawkins PN (2007). Natural history and outcome in systemic AA amyloidosis. N Engl J Med 356:2361-2371.

Laine A, Kääriäinen H, Notkola I, Kiuru-Enari S (2010). Hereditary gelsolin amyloidosis: questionnaire survey of clinical manifestations and satisfaction with life. Suomen Lääkärilehti 65:1417-1424.

Lamb JA, Allen PG, Tuan BY, Janmey PA (1993). Modulation of gelsolin function. Activation at low pH overrides Ca^{2+} requirement. J Biol Chem 268:8999-9004.

Lankarani KB, Sivandzadeh GR, Hassanpour S (2013). Oral manifestation in inflammatory bowel disease: a review. World J Gastroenterol 19:8571-8579.

Larsen CP, Walker PD, Weiss DT, Solomon A (2010). Prevalence and morphology of leukocyte chemotactic factor 2-associated amyloid in renal biopsies. Kidney Int 77:816-819.

Larsen CP, Kossmann RJ, Beggs ML, Solomon A, Walker PD (2014). Clinical, morphologic, and genetic features of renal leukocyte chemotactic factor 2 amyloidosis. Kidney Int 86:378-382.

Larsson SG, Benson L, Westermark P (1986). Computed tomography of the tongue in primary amyloidosis. J Comput Assist Tomogr 10:836-840.

Lavatelli F, Valentini V, Palladini G, Verga L, Russo P, Foli A, Obici L, Sarais G, Perfetti V, Casarini S, Merlini G (2011). Mass spectrometry-based proteomics as a diagnostic tool when immunoelectron microscopy fails in typing amyloid deposits. Amyloid 18 Suppl 1:64-66.

Leite RS, Marlow NM, Fernandes JK (2013). Oral health and type 2 diabetes. Am J Med Sci 345:271-273.

Levy E, Haltia M, Fernandez-Madrid I, Koivunen O, Ghiso J, Prelli F, Frangione B (1990). Mutation in gelsolin gene in Finnish hereditary amyloidosis. J Exp Med 172:1865-1867.

Li GH, Arora PD, Chen Y, McCulloch CA, Liu P (2012). Multifunctional roles of gelsolin in health and diseases. Med Res Rev 32:999-1025.

Lind SE, Smith DB, Janmey PA, Stossel TP (1986). Role of plasma gelsolin and the vitamin D-binding protein in clearing actin from the circulation. J Clin Invest 78:736-742.

Lindhe J, Karring T, Araujo M (2003). Anatomy of the Periodontium. In: Lindhe J, Karring T, Lang NP (eds) Clinical Periodontology and Implant Dentistry, 4th edn, Blackwell Munksgaard, pp. 3-48.

Llambes F, Arias-Herrera S, Caffesse R (2015). Relationship between diabetes and periodontal infection. World J Diabetes 6:927-935.

Lu M, Witke W, Kwiatkowski DJ, Kosik KS (1997). Delayed retraction of filopodia in gelsolin null mice. J Cell Biol 138:1279-1287.

Löe H (1981). The role of bacteria in periodontal diseases. Bull World Health Organ 59:821-825.

Magy N, Liepnieks JJ, Yazaki M, Kluve-Beckerman B, Benson MD (2003). Renal transplantation for apolipoprotein AII amyloidosis. Amyloid 10:224-228.

Mandel ID (1989). The role of saliva in maintaining oral homeostasis. J Am Dent Assoc 119:298-304.

Maramattom BV, Chickabasaviah YT (2013). A new Indian family affected by gelsolin amyloidosis. Neurol India 61:673-675.

Mattila JS, Krootila K, Kivelä T, Holopainen JM (2015). Penetrating keratoplasty for corneal amyloidosis in familial amyloidosis, Finnish type. Ophthalmology 122:457-463.

Maury CP, Baumann M (1990). Isolation and characterization of cardiac amyloid in familial amyloid polyneuropathy type IV (Finnish): relation of the amyloid protein to variant gelsolin. Biochim Biophys Acta 1096:84-86.

Maury CP, Kere J, Tolvanen R, de la Chapelle A (1990). Finnish hereditary amyloidosis is caused by a single nucleotide substitution in the gelsolin gene. FEBS Lett 276:75-77.

Maury CP (1991a). Immunohistochemical localization of amyloid in Finnish hereditary amyloidosis with antibodies to gelsolin peptides. Lab Invest 64:400-404.

Maury CP (1991b). Gelsolin-related amyloidosis. Identification of the amyloid protein in Finnish hereditary amyloidosis as a fragment of variant gelsolin. J Clin Invest 87:1195-1199.

Maury CP, Kere J, Tolvanen R, de la Chapelle A (1992). Homozygosity for the Asn187 gelsolin mutation in Finnish-type familial amyloidosis is associated with severe renal disease. Genomics 13:902-903.

Maury CP, Liljeström M, Boysen G, Törnroth T, de la Chapelle A, Nurmiaho-Lassila EL (2000). Danish type gelsolin related amyloidosis: 654G-T mutation is associated with a disease pathogenetically and clinically similar to that caused by the 654G-A mutation (familial amyloidosis of the Finnish type). J Clin Pathol 53:95-99.

Mellanen L, Sorsa T, Lähdevirta J, Helenius M, Kari K, Meurman JH (2001). Salivary albumin, total protein, IgA, IgG and IgM concentrations and occurrence of some periodontopathogens in HIV-infected patients: a 2-year follow-up study. J Oral Pathol Med 30:553-559.

Meretoja J (1969). Familial systemic paramyloidosis with lattice dystrophy of the cornea, progressive cranial neuropathy, skin changes and various internal symptoms. A previously unrecognized heritable syndrome. Ann Clin Res 1:314-324.

Meretoja J, Teppo L (1971). Histopathological findings of familial amyloidosis with cranial neuropathy as principal manifestation. Report on three cases. Acta Pathol Microbiol Scand [A] 79:432-440.

Meretoja J (1972). Comparative histopathological and clinical findings in eyes with lattice corneal dystrophy of two different types. Ophthalmologica 165:15-37.

Meretoja J (1973). Genetic aspects of familial amyloidosis with corneal lattice dystrophy and cranial neuropathy. Clin Genet 4:173-185.

Meretoja J (1976). Tautiperintömme: Suomalainen amyloiditauti. J Finn Med Assoc 31:2234-2236.

Meretoja J, Natvig JB, Husby G (1976). Amyloid-related serum protein (SAA) in patients with inherited amyloidosis and certain viral conditions. Scand J Immunol 5:169-174.

Merlini G, Stone MJ (2006). Dangerous small B-cell clones. Blood 108:2520-2530.

Meurman JH, Rantonen P, Pajukoski H, Sulkava R (2002). Salivary albumin and other constituents and their relation to oral and general health in the elderly. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 94:432-438.

Meurman JH, Toskala J, Nuutinen P, Klemetti E (1994). Oral and dental manifestations in gastroesophageal reflux disease. Oral Surg Oral Med Oral Pathol 78:583-589.

Miyamoto S, Wu JM (1990). Effect of staurosporine on the induction of actin/gelsolin in PMA-treated HL-60 cells. Biochem Int 22:427-433.

Morris AJ, Steele J, White DA (2001). Adult dental health survey: The oral cleanliness and periodontal health of UK adults in 1998. Br Dent J 191:186-192.

Mucci LA, Bjorkman L, Douglass CW, Pedersen NL (2005). Environmental and heritable factors in the etiology of oral diseases--a population-based study of Swedish twins. J Dent Res 84:800-805.

Myssiorek D, Alvi A, Bhuiya T (1992). Primary salivary gland amyloidosis causing sicca syndrome. Ann Otol Rhinol Laryngol 101:487-490.

Nagler RM, Hershkovich O (2005). Relationships between age, drugs, oral sensorial complaints and salivary profile. Arch Oral Biol 50:7-16.

Nanci A (2007). Structure of Oral Tissues. In: Nanci A (eds). Ten Cate's Oral Histology: Development, Structure, and Function, 7th ed. edn, Elsevier Health Sciences, pp. 1-15.

Nandapalan V, Jones TM, Morar P, Clark AH, Jones AS (1998). Localized amyloidosis of the parotid gland: a case report and review of the localized amyloidosis of the head and neck. Head Neck 20:73-78.

Närhi TO, Ainamo A, Meurman JH (1993). Salivary yeasts, saliva, and oral mucosa in the elderly. J Dent Res 72:1009-1014.

Nederfors T (2000). Xerostomia and hyposalivation. Adv Dent Res 14:48-56.

Needleman I (2012). Aging and the Periodontium. In: Newman MG, Takei HH, Carranza FA (eds) Carranza's clinical periodontology, 11th ed. edn, Elsevier Saunders, pp. 28-32.

Neville BW (2009a). Oral manifestations of systemic diseases. In: Neville BW (ed). Oral and maxillofacial pathology, 3rd ed. edn, Saunders/Elsevier, pp. 816-858.

Neville BW (2009b). Hematological disorders. In: Neville BW (ed). Oral and maxillofacial pathology, 3rd ed. edn, Saunders/Elsevier, pp. 571-612.

Nichols WC, Dwulet FE, Liepnieks J, Benson MD (1988). Variant apolipoprotein AI as a major constituent of a human hereditary amyloid. Biochem Biophys Res Commun 156:762-768.

Nickles K, Schacher B, Ratka-Kruger P, Krebs M, Eickholz P (2013). Long-term results after treatment of periodontitis in patients with Papillon-Lefevre syndrome: success and failure. J Clin Periodontol 40:789-798.

Niemietz C, Chandhok G, Schmidt H (2015). Therapeutic Oligonucleotides Targeting Liver Disease: TTR Amyloidosis. Molecules 20:17944-17975.

Nikoskinen T, Schmidt EK, Strbian D, Kiuru-Enari S, Atula S (2015). Natural course of Finnish gelsolin amyloidosis. Ann Med 47:506-511.

Norio R (2003a). Finnish Disease Heritage I: characteristics, causes, background. Hum Genet 112:441-456.

Norio R (2003b). The Finnish Disease Heritage III: the individual diseases. Hum Genet 112:470-526.

Novak KF, Takei HH (2012). Determination of Prognosis. In: Newman MG, Takei HH, Carranza FA (eds). Carranza's clinical periodontology, 11th ed. edn, Elsevier Saunders, pp. 33-65.

Nuvolone M, Palladini G, Merlini G (2012). Amyloid Diseases at the Molecular Level: General Overview and Focus on AL Amyloidosis. In: Picken MM, Dogan A, Herrera GA (eds). Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 9-29.

Obici L, Palladini G, Giorgetti S, Bellotti V, Gregorini G, Arbustini E, Verga L, Marciano S, Donadei S, Perfetti V, Calabresi L, Bergonzi C, Scolari F, Merlini G (2004). Liver biopsy discloses a new apolipoprotein A-I hereditary amyloidosis in several unrelated Italian families. Gastroenterology 126:1416-1422.

Ombrello AK, Aksentijevich I (2012). Amyloid Diseases at the Molecular Level: General Overview and Focus on AL Amyloidosis. In: Picken MM, Dogan A, Herrera GA (eds). Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 30-52.

Oliver RC, Tervonen T (1994). Diabetes--a risk factor for periodontitis in adults?. J Periodontol 65:530-538.

Oppenheim FG (1970). Preliminary observations on the presence and origin of serum albumin in human saliva. Helv Odontol Acta 14:10-17.

O'Reilly A, D'Souza A, Lust J, Price D (2013). Localized tongue amyloidosis: a single institutional case series. Otolaryngol Head Neck Surg 149:240-244.

Paccalin M, Hachulla E, Cazalet C, Tricot L, Carreiro M, Rubi M, Grateau G, Roblot P (2005). Localized amyloidosis: a survey of 35 French cases. Amyloid 12:239-245.

Page LJ, Suk JY, Huff ME, Lim HJ, Venable J, Yates J, Kelly JW, Balch WE (2005). Metalloendoprotease cleavage triggers gelsolin amyloidogenesis. EMBO J 24:4124-4132.

Paju S, Scannapieco FA (2007). Oral biofilms, periodontitis, and pulmonary infections. Oral Dis 13:508-512.

Parvinen T, Larmas M (1981). The relation of stimulated salivary flow rate and pH to Lactobacillus and yeast concentrations in saliva. J Dent Res 60:1929-1935.

Paster BJ, Boches SK, Galvin JL, Ericson RE, Lau CN, Levanos VA, Sahasrabudhe A, Dewhirst FE (2001). Bacterial diversity in human subgingival plaque. J Bacteriol 183:3770-3783.

Pau M, Reinbacher KE, Feichtinger M, Karcher H (2013). Surgical treatment of macroglossia caused by systemic primary amyloidosis. Int J Oral Maxillofac Surg 42:294-297.

Paunio T, Kiuru S, Hongell V, Mustonen E, Syvänen AC, Bengström M, Palo J, Peltonen L (1992). Solid-phase minisequencing test reveals Asp187----Asn (G654----A) mutation of gelsolin in all affected individuals with Finnish type of familial amyloidosis. Genomics 13:237-239.

Paunio T, Sunada Y, Kiuru S, Makishita H, Ikeda S, Weissenbach J, Palo J, Peltonen L (1995). Haplotype analysis in gelsolin-related amyloidosis reveals independent origin of identical mutation (G654A) of gelsolin in Finland and Japan. Hum Mutat 6:60-65.

Paunio T, Kangas H, Kiuru S, Palo J, Peltonen L, Syvänen AC (1997). Tissue distribution and levels of gelsolin mRNA in normal individuals and patients with gelsolin-related amyloidosis. FEBS Lett 406:49-55.

Paunio T, Kangas H, Heinonen O, Buc-Caron MH, Robert JJ, Kaasinen S, Julkunen I, Mallet J, Peltonen L (1998). Cells of the neuronal lineage play a major role in the generation of amyloid precursor fragments in gelsolin-related amyloidosis. J Biol Chem 273:16319-16324.

Pentenero M, Davico Bonino L, Tomasini C, Conrotto D, Gandolfo S (2006). Localized oral amyloidosis of the palate. Amyloid 13:42-46.

Pepys MB, Dash AC (1977). Isolation of amyloid P component (protein AP) from normal serum as a calcium-dependent binding protein. Lancet 1:1029-1031.

Pepys MB, Dyck RF, de Beer FC, Skinner M, Cohen AS (1979). Binding of serum amyloid P-component (SAP) by amyloid fibrils. Clin Exp Immunol 38:284-293.

Pepys MB, Hawkins PN, Booth DR, Vigushin DM, Tennent GA, Soutar AK, Totty N, Nguyen O, Blake CC, Terry CJ (1993). Human lysozyme gene mutations cause hereditary systemic amyloidosis. Nature 362:553-557.

Percival RS, Challacombe SJ, Marsh PD (1994). Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. J Dent Res 73:1416-1420.

Perfetti V, Coluccia AM, Intini D, Malgeri U, Vignarelli MC, Casarini S, Merlini G, Neri A (2001). Translocation T(4;14)(p16.3;q32) is a recurrent genetic lesion in primary amyloidosis. Am J Pathol 158:1599-1603.

Perlat A, Decaux O, Gervais R, Rioux N, Grosbois B (2009). Systemic light chain amyloidosis and Sjogren syndrome: an uncommon association. Amyloid 16:181-182.

Persson R, Hitti J, Verhelst R, Vaneechoutte M, Persson R, Hirschi R, Weibel M, Rothen M, Temmerman M, Paul K, Eschenbach D (2009). The vaginal microflora in relation to gingivitis. BMC Infect Dis 9:6-2334-9-6.

Peruzzo DC, Benatti BB, Ambrosano GM, Nogueira-Filho GR, Sallum EA, Casati MZ, Nociti FH Jr (2007). A systematic review of stress and psychological factors as possible risk factors for periodontal disease. J Periodontol 78:1491-1504.

Pianta A, Liu K, Lundberg P, Shimizu T, Hao-Shen H, Dirnhofer S, Kralovics R, Shapiro AD, Skoda RC (2013). Hereditary Thrombocytosis Caused By a Novel Germ-Line Mutation in Gelsolin Gene. 55th ASH Annual Meeting and Exposition, New Orleans, LA.

Picciani BLS, Souza TT, Santos Vde CB, Domingos TA, Carneiro S, Avelleira JC, Azulay DR, Pinto JMN, Dias EP (2015). Geographic Tongue and Fissured Tongue in 348 Patients with Psoriasis: Correlation with Disease Severity. Scientific World Journal, ePub.

Pihlamaa T, Rautio J, Kiuru-Enari S, Suominen S (2011). Gelsolin amyloidosis as a cause of early aging and progressive bilateral facial paralysis. Plast Reconstr Surg 127:2342-2351.

Pihlamaa T, Salmi T, Suominen S, Kiuru-Enari S (2015). Progressive cranial nerve involvement and grading of facial paralysis in gelsolin amyloidosis. Muscle Nerve 2015 Sep 30, epub ahead of print, doi: 10.1002/mus.24922.

Pinney JH, Smith CJ, Taube JB, Lachmann HJ, Venner CP, Gibbs SD, Dungu J, Banypersad SM, Wechalekar AD, Whelan CJ, Hawkins PN, Gillmore JD (2013). Systemic amyloidosis in England: an epidemiological study. Br J Haematol 161:525-532.

Plante-Bordeneuve V, Said G (2011). Familial amyloid polyneuropathy. Lancet Neurol 10:1086-1097.

Pöllänen MT, Laine MA, Ihalin R, Uitto VJ (2012). Host-bacteria crosstalk at the dentogingival junction. Int J Dent 2012:821383.

Price EJ, Venables PJ (2002). Dry eyes and mouth syndrome--a subgroup of patients presenting with sicca symptoms. Rheumatology 41:416-422.

Puchtler H, Sweat F, Kuhns JG (1964). On the Binding of Direct Cotton Dyes by Amyloid. J Histochem Cytochem 12:900-907.

Puchtler H, Sweat F (1962). Amidoblack as a stain for hemoglobin. Arch Pathol 73:245-249.

Qiu C, Kivipelto M, von Strauss E (2009). Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues Clin Neurosci 11:111-128.

Rajkumar SV, Gertz MA, Kyle RA (1998). Primary systemic amyloidosis with delayed progression to multiple myeloma. Cancer 82:1501-1505.

Real de Asua D, Costa R, Galvan JM, Filigheddu MT, Trujillo D, Cadinanos J (2014). Systemic AA amyloidosis: epidemiology, diagnosis, and management. Clin Epidemiol 6:369-377.

Research, Science and Therapy Committee of American Academy of Periodontology (2005). Informational paper: implications of genetic technology for the management of periodontal diseases. J Periodontol 76:850-857.

Richey TK, Bennion SD (1996). Etiologies of the sicca syndrome: primary systemic amyloidosis and others. Int J Dermatol 35:553-557.

Rocken C, Shakespeare A (2002). Pathology, diagnosis and pathogenesis of AA amyloidosis. Virchows Arch 440:111-122.

Romanos GE, Schroter-Kermani C, Hinz N, Wachtel HC, Bernimoulin JP (1992). Immunohistochemical localization of collagenous components in healthy periodontal tissues of the rat and marmoset (Callithrix jacchus). I. Distribution of collagen types I and III. J Periodontal Res 27:101-110.

Ronderos M, Jacobs DR, Himes JH, Pihlström BL (2000). Associations of periodontal disease with femoral bone mineral density and estrogen replacement therapy: cross-sectional evaluation of US adults from NHANES III. J Clin Periodontol 27:778-786.

Rothstein A, Auran JD, Wittpenn JR, Koester CJ, Florakis GJ (2002). Confocal microscopy in Meretoja syndrome. Cornea 21:364-367.

Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins PN, Obici L, Westermark P, Grateau G, Wechalekar AD (2014). Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. Hum Mutat 35:E2403-12.

Saito T, Shimazaki Y, Sakamoto M (1998). Obesity and periodontitis. N Engl J Med 339:482-483.

Schima W, Amann G, Steiner E, Steurer M, Vormittag W, Steurer L (1994). Case report: sicca syndrome due to primary amyloidosis. Br J Radiol 67:1023-1025.

Schiodt M, Pindborg JJ (1987). AIDS and the oral cavity. Epidemiology and clinical oral manifestations of human immune deficiency virus infection: a review. Int J Oral Maxillofac Surg 16:1-14.

Sekijima Y (2015). Transthyretin (ATTR) amyloidosis: clinical spectrum, molecular pathogenesis and disease-modifying treatments. J Neurol Neurosurg Psychiatry.

Sethi S, Theis JD, Quint P, Maierhofer W, Kurtin PJ, Dogan A, Highsmith EW Jr (2013). Renal amyloidosis associated with a novel sequence variant of gelsolin. Am J Kidney Dis 61:161-166.

Sheiham A, Netuveli GS (2002). Periodontal diseases in Europe. Periodontol 2000 29:104-121.

Sipe JD, Cohen AS (2000). Review: history of the amyloid fibril. J Struct Biol 130:88-98.

Sipe JD, Benson MD, Buxbaum JN, Ikeda S, Merlini G, Saraiva MJ, Westermark P (2014). Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis. Amyloid 21:221-224.

Smith DJ, Taubman MA (1992). Ontogeny of immunity to oral microbiota in humans. Crit Rev Oral Biol Med 3:109-133.

Snow AD, Willmer J, Kisilevsky R (1987). Sulfated glycosaminoglycans: a common constituent of all amyloids? Lab Invest 56:120-123.

Socransky SS, Haffajee AD, Goodson JM, Lindhe J (1984). New concepts of destructive periodontal disease. J Clin Periodontol 11:21-32.

Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, Levin AE (1994). "Checkerboard" DNA-DNA hybridization. BioTechniques 17:788-792.

Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL Jr (1998). Microbial complexes in subgingival plaque. J Clin Periodontol 25:134-144.

Socransky SS, Haffajee AD (2003). Microbiology of Periodontal Disease. In: Lindhe J, Karring T, Lang NP (eds) Clinical Periodontology and Implant Dentistry, 4th edn, Blackwell, Munksgaard, pp 106-149.

Socransky SS, Haffajee AD, Smith C, Martin L, Haffajee JA, Uzel NG, Goodson JM (2004). Use of checkerboard DNA-DNA hybridization to study complex microbial ecosystems. Oral Microbiol Immunol 19:352-362.

Solomon JP, Page LJ, Balch WE, Kelly JW (2012). Gelsolin amyloidosis: genetics, biochemistry, pathology and possible strategies for therapeutic intervention. Crit Rev Biochem Mol Biol 47:282-296.

Stabholz A, Soskolne WA, Shapira L (2010). Genetic and environmental risk factors for chronic periodontitis and aggressive periodontitis. Periodontol 2000 53:138-153.

Stangou AJ, Banner NR, Hendry BM, Rela M, Portmann B, Wendon J, Monaghan M, Maccarthy P, Buxton-Thomas M, Mathias CJ, Liepnieks JJ, O'Grady J, Heaton ND, Benson MD (2010). Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood 115:2998-3007.

Su Y, Misumi Y, Ueda M, Shono M, Tasaki M, Guo J, Jono H, Obayashi K, Senokuchi T, Yamagata K, Ando Y (2012). The occurrence of islet amyloid polypeptide amyloidosis in Japanese subjects. Pancreas 41:971-973.

Sun HQ, Yamamoto M, Mejillano M, Yin HL (1999). Gelsolin, a multifunctional actin regulatory protein. J Biol Chem 274:33179-33182.

Suominen-Taipale L, Nordblad A, Vehkalahti M, Aromaa A (2004). Suomalaisten aikuisten suun terveys, Terveys 2000 -tutkimus. Kansanterveyslaitoksen julkaisuja, B16.

Tamaoki T, Nakano H (1990). Potent and specific inhibitors of protein kinase C of microbial origin. Biotechnology 8:732-735.

Tanskanen M, Paetau A, Salonen O, Salmi T, Lamminen A, Lindsberg P, Somer H, Kiuru-Enari S (2007). Severe ataxia with neuropathy in hereditary gelsolin amyloidosis: a case report. Amyloid 14:89-95.

Tanskanen M, Peuralinna T, Polvikoski T, Notkola IL, Sulkava R, Hardy J, Singleton A, Kiuru-Enari S, Paetau A, Tienari PJ, Myllykangas L (2008). Senile systemic amyloidosis affects 25% of the very aged and associates with genetic variation in alpha2-macroglobulin and tau: a population-based autopsy study. Ann Med 40:232-239.

Teeuw WJ, Gerdes VE, Loos BG (2010). Effect of periodontal treatment on glycemic control of diabetic patients: a systematic review and meta-analysis. Diabetes Care 33:421-427.

Tenovuo J, Lagerlöf F (1999). Saliva. In: Thylstrup A, Fejerskov O (eds). Textbook of Clinical Cariology, 2th edn, Munksgaard, pp 17-42.

Touitou I, Sarkisian T, Medlej-Hashim M, Tunca M, Livneh A, Cattan D, Yalcinkaya F, Ozen S, Majeed H, Ozdogan H, Kastner D, Booth D, Ben-Chetrit E, Pugnere D, Michelon C, Seguret F, Gershoni-Baruch R, International Study Group for Phenotype-Genotype Correlation in Familial Mediterranean Fever (2007). Country as the primary risk factor for renal amyloidosis in familial Mediterranean fever. Arthritis Rheum 56:1706-1712.

Ueda M, Ando Y (2014). Recent advances in transthyretin amyloidosis therapy. Transl Neurodegener Sep 13, doi: 10.1186/2047-9158-3-19.

Uemichi T, Liepnieks JJ, Benson MD (1994). Hereditary renal amyloidosis with a novel variant fibrinogen. J Clin Invest 93:731-736.

Uhlar CM, Whitehead AS (1999). Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 265:501-523.

Valleix S, Drunat S, Philit JB, Adoue D, Piette JC, Droz D, MacGregor B, Canet D, Delpech M, Grateau G (2002). Hereditary renal amyloidosis caused by a new variant lysozyme W64R in a French family. Kidney Int 61:907-912.

Valleix S, Gillmore JD, Bridoux F, Mangione PP, Dogan A, Nedelec B, Boimard M, Touchard G, Goujon JM, Lacombe C, Lozeron P, Adams D, Lacroix C, Maisonobe T, Plante-Bordeneuve V, Vrana JA, Theis JD, Giorgetti S, Porcari R, Ricagno S, Bolognesi M, Stoppini M, Delpech M, Pepys MB, Hawkins PN, Bellotti V (2012). Hereditary systemic amyloidosis due to Asp76Asn variant beta2-microglobulin. N Engl J Med 366:2276-2283.

van der Hilst JC (2011). Recent insights into the pathogenesis of type AA amyloidosis. Scientific World Journal 11:641-650.

Van Dyke TE, Taubman MA, Ebersole JL, Haffajee AD, Socransky SS, Smith DJ, Genco RJ (1984). The Papillon-Lefevre syndrome: neutrophil dysfunction with severe periodontal disease. Clin Immunol Immunopathol 31:419-429.

Van Overbeke W, Verhelle A, Everaert I, Zwaenepoel O, Vandekerckhove J, Cuvelier C, Derave W, Gettemans J (2014). Chaperone nanobodies protect gelsolin against MT1-MMP degradation and alleviate amyloid burden in the gelsolin amyloidosis mouse model. Mol Ther 22:1768-1778.

Van Overbeke W, Wongsantichon J, Everaert I, Verhelle A, Zwaenepoel O, Loonchanta A, Burtnick LD, De Ganck A, Hochepied T, Haigh J, Cuvelier C, Derave W, Robinson RC, Gettemans J (2015). An ER-directed gelsolin nanobody targets the first step in amyloid formation in a gelsolin amyloidosis mouse model. Hum Mol Genet 24:2492-2507.

Vasconcellos CA, Allen PG, Wohl ME, Drazen JM, Janmey PA, Stossel TP (1994). Reduction in viscosity of cystic fibrosis sputum in vitro by gelsolin. Science 263:969-971.

Vassar PS, Culling CF (1959). Fluorescent stains, with special reference to amyloid and connective tissues. Arch Pathol 68:487-498.

Verga L, Morbini P, Palladini G, Obici L, Necchi V, Paulli M, Merlini G (2012). Amyloid Typing: Immunoelectron Microscopy. In: Picken MM, Dogan A, Herrera GA (eds). Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 249-259.

Vidal R, Frangione B, Rostagno A, Mead S, Revesz T, Plant G, Ghiso J (1999). A stop-codon mutation in the BRI gene associated with familial British dementia. Nature 399:776-781.

Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH, European Study Group on Classification Criteria for Sjogren's Syndrome (2002). Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554-558.

Vouyiouklis DA, Brophy PJ (1997). A novel gelsolin isoform expressed by oligodendrocytes in the central nervous system. J Neurochem 69:995-1005.

Watanabe K, Cho YD (2014). Periodontal disease and metabolic syndrome: a qualitative critical review of their association. Arch Oral Biol 59:855-870.

Weber FP, Smith FB, Cooke RT (1947). Amyloid macroglossia. Lancet 1:347.

Wechalekar AD, Gillmore JD, Bird J, Cavenagh J, Hawkins S, Kazmi M, Lachmann HJ, Hawkins PN, Pratt G, BCSH Committee (2015). Guidelines on the management of AL amyloidosis. Br J Haematol 168:186-206.

Weeds AG, Gooch J, McLaughlin P, Maury CP (1993). Variant plasma gelsolin responsible for familial amyloidosis (Finnish type) has defective actin severing activity. FEBS Lett 335:119-123.

Westberg JA, Zhang KZ, Andersson LC (1999). Regulation of neural differentiation by normal and mutant (G654A, amyloidogenic) gelsolin. FASEB J 13:1621-1626.

Westermark P (2012a). Aspects of the History and Nomenclature of Amyloid and Amyloidosis. In: Picken MM, Dogan A, Herrera GA (eds). Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humana Press, pp. 3-8.

Westermark P (2012b). Localized Amyloidoses and Amyloidoses Associated with Aging Outside the Central Nervous System. In: Picken MM, Dogan A, Herrera GA (eds). Amyloid and Related Disorders, Surgical Pathology and Clinical Correlations, Humanan Press, pp. 81-103.

Wisniewski T, Frangione B (1992). Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neurosci Lett 135:235-238.

Witke W, Sharpe AH, Hartwig JH, Azuma T, Stossel TP, Kwiatkowski DJ (1995). Hemostatic, inflammatory, and fibroblast responses are blunted in mice lacking gelsolin. Cell 81:41-51.

Wolff A, Zuk-Paz L, Kaplan I (2008). Major salivary gland output differs between users and non-users of specific medication categories. Gerodontology 25:210-216.

Worster-Drought C, Hill TR, McMenemey WH (1933). Familial Presenile Dementia with Spastic Paralysis. J Neurol Psychopathol 14:27-34.

Xu Y, Lindemann P, Vega-Ramos J, Zhang JG, Villadangos JA (2014). Developmental regulation of synthesis and dimerization of the amyloidogenic protease inhibitor cystatin C in the hematopoietic system. J Biol Chem 289:9730-9740.

Yamanaka S, Miyazaki Y, Kasai K, Ikeda S, Kiuru-Enari S, Hosoya T (2013). Hereditary renal amyloidosis caused by a heterozygous G654A gelsolin mutation: a report of two cases. Clin Kidney J 6:189-193.

Yazaki M, Farrell SA, Benson MD (2003a). A novel lysozyme mutation Phe57Ile associated with hereditary renal amyloidosis. Kidney Int 63:1652-1657.

Yazaki M, Liepnieks JJ, Barats MS, Cohen AH, Benson MD (2003b). Hereditary systemic amyloidosis associated with a new apolipoprotein AII stop codon mutation Stop78Arg. Kidney Int 64:11-16.

Yin HL, Stossel TP (1979). Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. Nature 281:583-586.

Yin HL, Zaner KS, Stossel TP (1980). Ca^{2+} control of actin gelation. Interaction of gelsolin with actin filaments and regulation of actin gelation. J Biol Chem 255:9494-9500.

Yin HL, Albrecht JH, Fattoum A (1981). Identification of gelsolin, a Ca^{2+}-dependent regulatory protein of actin gel-sol transformation, and its intracellular distribution in a variety of cells and tissues. J Cell Biol 91:901-906.

Yin HL, Kwiatkowski DJ, Mole JE, Cole FS (1984). Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin. J Biol Chem 259:5271-5276.

Yokota E, Etoh H, Araki Y, Kato E, Nagamine M, Kawachi H, Seita M (1984). A case of sicca syndrome due to primary amyloidosis. Jpn J Med 23:250-253.

Yu JC, Gotlieb AI (1992). Disruption of endothelial actin microfilaments by protein kinase C inhibitors. Microvasc Res 43:100-111.

Zambon JJ (1985). Actinobacillus actinomycetemcomitans in human periodontal disease. J Clin Periodontol 12:1-20.

Zeng J, Williams SM, Fletcher DJ, Cameron CM, Broadbent JM, Shearer DM, Thomson WM (2014). Reexamining the association between smoking and periodontitis in the dunedin study with an enhanced analytical approach. J Periodontol 85:1390-1397.

Zhen DB, Swiecicki PL, Zeldenrust SR, Dispenzieri A, Mauermann ML, Gertz MA (2015). Frequencies and geographic distributions of genetic mutations in transthyretin- and non-transthyretin-related familial amyloidosis. Clin Genet. 88:396-400.

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).

AApoAI amyloidosis (Nichols et al. 1988; Brewer and Rader 1991; Hamidi Asl et al. 1999; Gillmore et al. 2006; Obici et al. 2004).

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

ALys amyloidosis (Pepys et al. 1993; Yazaki et al. 2003a; Girnius et al. 2012; Jean et al. 2014).

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).

ACys amyloidosis (Abrahamson et al. 1987; Ghiso et al. 1986; Gudmundsson et al. 1972; Jensson et al. 1987; Xu et al. 2014).

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

Appendix 2: Questionnaire

Hampaiston ja suun alueen ongelmat perinnöllisessä gelsoliiniamyloidoosissa

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

Nimi: _____

Sosiaaliturvatunnus: _____

Sairaudet: _____

_____

Lääkitykset: _____

_____

<u>Rengastakaa oikea vaihtoehto:</u>

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

Kuvailkaa 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
Hammasvälien puhdistus:	ei lainkaan	pari krt/vko	joka päivä
	hammastikulla	hammaslangalla	väliharjoilla

Vaikeuttaako sairautenne mielestänne hampaiden puhdistusta?

Kyllä Ei

Kiitos vaivannäöstä! Ottakaa tämä paperi mukaanne kun tulette tutkimukseen.