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# AGel amyloidosis: Genetic background and natural history

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*To gelsolin amyloidosis patients*

# Abstract

Amyloidosis is a disease that results from systemic or localised deposition of amyloid fibrils in organs and tissues causing disruption to their normal function. Gelsolin (AGel) amyloidosis is a monogenic, autosomal dominantly inherited disorder that was described for the first time by the Finnish ophthalmologist Jouko Meretoja in 1969 (Meretoja, 1969). Most AGel amyloidosis patients have been reported in Finland. To date, four different gelsolin gene variants have been associated with amyloidosis, c.640G > A being the most common and the only so far reported mutation in Finland. The characteristic triad of progressive ophthalmological, neurological and dermatological manifestations is known to be frequent in AGel amyloidosis patients.

The purpose of this study was to investigate and broaden the knowledge on the genetic background and natural course of AGel amyloidosis. Firstly, we aimed to identify Finnish AGel amyloidosis patients and families, and to create a national patient registry of Finnish AGel amyloidosis patients. Based on information from the patient registry, we wanted to describe and verify the characteristic natural course of AGel amyloidosis in a representative patient group. Through conducting a haplotype analysis, we aimed to answer the question of whether all the Finnish AGel amyloidosis patients share the same gelsolin gene variant associated haplotype and thus possibly have a common ancestor. We pursued investigating the severity and frequency of pathological inner organ, especially cardiac, involvement in AGel amyloidosis. A further goal of this study was to recognise the causes of death of AGel amyloidosis patients, compare them with those of the normal Finnish population, and increase understanding of the disease's influence on patients' lifespan.

As part of this study, we founded and later updated the National Finnish Gelsolin Amyloidosis Patient Registry (FIN-GAR). In the first phase, a total of 227 AGel amyloidosis patients were included in the FIN-GAR registry, and five years later the registry was updated to include 261 patients, that is approximately 25-40% of all Finnish patients. Based on the FIN-GAR registry and genealogical study, we identified 62 Finnish AGel amyloidosis families and conducted a haplotype analysis.

Further, we collected and systematically analysed autopsy reports and tissue samples of 25 deceased patients to better understand the inner organ involvement of the disease. Finally, we collected the death certificates of 272 deceased patients and investigated their causes of death. The life span of the patients was calculated both based on the data from the death certificates and utilising the relative-survival method.

The study confirmed that the 62 studied Finnish AGel amyloidosis families share a gelsolin gene variant c.640G > A associated haplotype that is not otherwise common in the Finnish population, implicating that all the Finnish patients are likely to descend from a common ancestor. The natural course of AGel amyloidosis was fortified and it was shown that the prevalence of a variety of symptoms belonging to the characteristic triad of ophthalmological, dermatological and neurological symptoms is high. A significant portion of patients need repeated plastic surgery as symptomatic treatment, and the incidence of cataract and carpal tunnel syndrome operations is high. Amyloid deposits in the myocardium and cardiac blood vessels are common in middle-aged and elderly patients, possibly leading to clinical cardiac manifestations. As it is a systemic disease, amyloid deposits are also frequently found in the kidneys, lungs, thyroid gland, liver, spleen and pancreas. AGel amyloidosis was demonstrated to be the underlying cause of death in 20% of the patients, underlining the severity of the disease. Renal diseases may prove lethal, even in heterozygotes, even though renal manifestations are usually mild. Interestingly, for an unknown reason, malignancies were significantly less common as causes of death in AGel amyloidosis patients than in the general Finnish population. Even though the disease burden, visible in the high prevalence of diverse symptoms and high number of operations, is significant, the life span is comparable with that of the normal population, and patients do not seem to retire earlier than the general population.

# Tiivistelmä

Amyloidoosit ovat sairauksia, jotka johtuvat amyloidoosisäikeiden systeemisestä tai paikallisesta kertymisestä eri elimiin ja kudoksiin, mikä aiheuttaa haittaa näiden normaalille toiminnalle. Gelsoliiniamyloidoosi (AGel-amyloidoosi) on yhden geenin mutaation aiheuttama, autosomalisesti vallitsevasti periytyvä sairaus, jonka kuvasi ensimmäisenä suomalainen silmälääkäri Jouko Meretoja vuonna 1969 (Meretoja, 1969). Suurin osa raportoiduista gelsoliiniamyloidoosipotilaista asuu Suomessa. Tähän mennessä neljän eri gelsoliinigeenissä tapahtuneen mutaation on todettu aiheuttavan amyloidoosisairauden. Tunnetuista mutaatioista c.640G > A on yleisin, ja mutaatioista toistaiseksi ainoa Suomessa todettu. Gelsoliiniamyloidoosin tyypillisiä ilmenemismuotoja ovat etenevät silmien, ihon ja hermoston oireet.

Tämän tutkimuksen tavoitteena oli laajentaa gelsoliiniamyloidoosin geneettistä taustaa ja luonnollista kulkua koskevaa tietämystä. Tutkimuksen ensimmäisenä tavoitteena oli tunnistaa suomalaisia gelsoliiniamyloidoosipotilaita ja -sukuja ja perustaa kansallinen potilasrekisteri. Perustuen potilasrekisteristä saatuihin tietoihin tavoitteena oli kuvailla gelsoliiniamyloidoosin luonnollinen kulku edustavassa potilasjoukossa. Teimme haplotyyppianalyysin sen selvittämiseksi, onko kaikilla tunnistetuilla suomalaispotilailla yhteinen gelsoliinigeenimutaatioon yhdistyvä haplotyyppi ja siten todennäköisesti yhteinen esi-isä. Lisäksi tavoitteenamme oli selvittää, kuinka merkittäviä ja yleisiä patologiset amyloidoosikertymät ovat potilaiden sisäelimissä, erityisesti sydämessä. Työn viidentenä tavoitteena oli selvittää suomalaisten gelsoliiniamyloidoosipotilaiden kuolinsyyt ja verrata niitä muun väestön kuolinsyihin sekä selvittää sairauden vaikutus potilaiden elinikään.

Osana tätä tutkimusta perustimme kansallisen potilasrekisterin (FIN-GAR). Ensimmäisessä vaiheessa 227 potilaan tiedot tallennettiin rekisteriin, ja viisi vuotta myöhemmin tehdyn päivityksen yhteydessä rekisterin jäsenmäärä kasvoi 261 potilaaseen, mikä kattaa noin 25-40 % kaikista suomalaisista potilaista. Rekisterin ja sukututkimuksen perusteella tunnistimme 62 suomalaista gelsoliiniamyloidoosiperhettä ja suoritimme haplotyyppianalyysin. Keräsimme ja analysoimme 25 potilaan ruumiinavauskertomukset ja ruumiinavauksissa otetut

kudosnäytteet saadaksemme paremman käsityksen sairauden sisäelimiin aiheuttamista muutoksista. Lisäksi keräsimme 272 edesmenneen potilaan kuolintodistukset potilaiden kuolinsyiden selvittämiseksi. Potilaiden elinikä laskettiin sekä kuolintodistuksista saatujen tietojen perusteella että käyttäen relative survival -menetelmää.

Tutkimus osoitti, että 62 tunnistetulla gelsoliiniamyloidoosia sairastavalla suomalaissuvulla on yhteinen gelsoliinigeenin mutaatioon c.640G > A yhdistyvä haplotyyppi. Kyseinen haplotyyppi ei ole yleinen muussa suomalaisväestössä, mikä viittaa siihen, että kaikilla suomalaispotilailla on todennäköisesti yhteinen esi-isä. Tutkimus vahvisti kokonaiskuvan sairauden luonnollisesta kulusta ja osoitti, että moninaiset silmiin, ihoon ja hermostoon liittyvät oireet ovat hyvin yleisiä. Merkittävä osa potilaista tarvitsee oireen mukaiseksi hoidoksi toistettuja plastiikkakirurgisia toimenpiteitä. Näiden lisäksi kaihi- ja rannekanavaoireyhtymäleikkausten esiintyvyys on suurta. Amyloidia kertyy keski-ikäisillä ja iäkkäillä potilailla sydänlihakseen ja sydämen verisuoniin, mikä voi johtaa kliinisesti merkittävien sydänoireiden kehittymiseen. Kyseessä on systeemisairaus, ja amyloidikertymiä on sydämen lisäksi myös munuaisissa, keuhkoissa, kilpirauhasessa, maksassa, pernassa ja haimassa. Potilaista 20 %:lla kuolinsyynä on gelsoliiniamyloidoosi, mikä osoittaa kyseessä olevan vakavan sairauden. Munuaisten ongelmat voivat olla kuolemaan johtavia myös heterotsygooteilla potilailla, vaikka heillä munuaisoireet ovat tavallisesti lieviä. Toistaiseksi tuntemattomasta syystä syöpätaudit kuolinsyynä olivat harvinaisempia gelsoliiniamyloidoosipotilailla kuin muulla väestöllä. Vaikka potilaat kärsivätkin merkittävästä tautitaakasta, joka ilmenee yleisinä monien elinjärjestelmien oireina ja toistuvina leikkauksina, potilaat eivät eläköidy tai kuole muuta väestöä varhaisemmin.

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# List of abbreviations

| | |
|---|---|
| AA | Amyloid A |
| A beta | Amyloid beta |
| AGel | Gelsolin amyloid |
| AL | Immunoglobulin light chain amyloid |
| ACys | Cystatin C related amyloid |
| AFib | Fibrinogen A α-chain amyloid |
| ALys | Lysozyme amyloid |
| ApoAI | Apolipoprotein AI amyloid |
| ATTRv | Hereditary transthyretin amyloid |
| ATTRwt | Wild type transthyretin amyloid |
| CI | Confidence interval |
| CLD | Corneal lattice dystrophy |
| CNS | Central nervous system |
| CoD | Cause of death |
| ECM | Extracellular matrix |
| EMA | European Medicines Agency |
| FDH | Finnish Disease Heritage |
| FIN-GAR | National Finnish Gelsolin Amyloidosis Patient Registry |
| GI | Gastrointestinal |
| *GSN* | Gelsolin gene |
| GSN | Gelsolin protein |
| HE | Haematoxylin and eosin |
| HUS | Helsinki University Hospital |
| Ig | Immunoglobulin |

| | |
|---|---|
| MMP | Matrix metalloprotease |
| PMR | Proportional mortality ratio |
| RNAi | RNA interference |
| SAA | Serum amyloid A protein |
| SAMY | Suomen Amyloidoosiyhdistys ry |
| SNP | Single nucleotide polymorphism |
| SSA | Senile systemic amyloidosis |
| TMD | Tibial muscular dystrophy |
| TTR | Transthyretin |

# List of publications

This thesis is based on the following publications:

I. Mustonen T*, Schmidt EK*, Valori M, Tienari PJ, Atula S, Kiuru-Enari S. Common origin of the gelsolin gene variant in 62 Finnish AGel amyloidosis families. European Journal of Human Genetics. 2018 Jan;26(1):117-123.

II. Nikoskinen T, Schmidt EK, Strbian D, Kiuru-Enari S, Atula S. Natural course of Finnish gelsolin amyloidosis. Ann Med. 2015;47(6):506-11.

III. Schmidt EK, Mustonen T, Kiuru-Enari S, Kivelä T, Atula S. Finnish gelsolin amyloidosis causes significant disease burden but does not affect survival: FIN-GAR phase II study. Orphanet J Rare Dis. 2020;15.1: 19.

IV. Schmidt EK, Kiuru-Enari S, Atula S, Tanskanen M. Amyloid in parenchymal organs in gelsolin (AGel) amyloidosis. Amyloid. 2019;26(3):118-124.

V. Schmidt EK, Atula S, Tanskanen M, Nikoskinen T, Notkola IL, Kiuru-Enari S. Causes of death and life span in Finnish gelsolin amyloidosis. Ann Med. 2016;48(5):352-8.

The publications are referred to in the text by their roman numerals.

# 1. Introduction

Gelsolin (AGel) amyloidosis is a monogenic, autosomal dominantly inherited deposition disease that was described for the first time by the Finnish ophthalmologist Jouko Meretoja in 1969 ((Meretoja, 1969). AGel amyloidosis has also been known as Meretoja disease, gelsolin-related amyloidosis, amyloid polyneuropathy IV, familial amyloidosis Finnish (FAF), amyloidosis V and corneal lattice dystrophy type II (CLD II) (Kiuru-Enari & Haltia, 2013). It belongs to the group of amyloid diseases, which are either acquired or inherited and systemic or local disorders in which amyloid material of different origin accumulates in organs and tissues, causing disruption to their normal function.

Most AGel amyloidosis patients have been reported in Finland but during the past years increasingly many single patients and kindred groups have been reported worldwide. To date, four different gelsolin gene variants have been associated with amyloidosis, c.640G > A being the most common and the only so far reported mutation in Finland. It has been speculated whether the disorder is caused by independent mutations in a known mutational hot spot in the gelsolin gene, or whether at least the Finnish patients descend from a common ancestor (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995). The number of Finnish AGel amyloidosis patients has been estimated to vary between 400 patients to 1000 gene carriers, but precise epidemiological information on the patients and their disease course has not been collected systematically (Meretoja, 1976; Kiuru, 1992; Kiuru-Enari & Haltia, 2013).

The characteristic triad of progressive ophthalmological, neurological and dermatological involvement is known to be frequent in AGel amyloidosis patients. Renal manifestation may be severe, especially in homozygous patients, but comprehensive information on other inner organ involvement has not been available. Autopsy studies with a limited number of subjects (Meretoja & Teppo, 1971; Meretoja, 1973b; Kiuru *et al.*, 1994), case reports and clinical experience have indicated that cardiac involvement in particular may be more frequent than previously thought. In contrast to many other systemic amyloidoses, AGel amyloidosis patients have been assumed to have a lifespan that is comparable with the rest of the population

(Meretoja, 1973b). Exact information on the age at death and the causes of death has not, however, been available.

The starting point of this study was founding the National Finnish Gelsolin Amyloidosis Patient Registry (FIN-GAR). We wanted to collect data on a representative number of Finnish AGel amyloidosis patients, and to describe and particularise the natural course of the disease. The FIN-GAR registry also enabled us to identify Finnish AGel amyloidosis families and to conduct a haplotype analysis to explore the genetic background of the Finnish patients. Further, we wanted to investigate systematically the inner organ involvement through a histological study. Finally, as part of the natural course of AGel amyloidosis, we wanted to increase the understanding of the severity of the disease by investigating the age at retirement, the causes of death and the lifespan of the patients.

# 2. Literature review

## 2.1. Amyloid and amyloid diseases

The term “amyloid” was first used by Matthias Schleiden, a German botanist, in 1838 to describe a normal amylaceous component of plants. The pathologist Rudolf Virchow used the term amyloid, meaning “starch-like”, in 1854 in an autopsy referring to tissue deposits of insoluble material that stained like cellulose when exposed to iodine (Aterman, 1976; Kyle, 2001; Benson & Kincaid, 2007). Amyloid is a proteinaceous material exhibiting a β-sheet structure. Amyloid aggregations look homogeneous and amorphous under the light microscope, and under polarised light, amyloid that is stained with Congo red shows typical birefringence (Kyle & Gertz, 1990).

Amyloid disease results from systemic or localised amyloid fibril depositions in the extracellular spaces of organs and tissues, causing disruption to their normal function. In some cases, intracellular protein inclusions have been reported to have fibrillar structures similar to amyloid (Sipe *et al.*, 2016). Amyloidosis may be acquired or inherited, and it may lead to a lethal disease or amyloid aggregation may be an incidental finding without known clinical significance. The disorders are classified according to the fibril proteins that build up the amyloid material. Proteins that are involved in human amyloidogenesis have distinguishable native structures and functions but they all form a similar cross beta-sheet conformation (Page *et al.*, 2005; Lachmann & Hawkins, 2006).

According to Page (Page *et al.*, 2005), amyloidoses emerge from three classes of proteins: 1) native folds that undergo amyloidogenic conformational changes, 2) proteins that are normally endoproteolytically cleaved to an amyloidogenic fragment, 3) and proteins where a mutation changes the fold, leading to exceptional endoproteolytic release of an amyloidogenic fragment(s). To date, 36 different amyloidogenic proteins are known, presented in Table 1.

| Fibril protein | Precursor protein | Systemic (S), localised (L) |
|---|---|---|
| AL | Immunoglobulin light chain | S, L |
| AH | Immunoglobulin heavy chain | S, L |
| AA | (Apo) Serum amyloid A | S |
| ATTR | Transthyretin, wild type | S |
| | Transthyretin, variants | S |
| Aβ2M | β2-Microglobulin, wild type | S |
| | β2-Microglobulin, variant | S |
| AApoAI | Apolipoprotein A I, variants | S |
| AApoAII | Apolipoprotein A II, variants | S |
| AApoAIV | Apolipoprotein A IV, wild type | S |
| AApoCII | Apolipoprotein C II, variants | S |
| AApoCIII | Apolipoprotein C III, variants | S |
| AGel | Gelsolin, variants | S |
| ALys | Lysozyme, variants | S |
| ALECT2 | Leukocyte chemotactic factor-2 | S |
| AFib | Fibrinogen a, variants | S |
| ACys | Cystatin C, variants | S |
| ABri | ABriPP, variants | S |
| Adan | ADanPP, variants | L |
| Aβ | Aβ protein precursor, wild type | L |
| | Aβ protein precursor, variant | L |
| AαSyn | α-Synuclein | L |
| ATau | Tau | L |
| APrP | Prion protein, wild type | L |
| | Prion protein variants | L |
| | Prion protein variant | S |
| ACal | (Pro)calcitonin | L |
| AIAPP | Islet amyloid polypeptide (amylin) | L |
| AANF | Atrial natriuretic factor | L |
| APro | Prolactin | L |
| AIns | Insulin | L |
| ASPC | Lung surfactant protein | L |
| AGal7 | Galectin 7 | L |
| ACor | Corneodesmosin | L |
| AMed | Lactadherin | L |
| AKer | Kerato-epithelin | L |
| ALac | Lactoferrin | L |
| AOAAP | Odontogenic ameloblast-associated protein | L |
| ASem1 | Semenogelin 1 | L |
| AEnf | Enfurvitide | L |

**Table 1 The 36 amyloid precursor proteins. The precursor proteins that cause hereditary amyloidosis diseases are marked with colour. Modified from Sipe *et al.* (2016).**

The following sections present the most important acquired and hereditary systemic amyloidoses. At the end of the chapter, localised amyloidoses are presented briefly.

### 2.1.1. Acquired systemic amyloidoses

#### *2.1.1.1. Immunoglobulin light chain (AL) amyloidosis*

Immunoglobulin light chain (AL) amyloidosis is a plasma cell disorder in which insoluble amyloid fibrils composed of immunoglobulin (Ig) light chains (kappa or lambda) accumulate in various tissues (Dispenzieri, Gertz & Buadi, 2012). AL amyloidosis is a rare disease but, at the same time, the most common systemic amyloidosis in the developed countries. Its annual incidence is estimated to be 1.2 per 100 000 inhabitants (Kyle *et al.*, 2019). It is usually a systemic disease but localised AL amyloidosis also exists (Desport *et al.*, 2012).

In AL amyloidosis, the monoclonal plasma cell population produces an aberrant paraprotein which misfolds to produce amyloid. AL amyloidosis is frequently found in association with multiple myeloma. When first diagnosed, 10-15% of myeloma patients have co-existing AL amyloidosis but only 1% of patients with active myeloma will also have AL amyloidosis over time (Desikan *et al.*, 1997; Madan *et al.*, 2010).

The median age of disease onset is 76 years (Kyle *et al.*, 2019) and less than 10% of patients are under 50 years old (Desport *et al.*, 2012). The most common clinical manifestations of AL amyloidosis are present in the heart, skin, liver, kidneys, peripheral nerves and autonomic nerves (Dispenzieri *et al.*, 2012). Approximately 20% of patients have peripheral neuropathy and an even larger number have carpal tunnel syndrome (Benson & Kincaid, 2007). Approximately 20% of patients are suffering from dominant symptomatic cardiac amyloidosis at the time of diagnosis (Dubrey *et al.*, 1998).

The more organs involved in AL amyloidosis, the poorer the prognosis of the disease (Gertz, Kyle & Greipp, 1991; Moreau *et al.*, 1998), and the presence of cardiac amyloidosis is indicative of the worst prognosis compared to other organ involvement (Kyle & Gertz, 1995). However, early diagnosis is very important as new therapeutic strategies, namely chemotherapy targeted at suppressing the underlying plasma cell clone secreting amyloid-forming immunoglobulin light chains, induce haematological remission in the majority of the patients, and their median survival time increases to

over five years (Desport *et al.*, 2012). Earlier, despite of therapy, most patients died within 2 years after diagnosis (Kyle, Greipp & O'Fallon, 1986; Gertz *et al.*, 1991; Skinner *et al.*, 1996; Kyle *et al.*, 1997).

#### *2.1.1.2. Serum amyloid A (AA) amyloidosis*

Serum amyloid A (AA) amyloidosis is a severe consequence of chronic inflammatory conditions (Obici & Merlini, 2012; Westermark, Fändrich & Westermark, 2015). AA amyloid fibrils originate from the acute-phase reactant serum amyloid A protein (SAA) and are formed through a process of cleavage, misfolding, and aggregation into a β-sheet conformation (Merlini & Bellotti, 2003). The chronic inflammatory condition that leads to the development of AA amyloidosis may be one of the following: chronic inflammatory arthritis (e.g. rheumatoid arthritis; 60%), chronic infections (e.g. tuberculosis, injection-drug abuse, osteomyelitis; 15%), autoinflammatory syndromes (9%), Crohn's disease (5%), other (e.g. neoplasia, vasculitis; 6%) and unknown causes (6%) (Lachmann *et al.*, 2007).

SAA is a protein synthesised in the liver under the transcriptional regulation of proinflammatory cytokines (Urieli-Shoval, Linke & Matzner, 2000), and its concentration in the blood also increases during acute-phase inflammatory responses in otherwise healthy persons. For some, yet unknown, reasons, patients with chronic inflammatory disorders may develop AA amyloidosis through sustained SAA overproduction, which is the prerequisite for the disease (Lachmann *et al.*, 2007). Renal manifestations (e.g. proteinuria) are the most common in AA amyloidosis, but the liver, spleen, adrenal glands and gut are also typical locations of AA amyloid aggregation (Lachmann *et al.*, 2007). Cardiac manifestations are rare (2%) but amyloid deposition in the heart may be massive and even lead to severe heart failure (Ishii *et al.*, 2011; Dubrey & Comenzo, 2012).

AA amyloidosis was formerly the most common systemic amyloidosis, but its incidence in the developed countries has steadily decreased in recent years due to improved treatment of the underlying inflammatory conditions, including biological drugs (Nuvolone & Merlini, 2017). The progression of the disease can be delayed or

even stopped by treatment of the underlying disease, and the amyloid deposits may even regress (Röcken & Shakespeare, 2002).

#### *2.1.1.3. Wild type transthyretin amyloidosis (ATTRwt)*

Wild type transthyretin (ATTRwt) amyloidosis, previously known as senile systemic amyloidosis (SSA), is an acquired systemic amyloidosis that typically manifests with old age (Gustavsson *et al.*, 1995). The prevalence of ATTRwt amyloidosis in the elderly (> 80 years) was found to be 12-25% in autopsy studies and as high as 37% in people over the age of 95 (Westermark, Johansson & Natvig, 1979; Cornwell *et al.*, 1983; Tanskanen *et al.*, 2006; Ueda *et al.*, 2011), but it is only rarely diagnosed during the lifetime (Westermark *et al.*, 2003; Nakagawa *et al.*, 2016). The reason for the low frequency of antemortem diagnosis might be that ATTR amyloid deposits discovered on autopsy are often asymptomatic and there is no effective treatment available for ATTRwt amyloidosis patients (Nakagawa *et al.*, 2016).

ATTRwt amyloidosis is caused by pathologic accumulation of transthyretin (TTR) protein based amyloid fibrils. In contrast to the hereditary transthyretin amyloidosis, TTR in ATTRwt amyloidosis is an unmutated, wild type protein (Gustavsson *et al.*, 1995; Westermark *et al.*, 2003).

ATTRwt amyloidosis has been thought to be a rather benign disease with small and scattered amyloid deposits (Cornwell 3rd, Johnson & Westermark, 1995). In some individuals, however, the disease can even be fatal, due to massive myocardial deposits resulting in heart failure, conduction disorders and arrhythmias (Pitkänen, Westermark & Cornwell 3rd, 1984; Johansson & Westermark, 1991; Westermark *et al.*, 2003). In addition to cardiac involvement, pathological deposits of TTRwt amyloid are found in blood vessels, the lungs and the renal medulla of the kidneys (Westermark *et al.*, 2003). If diagnosed while living, only symptomatic treatment is available.

### 2.1.2. Hereditary systemic amyloidoses

In the following, the most common hereditary systemic amyloidoses other than the topic of this study, AGel amyloidosis, are presented briefly.

#### *2.1.2.1. Hereditary transthyretin amyloidosis (ATTRv)*

Hereditary transthyretin amyloidosis (ATTRv) is an autosomal dominant disorder, reported for the first time in Portugal in 1952 (Andrade, 1952). More than 100 different variants of the primary structure of transthyretin have been reported, and the majority have been reported to cause amyloidosis (Benson & Uemichi, 1996; Connors *et al.*, 2003), some of them, however, only in single individuals or families (Benson & Kincaid, 2007). The most common of the ATTRv associated mutations is Val30Met (replacement of methionine for valine at position 30 in the TTR gene located on the short arm of chromosome 18), which is prominent in Portugal, Brazil, Sweden and Japan (Andrade, 1952; Benson & Cohen, 1977; Tawara *et al.*, 1983; Holmgren *et al.*, 1988; Planté-Bordeneuve & Said, 2011).

Hereditary TTR amyloidosis manifests typically as a polyneuropathy starting from the feet as numbness and spontaneous pain, which over the course of years gradually extends all the way to the thighs and upper limbs (Planté-Bordeneuve & Said, 2011). In addition, autonomic nervous dysfunction is common (Planté-Bordeneuve & Said, 2011). CNS manifestations in ATTRv amyloidosis are rare but leptomeningeal amyloid depositions may occur (Said & Planté-Bordeneuve, 2009). Extra-neurological manifestations include cardiac involvement, which in some cases is the main or only disease manifestation, ocular manifestations, renal manifestations, loss of body weight and cachexia (Ando *et al.*, 1997; Planté-Bordeneuve & Said, 2011). The symptoms and clinical course may vary significantly between patients, mutations and geographical locations.

Even though the variant TTR is present in gene carriers' blood from birth, the amyloid formation only starts at adult age (Harats, Worth & Benson, 1989). There is considerable variation in the age at disease onset and the penetrance, depending on the mutation but also on the geographical location. For an example, in Sweden, Val30Met mutation most commonly causes late-onset (mean age 55-60 years) disease, whereas in Portugal, the same gene variant is typically associated with earlier onset of clinical manifestations (mean age 30-35 years). However, sporadic cases of early-onset disease in Sweden and late-onset disease in Portugal have been reported. The disease

phenotype and time of onset depend on the ATTR fibril composition in the patient (Suhr, Lundgren & Westermark, 2017). ATTRv leads to death in 5 to 15 years on average (Benson *et al.*, 2011; Planté-Bordeneuve & Said, 2011).

The primary treatment of ATTRv amyloidosis is symptomatic, for example treatment of neuropathic pain (Said, Grippon & Kirkpatrick, 2012). Nearly all plasma transthyretin is synthesised in the liver, and liver transplantation is an established therapy for ATTRv and has been in use since 1990. Patients with the Val30Met mutation are the most likely to benefit from this treatment, and have an 80% survival rate at 5 years, whereas patients with some other transthyretin gene variant are more likely to suffer from continued disease progression after liver transplantation (Benson & Kincaid, 2007). Interestingly, the response to liver transplantation varies between Val30Met patients depending on the ATTR fibril composition: patients with the early-onset Val30Met phenotype have a better prognosis after liver transplantation than patients with the late-onset phenotype (Ericzon *et al.*, 2015), and patients with the early-onset phenotype are less likely to develop significant cardiomyopathy after liver transplantation (Gustafsson *et al.*, 2012).

The first specific pharmacological treatment for an amyloidosis disease, Tafamidis (2-(3,5-dichlorophenyl)-1,3-benzoxazole-6-carboxylic acid), which inhibits the dissociation of transthyretin tetramers, was approved by the European Medicines Agency (EMA) in 2011 and in Japan in 2013. It is authorised as a therapy for ATTRv in adult patients with stage 1 symptomatic polyneuropathy, and it has been reported to postpone the peripheral neurological impairment (Said *et al.*, 2012). A recent study shows that Tafamidis treatment reduced all-cause mortality and cardiovascular-related hospitalisations compared with a placebo in patients with transthyretin amyloid cardiomyopathy (Maurer *et al.*, 2018). The second pharmacological treatment option for ATTRv amyloidosis is an RNA interference (RNAi) therapeutic called patisiran, accepted by both the EMA and FDA in 2018. It has been shown to cause dose-dependent reduction of TTR protein in both healthy persons and ATTRv amyloidosis patients (Coelho *et al.*, 2013; Suhr *et al.*, 2015). A recent study shows that in ATTRv patients, patisiran may stop or even reverse the progression of the cardiac manifestations (Solomon *et al.*, 2019). Still another pharmacological treatment option,

inotersen, an antisense oligonucleotide inhibitor of mutant and wild type human transthyretin, has been approved in the EU for the treatment of stage 1 or 2 polyneuropathy in adult patients with hATTR (Keam, 2018).

#### *2.1.2.2. Apolipoprotein AI (AApoAI) amyloidosis*

The ApoAI protein has a particular role in lipid metabolism and is the major protein component of high-density lipoprotein (HDL) particles. ApoAI amyloidosis is an adult-onset autosomal dominant disease that is caused by different mutations in the ApoA1 gene, present as a single copy on chromosome 11 (11q23) (Nichols *et al.*, 1990). The disease phenotype varies according to the gene variant (Rowczenio *et al.*, 2011), but patients with hereditary ApoAI amyloidosis typically present with hypertension, proteinuria and renal impairment. They may also develop visceral amyloid deposits that affect the liver and spleen, and occasional involvement of the heart, nerves, larynx and gastrointestinal tract has been reported (Persey *et al.*, 1998). Only the ApoA1 gene variant Gly26Arg has been shown to cause peripheral neuropathy similar to that in ATTRv amyloidosis (Benson & Kincaid, 2007). Although even end-stage renal disease may develop, the disease progression is typically slow (Rowczenio *et al.*, 2011). Treatment is supportive but also long-term outcomes of liver, kidney, and heart transplantation have been good despite recurrent deposition of amyloid (Gillmore *et al.*, 2006).

#### *2.1.2.3. Lysozyme (ALys) amyloidosis*

Lysozyme is a bacteriolytic enzyme that is synthesised in the gastrointestinal (GI) tract by macrophages and in hepatocytes. Lysozyme (ALys) amyloidosis is an autosomal dominantly inherited, non-neuropathic systemic amyloidosis that manifests in the GI tract, skin, kidneys, lymph nodes and lacrimal and salivary glands (Pepys *et al.*, 1993; Benyamine *et al.*, 2017). Splenic and hepatic involvement may be histologically severe (Gillmore *et al.*, 1999) leading potentially to spontaneous hepatic or splenic rupture or haematoma, even though liver function may remain normal (Sattianayagam *et al.*, 2012). Several different lysozyme gene mutations have been reported (Pepys *et al.*, 1993; Valleix *et al.*, 2002; Yazaki, Farrell &

Benson, 2003; Röcken *et al.*, 2006; Wooliver *et al.*, 2008; Girnius *et al.*, 2012; Benyamine *et al.*, 2017).

ALys amyloidosis is an extremely uncommon disorder with only few families being reported. The prognosis of the disorder is remarkably variable: some patients have been reported to survive for 20 years from diagnosis while others may die within a year (Gillmore *et al.*, 1999). Treatment of this disorder is symptomatic, including liver and renal transplantation due to hepatic ruptures and renal dysfunction (Gillmore *et al.*, 1999; Sattianayagam *et al.*, 2012).

#### *2.1.2.4. Fibrinogen A α-chain (AFib) amyloidosis*

Fibrinogen is a plasma protein that is essential in the coagulation cascade through its conversion into fibrin. Fibrinogen A α-chain (AFib) amyloidosis is an autosomal dominantly inherited systemic amyloidosis, in which the patients develop chronic renal disease that proceeds to end-stage renal failure and death (Benson *et al.*, 1993; Gillmore *et al.*, 2009). In addition to the characteristic renal manifestation, there is evidence that AFib amyloidosis is a systemic disorder with multivisceral, neurological, cardiac, hepatic and splenic manifestations (Stangou *et al.*, 2010).

Several fibrinogen gene variants have been reported to cause the disorder, Glu526Val (c.1634A > T) being the most common. It has been identified in families from Canada, the United Kingdom, France, Germany, Brazil, the United States and China (Tavares *et al.*, 2017). The median age of onset is 58 years (range 33 to 83 years), sex distribution being equal. The penetrance of the disorder varies. Median time from disease onset to end-stage renal failure has been reported to be 4.6 years, and the approximated median patient survival time 15.2 years from disease manifestation (Gillmore *et al.*, 2009).

Symptomatic treatment is offered for patients, and good results in kidney transplantation have been reported (Gillmore *et al.*, 2009; Sethi *et al.*, 2010), even though amyloid depositions in the renal grafts are seen in 1 to 10 years (Benson, 2005). Two reports have shown that hepatic transplantation reverses the disease: the variant fibrinogen disappears from the serum and amyloid has been observed to be mobilised

from affected organs (Gillmore *et al.*, 2000; Zeldenrust *et al.*, 2003), which makes AFib amyloidosis the only hereditary amyloidosis that has so far been cured by medical means (Benson, 2005). This is interesting, noting that liver transplantation has not been reported to cure patients suffering from TTR amyloidosis. The explanation has been suggested to be that in TTR amyloidosis, both normal and variant proteins are deposited in the amyloid, and, once initiated, the variant is no longer required for continued fibril deposition. In AFib amyloidosis, the amyloid fibril protein is derived strictly from the variant gene product which has been proteolysed prior to incorporation into the fibril (Benson, 2005).

#### *2.1.2.5. Cystatin C related (ACys) amyloidosis*

In 1936, Arnason reported multiple Icelandic families that had a high incidence of fatal cerebral haemorrhage and came to the conclusion that they had an autosomal dominantly inherited disease (Arnason, 1936), but only decades later, in 1972, did Gudmundsson show that the patients were suffering from an amyloidosis (Gudmundsson *et al.*, 1972).

Cystatin C related (ACys) amyloidosis, also called hereditary cerebral haemorrhage with amyloidosis, Icelandic type (HCHWA-I) or hereditary cystatin C amyloid angiopathy, is essentially a cerebral amyloid angiopathy (CAA) (Gudmundsson *et al.*, 1972; Ólafsson, Thorsteinsson & Jensson, 1996). The disorder is caused by an amino acid substitution L68Q (glutamine for leucine at position 68) in the cystatin C gene (Ghiso, Jensson & Frangione, 1986), the gene variant being highly penetrative.

ACys amyloidosis is a rare disease, found exclusively in the Icelandic population with one reported exception from Croatia (Graffagnino *et al.*, 1995). Haplotype and genealogical analysis suggest that the mutation took place in a common ancestor born around 1550, most likely in Iceland. Today, 15 families with approximately 270 variant gene carriers are known (Palsdottir *et al.*, 2008).

In ACys amyloidosis, amyloid deposits are mainly found in cerebral and spinal arteries and arterioles, leading to recurrent haemorrhagic strokes causing serious brain damage and eventually death before the age of 40 (Gudmundsson *et al.*, 1972; Palsdottir *et al.*,

2008). Late-onset disease leading to death in the 7th or 8th decade of life has been reported in some patients (Jensson *et al.*, 1987). The disease may also manifest in other tissues, such as the skin and lymph nodes (Benedikz, Blöndal & Gudmundsson, 1990).

### 2.1.3. Localised amyloidoses

In localised amyloidoses, amyloid aggregates are found only in one organ or organ system. Like systemic amyloidosis, localised amyloidosis may either be acquired or inherited, and there are several different possible precursor proteins of which some can cause both systemic and localised amyloidosis and some only localised amyloidosis.

Probably the best known localised amyloidosis is Alzheimer's disease, in which amyloid beta (Aβ) derived plaques deposit in the brain causing dementia (Hardy & Higgins, 1992). Hereditary cerebral haemorrhage with amyloidosis Dutch type (HCHWA-D) is an example of a hereditary localised amyloidosis, caused by a mutation in the Aβ peptide, clinically characterised by recurrent strokes and dementia (Bornebroek *et al.*, 1996). Of the acquired systemic amyloidoses presented earlier in this chapter, AL amyloidosis in particular may manifest in a localised form (Desport *et al.*, 2012).

Localised amyloidosis has been described in almost every organ system: the brain, skin and soft tissues (subcutis, muscle), oropharynx, larynx, lung, bladder, urethra, seminal vesicles, colon, conjunctiva and lymph node (Rubinow *et al.*, 1978; Seidman *et al.*, 1989; Benson, 1991; Bennett & Chowdhury, 1994; Fugita, DeLatorre & Kavoussi, 2001; Ng *et al.*, 2002; Kyle *et al.*, 2003; Gibbaoui, Abouchacra & Yaman, 2004; Biewend, Menke & Calamia, 2006). In some cases surgical resection of the local amyloid deposit may be curative, but the deposit will possibly recur within the same site or elsewhere in the same tissue (Tirzaman *et al.*, 2000).

Amyloid deposits that appear to be localised may sometimes be a manifestation of systemic disease and it is important to screen patients in order to exclude systemic amyloidosis (Shah *et al.*, 2002). However, progression from localised to systemic amyloidosis is not common (Paccalin *et al.*, 2005; Biewend *et al.*, 2006)

### 2.1.4. Hereditary amyloidoses in Finland

AGel amyloidosis is the most prevalent hereditary amyloidosis in Finland. In addition, two families with hereditary ATTR amyloidosis have been reported. The first of these families has the Val30Met TTR mutation, which is the most common mutation to cause ATTRv amyloidosis in Sweden. However, no Swedish ancestors were found in genealogical studies tracing back to the 18$^{th}$ century (Drugge, Holmgren & Udd, 1992). The second family was reported to have a novel TTR Lys70Glu (p. Lys90Glu) mutation, that has not to current knowledge been reported anywhere else. Four members of this family carried the mutation, and three of them had symptoms: a 78-year old woman suffered from vitreous opacities in the eyes, a 58-year old woman had dry eye syndrome as the only manifestation, and a 68-year old male patient was reported to have carpal tunnel syndrome (Raivio *et al.*, 2016). In addition to the two above-mentioned families, there is known to be a third ATTRv family in Finland which has been verified to have the Val30Met TTR gene variant (personal communication, Sari Kiuru-Enari) but this case has not been published.

## 2.2. AGel amyloidosis

AGel amyloidosis is an autosomal dominantly inherited systemic amyloidosis, first presented by the Finnish ophthalmologist Jouko Meretoja in 1969 (Meretoja, 1969). Two independent groups reported in 1990 that the amyloid material that accumulates in patients' organs and tissues was composed of gelsolin fragments; previously gelsolin had not been known as an amyloidogenic protein (Maury, 1990; Haltia *et al.*, 1990b). Still later in 1990, the mutation causing the disorder was discovered by the same research groups (Levy *et al.*, 1990; Maury *et al.*, 1990). AGel amyloidosis is caused by a point mutation in the gelsolin gene (*GSN*), c.640G > A or c.640G > T; recently other gelsolin gene mutations associated with amyloidosis or other pathologies have also been reported from abroad. The characteristic signs include progressive corneal lattice amyloidosis, cranial and peripheral neuropathy and cutis laxa. To date, no specific treatment is available for the disease.

### 2.2.1. Finnish Disease Heritage

The Finnish Disease Heritage (FDH) includes rare hereditary, mostly autosomal recessive, diseases that are more frequent in Finland than anywhere else (Norio, 2003a). The first identified disorder of FDH was congenital nephrosis in the 1950s, and it is possible that not all suitable disorders have yet been identified (Norio, 2003a). The disorders are monogenic, and a known gene mutation causes the disease in most or even all cases (Kestilä, Ikonen & Lehesjoki, 2010). Currently, the FDH comprises of 36 diseases, of which 32 are autosomal recessive, 2 are autosomal dominant and 2 are X-chromosomal.

The diseases of FDH are overrepresented in Finland, but still very rare, and the incidence varies typically between 1:10 000 and 1:100 000 (Norio, 2003a). The enrichment of these diseases in Finland is probably due to the small size of the original population and its gene mutations, genetic bottlenecks caused, for example, by wars, and the geographical and cultural isolation in which people in Finland lived for several hundred years (Norio, 2003a; Norio, 2003b).

AGel amyloidosis, the topic of the present study, is one of the most common disorders of the FDH (Norio, 2000).

### 2.2.2. Epidemiology

AGel amyloidosis seems to be more common in Finland than anywhere else. Moreover, there has been a clear clustering of the disease in south-eastern Finland (Kymenlaakso) and in South Tavastia (Meretoja, 1973a), which can, to some extent, still be seen. Previous studies have revealed that all the Finnish patients have the same mutation *GSN* c.640G > A, previously known as c.654G > A, (Kiuru, 1998), and uniformity of the haplotype associated with the disease has been detected in the occasional Finnish families that have been studied (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995). This may have resulted from a single ancestor (Kiuru-Enari & Haltia, 2013), but a hypothesis of a mutational hot spot in the gelsolin gene has been presented (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995) The number of Finnish patients has been estimated to be between 400 and 1000 (Meretoja, 1976; Kiuru, 1992), but exact epidemiological data are not available and the estimates may be outdated.

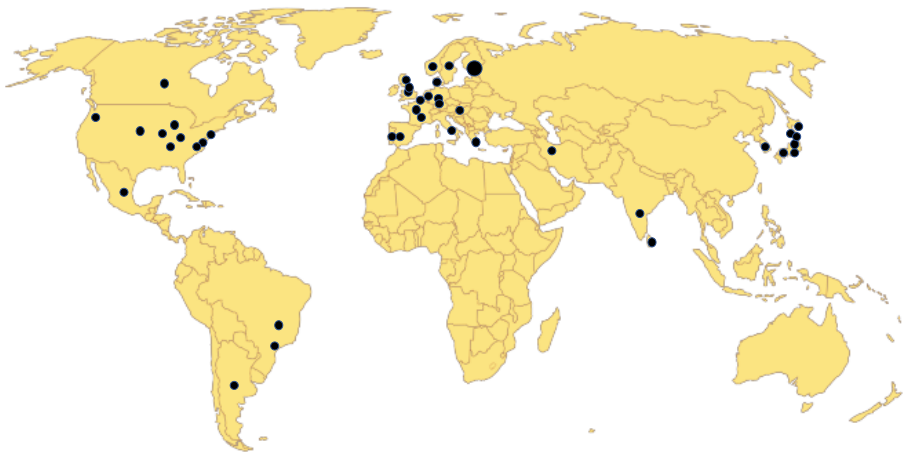


**Figure 1 The world map of AGel amyloidosis, updated and modified from Kiuru-Enari & Haltia (2013).**

In addition to Finland, AGel amyloidosis has been reported from several European, American and Asian countries (Figure 1, Table 2). A case in Greece with corneal lattice amyloidosis has been reported, but genetic testing was not done (Papathanassiou *et al.*, 2009) as is the case with an Indian family with typical clinical signs and symptoms (Maramattom & Chickabasaviah, 2013). There is one known AGel amyloidosis case in Norway, but this has not been published yet (Sari Kiuru-Enari, personal communication). Another gelsolin gene mutation in the same locus c.640G > T has also been reported to cause AGel amyloidosis, but this variant is remarkably less common than c.640G > A (Figure 1, Table 2). In addition to the two long-known mutations, two recently identified *GSN* variants (c.633C > A and c.580G > A) have been reported to cause renal amyloidosis in North America (Sethi *et al.*, 2013; Efebera *et al.*, 2014). Finally, a novel c.1732G > C gelsolin gene variant was found in a patient who was histologically verified to have both ATTRv and AGel derived amyloid deposits (Sridharan *et al.*, 2018).

Mutations in the gelsolin gene may lead to pathologies other than amyloidosis, and a heterozygous G duplicate in exon 1, c.100dupG, has recently been reported in a patient with seizures, multiple cerebral cavernous malformations and haemorrhagic lesions in the brain. In this case, histological or immunohistochemical staining was not done and therefore it is not known whether there was gelsolin-based amyloid in the patient's tissues (Feng *et al.*, 2018). In another recent study, a male of African descent with clinical manifestation including fevers, anaemia, skin rash and polyneuropathy was confirmed to have the 1375C > G mutation in exon 10 in the gelsolin gene (Oregel *et al.*, 2018). In this case, Congo red positive amyloid was found in the vascular walls but the publication does not report how the researchers concluded that the amyloid was derived from gelsolin.

**Table 2 AGel amyloidosis patients, families and mutations reported worldwide, updated and modified from Kiuru-Enari & Haltia (2013).**

| Country of origin | Patients (families) | Type of mutation | Reference |
|---|---|---|---|
| Czechoslovakia | 3 (1) | 640G > T | Klaus *et al.* (1959), de la Chapelle *et al.* (1992b) |
| Finland | 5 (5) | 640G > A | Levy *et al.* (1990) |
| Finland | 5 (5) | 640G > A | Maury *et al.* (1990) |
| Finland | 31 (3) | 640G > A | Hiltunen *et al.* (1991) |
| Finland | 6 (6) | 640G > A | de la Chapelle *et al.* (1992a) |
| Finland | 15 (7) | 640G > A | de la Chapelle *et al.* (1992b) |
| Finland | 23 (13) | 640G > A | Haltia *et al.* (1992) |
| Finland | 30 (17) | 640G > A | Kiuru (1992), Paunio *et al.* (1992) |
| Finland | 2 (1) | 640G > A | Maury *et al.* (1992) |
| Finland | 94 (55) | 640G > A | Paunio *et al.* (1992) |
| The Netherlands | 2 (1) | 640G > A | Winkelman, Delleman & Ansink (1971), de la Chapelle *et al.* (1992b) |
| Denmark | 5 (1) | 640G > T | Boysen *et al.* (1979), de la Chapelle *et al.* (1992b) |
| USA | 2 (1) | 640G > A | Sack *et al.* (1981) |
| USA | 2 (1) | 640G > A | Purcell *et al.* (1983), Gorevic *et al.* (1991) |
| USA* | 3 (1) | 640G > A | Steiner *et al.* (1995) |
| USA | 8 (1) | 640G > A | Rothstein *et al.* (2002) |
| Japan | 14 (1) | 640G > A | Sunada *et al.* (1993) |
| Japan | 17 (1) | 640G > A | Makishita *et al.* (1994) |
| Japan | 2 (1) | 640G > A | Akiya *et al.* (1996) |
| Japan | 1 (1) | 640G > A | Makishita *et al.* (1994) |
| Japan | 1 (1) | 640G > A | Makioka *et al.* (2010) |
| Japan | 5 (1) | 640G > A | Asahina *et al.* (2011) |
| Great Britain | 1(1) | 640G > A | Reilly, Brueton & Sweeney (1998) |
| Great Britain | 6 (1) | 640G > A | Stewart *et al.* (2000) |
| Great Britain | 1 (1) | 640G > A | Hornigold *et al.* (2006) |
| Portugal | 16 (1) | 640G > A | Conceição *et al.* (2003) |
| France | 4 (1) | 640G > T | Chastan *et al.* (2006) |
| France | 1 (1) | 640G > T | Contégal *et al.* (2006) |
| Brazil | 1 (1) | N/A | Felix *et al.* (2008) |
| Iran | 9 (1) | 640G > A | Ardalan *et al.* (2007a), (2007b) |
| Spain | 3 (1) | 640G > A | Huerva *et al.* (2007) |
| Sweden | 1 (1) | 640G > A | Carrwik & Stenevi (2009) |
| Greece | 1 (1) | N/A | Papathanassiou *et al.* (2009) |
| Germany | 22 (1) | 640G > A | Lüttmann *et al.* (2010) |
| Germany | 3 (1) | 640G > A | Bürmann *et al.* (2011) |
| Brazil | 3 (1) | 640G > T | Solari *et al.* (2011) |
| USA | 1 (1) | 580G > A | Sethi *et al.* (2013) |
| India | 7 (1) | N/A | Maramattom & Chickabasaviah (2013) |
| Mexico | 1 (1) | 640G > A | Gonzalez-Rodriguez *et al.* (2014) |
| Sri Lanka | 2 (1) | 640G > A | Chardon & Massie (2014) |
| USA | 5 (1) | 633C > A | Efebera *et al.* (2014) |
| USA | 8-11 (1) | 640G > T | Caress *et al.* (2017) |
| Canada** | 1 (1) | 640G > A | Alabdali *et al.* (2015) |
| South Korea | 5 (1) | 640G > T | Park *et al.* (2016) |
| Argentina | 4 (1) | 640G > A | Saá *et al.* (2017) |
| Brazil** | 5 (1) | 640G > A | de Souza *et al.* (2017) |
| Italy | 6 (1) | 640G > A | Sagnelli *et al.* (2017) |
| USA | 1 (1) | 1732G > C | Sridharan *et al.* (2018) |

*Ancestors from Sweden and Åland, an archipelago belonging to Finland

**Family known to have a Finnish ancestor

### 2.2.3. The gelsolin gene variants and genealogical considerations

AGel amyloidosis is caused by a mutation in the gelsolin gene, located on chromosome 9 at q33.2 (Kwiatkowski *et al.*, 1988b) where guanine is replaced by adenosine (NM_000177.4:c.640G > A, NP_000168.1:p. Asp214Asn) (Maury *et al.*, 1990; Levy *et al.*, 1990). All the Finnish patients examined so far have the same gelsolin c.640G > A variant but, as presented in the previous chapter, other disease-causing gelsolin gene variants, c.640G > T, c.633C > A and c.580G > A, have been reported from other countries. Because the disease-causing mutation is dominant, the large majority of the patients are heterozygote and only few homozygote patients have been reported. Patients and families with AGel amyloidosis reported worldwide so far are presented in Table 2.

It is not known whether the homogeneity of the mutation in the Finnish patients is due to a mutational hot spot in the gelsolin gene, which has been the prevailing hypothesis concerning the gelsolin gene variant reported worldwide (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995), or due to a common ancestor. The base preceding the 640 nucleotide guanine (G) is cytosine (C), and transitional mutations in the CpG dinucleotide (C > T or G > A), such as the c.640 G > A mutation, are known to take place 12 times more often than expected (Cooper & Krawczak, 1990). This would support the theory of a mutational hot spot as the origin of the gene variants leading to AGel amyloidosis. Moreover, two Japanese families have been reported to have a different c.640 G > A associated haplotype than 10 Finnish families that share an identical haplotype (Paunio *et al.*, 1995). Finally, two unequal haplotypes were also found in the Danish and Czech families with c.640A > T mutation (de la Chapelle *et al.*, 1992b).

Meretoja suggested that all the Finnish patients may have a common ancestor who lived over 600 years ago (Meretoja, 1976). The patients were clustered in South-East Finland and in southern Tavastia in certain villages, their progenitors were static farmers, and no foreign members had entered these families (Meretoja, 1973a). The theory of a common ancestor of all Finnish patients has been strengthened by the homogeneity of the mutation (Kiuru, 1998). Moreover, two haplotype studies (n = 7

and n = 10) show that these Finnish families share an identical haplotype (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995). Finally, an American family with a Scandinavian ancestor has been reported to share the same haplotype as the families in Paunio's study (Steiner *et al.*, 1995; Paunio *et al.*, 1995).

In addition to the above-mentioned haplotype studies, a haplotype analysis of five Japanese families manifesting with cutis laxa, lattice corneal amyloidosis, multiple cranial neuropathies, blepharoptosis and facial weakness has been completed. All of the families were shown to have the c.640G > A gelsolin gene variant, and the study revealed that four of them shared the same mutation-associated haplotype, but these haplotypes were not compared with the Finnish haplotype (Taira *et al.*, 2012).

#### *2.2.3.1. Normal function of gelsolin protein*

Gelsolin protein is the founding member of gelsolin superfamily that consists of gelsolin and at least six other proteins: villin, adseverin, capG, advillin, supervillin and flightless I (Silacci *et al.*, 2004). Gelsolin is a principal actin-modulating protein that plays a role in cell motility and in the control of cell growth, morphology, ion-channel regulation and apoptosis (Sun *et al.*, 1999; Kwiatkowski, 1999). Three isoforms of gelsolin protein are encoded by the gelsolin gene as the result of alternative splicing: cytoplasmic gelsolin (81 kDa), plasma gelsolin (83 kDa) (Kwiatkowski *et al.*, 1986) and gelsolin-3, which is present in oligodendrocytes, the lungs and testes (Vouyiouklis & Brophy, 1997). Smooth and striated muscle cells are the main source of secreted gelsolin, but gelsolin is also expressed in other cells and tissues such as ocular, dermal and renal cells as well as the cells of the peripheral and central nervous systems (Kwiatkowski *et al.*, 1988a; Kwiatkowski *et al.*, 1988b; Paunio *et al.*, 1997).

Gelsolin severs and caps actin filaments, an interaction that is regulated by the calcium ion and pH, which enable gelsolin's binding to actin (Kwiatkowski, 1999). Actin can be cross-linked into a gel by actin cross-linking proteins, and gelsolin can change this gel into a sol, gel-sol – thus the name gelsolin. Gelsolin-null mice in mixed-strain backgrounds that express no cytoplasmic or plasma gelsolin are completely viable and have a normal life span (Witke *et al.*, 1995).

In addition to its other functions, gelsolin has a role in carcinogenesis, and gelsolin expression is downregulated in several types of human cancers (Chaponnier & Gabbiani, 1989; Asch *et al.*, 1996; Kuzumaki *et al.*, 1998; Afify & Werness, 1998; Lee *et al.*, 1999). The exact mechanism is not yet understood but it might relate to its role in motility (Kwiatkowski, 1999). On the other hand, high expression of gelsolin has been shown in colorectal tumour borders infiltrating into adjacent liver tissues (Zhuo *et al.*, 2012), and in patients with Stage I non-small cell lung cancer, high expression of gelsolin has been shown to indicate an increased risk for cancer recurrence (Shieh *et al.*, 1999).

#### *2.2.3.2. Pathogenesis*

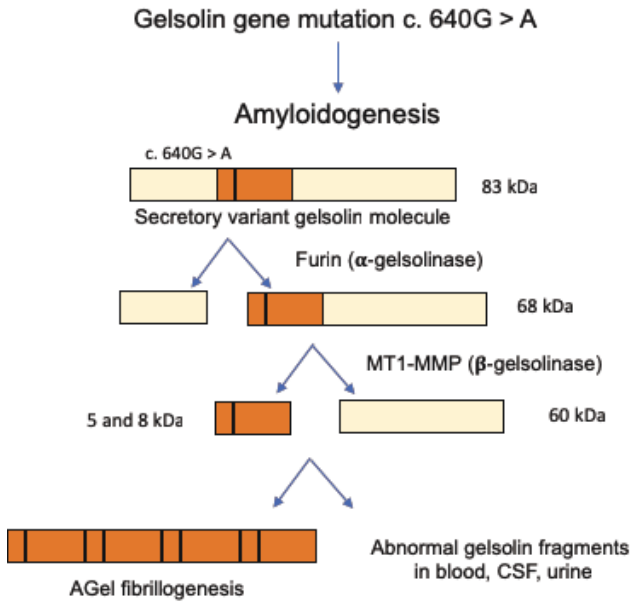



**Figure 2 Molecular pathogenesis of hereditary gelsolin amyloidosis. Modified from Kiuru-Enari & Haltia (2013)**

The mutation (c. 640G > A or c.640G > T) in the gelsolin gene alters the primary structure of the gelsolin protein. Subsequently, it loses its stabilising calcium binding site and makes the variant gelsolin molecule susceptible to cleavage by the protease furin (α-gelsolinase) (Chen *et al.*, 2001), a process illustrated in Figure 2. The proteolysis of the variant gelsolin molecule takes place in the *trans*-Golgi network and

leads to the formation of a 68 kDa gelsolin fragment (Chen *et al.*, 2001). This 68 kDa gelsolin fragment is further processed in the extracellular matrix (ECM) by the MT1 matrix metalloprotease (MT1-MMP) which has β-gelsolinase activity, leading to the formation of 8 kDa and 5 kDa amyloidogenic peptides (Page *et al.*, 2005). 8 kDa and 5 kDa protein fragments are found in the amyloid deposits (Ghiso *et al.*, 1990; Haltia *et al.*, 1990a; Levy *et al.*, 1990; Maury, 1990; Maury *et al.*, 1990).

While both the cytosolic and secreted forms of gelsolin could potentially lead to the formation of amyloidogenic fragments, evidence strongly shows that it is the secreted form of gelsolin that builds amyloid fibrils that accumulate in the organs of patients (Kangas *et al.*, 1996) Local synthesis seems to be the source of the amyloidogenic protein fragments, and the MT1-MMP proteolysis of the 68 kDa variant gelsolin fragment is thought to take place very close to the cells producing the 68 kDa fraction, while the circulating 68 kDa fraction and full-length gelsolin protein are unlikely to be part of amyloidogenesis (Page *et al.*, 2009).

The major (8 kDa) and minor (5 kDa) amyloidogenic fragments aggregate in different tissues such as the blood vessel walls, skin, neural sheaths, autonomic nervous system and eye, disturbing their structure and function and causing the characteristic clinical manifestations of the disease (Kiuru, 1998; Kiuru-Enari *et al.*, 2002). Homozygote patients typically have a more severe disease variant, and the symptoms may begin earlier than in heterozygotes (Maury *et al.*, 1992; Ardalan, Shoja & Kiuru-Enari, 2007b). Cutaneous AGel amyloid deposits have been demonstrated to increase along the disease progression and spread to deeper dermis and subcutaneous adipose tissue over time (Pihlamaa *et al.*, 2016b). Moreover, the clinical disease severity has been shown to correlate with AGel amyloid accumulation (Pihlamaa *et al.*, 2016b). Secondly, it has been suggested that the formation of oligomeric aggregates during the amyloidogenesis process itself brings about the proteotoxicity and cytotoxicity characteristic of the disease (Solomon *et al.*, 2012). Finally, tissue damage and clinical phenotype may to some extent be a consequence of abnormal functioning of the variant gelsolin molecule prior to amyloidogenesis (Kiuru-Enari & Haltia, 2013).

### 2.2.4. Disease manifestations on organ level

#### *2.2.4.1. Ophthalmological manifestations*

The corneal manifestations are typically the first clinical signs in AGel amyloidosis. They are already visible in patients in their 3rd decade or even younger (Meretoja, 1969; Meretoja, 1973a; Kiuru, 1998) and in homozygous patients as early as at 12 to 13 years (Meretoja, 1972). AGel amyloidosis can be diagnosed based on the corneal manifestations and positive family history, which was, besides biopsies, the diagnostic method used by Meretoja himself (Meretoja, 1969; Meretoja, 1972).

The corneal manifestation of AGel amyloidosis, to date called corneal lattice amyloidosis, was previously known as corneal lattice dystrophy (CLD), lattice corneal dystrophy, type II (LD II) or gelsolin type lattice corneal dystrophy. According to the consensus of ophthalmologists, the previously used term dystrophy is considered to be a misnomer and is not to be used in the case of AGel amyloidosis, which is a systemic amyloidosis plus corneal lattice lines (Weiss *et al.*, 2015).

Immunohistochemical staining of the cornea shows amyloid strands in the anterior and mid-stroma, a continuous layer of amyloid underneath the Bowman's layer and occasionally in the epithelial basement membranes, and secondary scarring with sporadic amyloid deposits penetrating the subepithelial space (Meretoja, 1972; Loeffler, Edward & Tso, 1992; Kivelä *et al.*, 1993; Kivelä *et al.*, 1994). In addition, amyloid is seen in the conjunctiva, anterior sclera, perineurium of ciliary nerves, walls of ciliary vessels, optic nerve sheaths and stroma of the ciliary body, and along the choriocapillaris (Kivelä *et al.*, 1994). The subepithelial deposits rather than the lattice lines lead to recurrent corneal erosions and eventual loss of vision requiring corneal transplantation (Mattila *et al.*, 2015).

In addition to the corneal manifestations, blepharochalasis (recurrent eyelid oedema possibly resulting in an acquired ptosis) is frequently seen in patients with AGel amyloidosis, and elderly patients in particular may suffer from eyelid problems such as entropium (inward turning of the eyelid margin), ectropium (outward turning of the

eyelid margin) and lagophthalmos (inability to fully close the eyelids) (Kiuru, 1998; Kiuru-Enari & Haltia, 2013).

Patients report several kind of eye-related symptoms: dryness, photophobia and periodical visual impairment, which may be disturbing, especially in the morning, and progress over time (Meretoja, 1969; Winkelman *et al.*, 1971; Meretoja, 1972; Purcell *et al.*, 1983; Darras *et al.*, 1986; Kiuru, 1992; Stewart *et al.*, 2000; Rothstein *et al.*, 2002; Carrwik & Stenevi, 2009; Laine *et al.*, 2010). Loss of vision and even blindness is a possible consequence of ophthalmological changes (Meretoja, 1972; Akiya *et al.*, 1996; Ardalan *et al.*, 2007a; Lüttmann *et al.*, 2010). Open-angle glaucoma and cataract may further complicate the situation (Winkelman *et al.*, 1971; Meretoja, 1972; Purcell *et al.*, 1983; Starck *et al.*, 1991; Kiuru, 1992; Akiya *et al.*, 1996; Stewart *et al.*, 2000; Carrwik & Stenevi, 2009; Papathanassiou *et al.*, 2009).

Regular check-ups by an ophthalmologist are recommended for AGel amyloidosis patients (Carrwik & Stenevi, 2009). Because of the dryness and general irritability of the eyes, patients use artificial tears as a symptomatic treatment. Corneal transplantation improves the vision in only a minority of cases, and is associated with a high risk of graft failure (Mattila *et al.*, 2015).

#### *2.2.4.2. Neurological manifestations*

Neurological manifestations are characteristic of AGel amyloidosis, and the peripheral, autonomous and central nervous systems may all be affected. It has been shown, *in vitro*, that cells of neuronal origin have particular importance in the generation of the AGel precursor protein (Paunio *et al.*, 1998), which may be one reason for the frequent neurological manifestations of the disease. The following presents the current view on the involvement of different neurological systems.

**Peripheral nervous system involvement**

Peripheral neuropathy associated with AGel amyloidosis is predominantly associated with sensory nerve involvement (Kiuru, 1992; Kiuru & Seppäläinen, 1994). The peripheral neuropathy is typically rather mild, and the symptoms start from the distal part of the legs as numbness, paraesthesia and the loss of vibration sense. Touch and

position sensation may become affected later and usually to a lesser degree. Similarly, the tendon reflexes get weaker first in the lower extremities, followed later by changes in the upper extremities. In elderly patients with advanced disease, areflexia is possible. Motor dysfunction is rare (Kiuru, 1992) but elderly patients may show manual clumsiness (Boysen *et al.*, 1979; Darras *et al.*, 1986; Kiuru, 1992; Lüttmann *et al.*, 2010). Electrophysical finding of carpal tunnel syndrome seems to be more common than actual clinical signs, and is equally prevalent in males and females (Kiuru & Seppäläinen, 1994). At advanced age, sensory neuropathy together with ataxia may cause balance problems and even make patients bedridden (Tanskanen *et al.*, 2007).

In the peripheral nervous system, significant endoneurial and vascular amyloid deposition is seen in anterior and posterior spinal nerve roots as well as in the sensory nerve ganglia (Kiuru, Salonen & Haltia, 1999b; Kiuru-Enari *et al.*, 2002). Amyloid is also seen along the whole length of peripheral nerves (Tanskanen *et al.*, 2007) in the peri-, epi- and endoneurial and vascular structures (Meretoja & Teppo, 1971; Makishita *et al.*, 1996; Kiuru-Enari *et al.*, 2002).

The most important neurological manifestation in AGel amyloidosis is slowly progressive cranial neuropathy, particularly, in most cases bilateral, facial palsy that involves typically first the upper and subsequently the lower nerve branches of the facial nerve (cranial nerve VII) (Meretoja, 1969; Kiuru & Seppäläinen, 1994; Kiuru-Enari & Haltia, 2013; Pihlamaa *et al.*, 2016a). Facial palsy manifests in AGel amyloidosis patients in their thirties or forties (Meretoja, 1973b; Kiuru & Seppäläinen, 1994; Kiuru-Enari & Haltia, 2013) but signs of partial denervation are already seen in electrophysiological studies before clinical symptoms become apparent (Kiuru & Seppäläinen, 1994). In homozygous AGel amyloidosis patients, bifacial paresis is already seen in their teenage years (Meretoja, 1973b). Cranial nerve involvement is extremely rare in other amyloid diseases (Pihlamaa *et al.*, 2016a).

Later on, other cranial nerves may also become affected, and in particular the involvement of the hypoglossal nerve (XII) becomes frequent, and may be combined with neuropathy of the glossopharyngeal (IX) and vagal (X) nerves (Kiuru, 1992;

Conceição *et al.*, 2003; Contégal *et al.*, 2006; Ardalan *et al.*, 2007a; Shoja *et al.*, 2009; Makioka *et al.*, 2010; Asahina *et al.*, 2011; Pihlamaa *et al.*, 2016a). A recent study with 29 patients demonstrated that the trigeminal nerve (V) was affected in all of the cases, and the oculomotor (III, 69%), hypoglossal (XII, 59%) and vestibulocochlear (VIII, 48%) nerves frequently, whereas the vagal (X) and accessory (XI) nerves were only sporadically affected (Pihlamaa *et al.*, 2016a). Atrophy and fasciculation of the tongue, dysarthria, drooling and dysphagia may complicate the daily functions and social life of elderly patients (Meretoja, 1969; Kiuru, Nieminen & Partinen, 1999a; Conceição *et al.*, 2003; Tanskanen *et al.*, 2007; Shoja *et al.*, 2009; Makioka *et al.*, 2010; Lüttmann *et al.*, 2010). For some yet unknown reason, bulbar dysfunction has subsequently been reported to manifest earlier and in more severe form in patients carrying the c.640G > T gelsolin gene variant than in patients with the c.640G > A variant (Boysen *et al.*, 1979; de la Chapelle *et al.*, 1992b; Chastan *et al.*, 2006; Caress *et al.*, 2017). Finally, hearing may be compromised more often than previously thought (Meretoja, 1969; Kiuru, 1992; Hornigold *et al.*, 2006; Shoja *et al.*, 2009; Ardalan *et al.*, 2007a; Makioka *et al.*, 2010).

**Autonomic nervous system involvement**

The main autonomic nervous system manifestations are orthostatic hypotension, postural dizziness, disturbance of the gastrointestinal tract, dysuria, impotence and decreased perspiration (Kiuru, 1992; Kiuru *et al.*, 1994; Makishita *et al.*, 1994; Akiya *et al.*, 1996; Chastan *et al.*, 2006; Makioka *et al.*, 2010; Bürmann *et al.*, 2011), but even grave cardiac conduction alterations due to cardiac sympathetic denervation may be associated with AGel amyloidosis (Chastan *et al.*, 2006).

In the autonomic nervous system amyloid deposition is seen in the superior cervical ganglion. In addition, vascular, perineural and, to a small extent, endoneurial AGel amyloid deposits in the cervical sympathetic trunk and vagal nerve have been reported (Kiuru *et al.*, 1994).

**Central nervous system involvement**

Clinically significant central nervous system involvement is not common in AGel amyloidosis. However, a histopathological study has shown that there are alterations of the white matter and amyloid deposition in the spinal and cerebral blood vessel walls, meninges, spinal nerve roots and sensory ganglia (Kiuru *et al.*, 1999b). Postmortem MRI performed in a 86-year old patient disclosed large areas of diffuse signal increase in the cerebral white matter, particularly in the frontal lobes and pons (Kiuru *et al.*, 1999b). Changes in visuoconstructional and visuospatial performance in AGel patients has been demonstrated in neuropsychological tests (Kiuru *et al.*, 1995; Kantanen *et al.*, 2014). AGel amyloidosis patients may have an increased risk of depression (Kantanen *et al.*, 2014).

#### *2.2.4.3. Cutaneous manifestations*

Abnormal skin laxity, cutis laxa, hanging of the skin, is one of the characteristic features of AGel amyloidosis and the most common dermatological finding in the disease (Kiuru-Enari, Keski-Oja & Haltia, 2005).

First, cutis laxa usually manifests as loose thickened scalp and forehead skin (Meretoja, 1969; Kiuru, 1992). Later on, the laxity of the skin becomes apparent on the lower parts of the face as well as in the back, hands, elbows and knees (Kiuru-Enari & Haltia, 2013). In addition, patients may suffer from skin atrophy, dryness, itching, increased skin vulnerability and scar building, occasionally excessive bruising and loss of hair (Kiuru, 1992; Stewart *et al.*, 2000; Kiuru *et al.*, 2000; Ikeda *et al.*, 2007).

The facial cutis laxa progresses with age, remarkably changes the facial appearance and complicates the speech and other oral functions (Kiuru-Enari & Haltia, 2013). The facial symptoms may be complicated by the frequent neuropathy of facial and trigeminal nerves and facial paresis (Pihlamaa *et al.*, 2016a). Facial cutis laxa together with facial paresis and bulbar palsy problems, typical especially of aged AGel amyloidosis patients, may increase the risk for aspiration pneumonia. Moreover, the typical facial appearance causes psychosocial stress for AGel amyloidosis patients and

may contribute to the increased risk of depression (Rintala *et al.*, 1988; Laine *et al.*, 2010; Kantanen *et al.*, 2014). Symptomatic plastic surgery treatment, which needs to be repeated over the years along the disease progression, can improve the lives of AGel amyloidosis patients (Pihlamaa *et al.*, 2011).

In AGel amyloidosis patients, cutaneous amyloid deposition is found under the epidermal basement membrane and in the basement membranes or basal laminae of various cutaneous structures such as the eccrine sweat glands, sebaceous glands ad hair follicles, as well as around the smooth muscle cells, blood vessel walls and nerve perineuria (Kiuru-Enari *et al.*, 2005; Pihlamaa *et al.*, 2016b). An increasing amount of amyloid is found in patients' skin with advancing age, and amyloid spreads even to the deeper dermis and subcutaneous adipose tissue, where amyloid is found in the stroma between fat cells, in the area of the collagen fibres (Pihlamaa *et al.*, 2016b). AGel amyloid is likely to contribute to elastolysis of the skin, as in the blood vessel walls (Koskelainen *et al.*, 2016). AGel amyloid fibrils that surround the elastic fibres may have a direct elastolytic influence on them (Kiuru-Enari *et al.*, 2005; Solomon *et al.*, 2012). The histopathological finding of dermal adnexal atrophy may represent the same underlying pathology as salivary gland atrophy, which was also demonstrated earlier in AGel amyloidosis patients (Juusela *et al.*, 2013). This probably contributes to the skin dryness and hypohidrosis reported by many patients (Pihlamaa *et al.*, 2016b).

#### *2.2.4.4. Renal manifestations*

Renal manifestation of AGel amyloidosis seems to be rather rare and mild in heterozygous patients (Purcell *et al.*, 1983; Kiuru, 1992; Steiner *et al.*, 1995), but in some cases patients have even developed nephrotic syndrome (Meretoja, 1973a; Papathanassiou *et al.*, 2009), which is treated with dialysis or kidney transplantation. The reason for this wide variation in renal manifestations is unknown, and information on the incidence of severe renal disease in AGel amyloidosis is yet not available. In contrast, renal manifestations in homozygous patients are known to be severe and they may suffer from nephrotic syndrome as early as in their early twenties, proceeding

progressively to renal failure, dialysis treatment and death (Meretoja, 1973a; Maury *et al.*, 1992; Ardalan *et al.*, 2007b).

Recently, two previously unknown gelsolin gene variants, c.580G > A and c.633C > A, have been reported to cause renal amyloidosis in heterozygous patients (Sethi *et al.*, 2013; Efebera *et al.*, 2014). A patient with the c.580G > A variant presented with kidney failure but only minimal proteinuria; a kidney biopsy showed amyloid deposits in the glomeruli (Sethi *et al.*, 2013). Four out of five patients with c.633C > A variant suffered from nephrotic range proteinuria (Efebera *et al.*, 2014). None of the reported patients had signs of systemic amyloidosis.

AGel amyloid deposits in kidneys are typically restricted to the glomeruli, where amyloid is evenly distributed along the glomerular basement membranes (Meretoja *et al.*, 1972; Haltia *et al.*, 1990a; Sethi *et al.*, 2017), and interstitial amyloid deposits seem to be rare (Sethi *et al.*, 2017). In the most grave cases, the glomeruli may even be hyalinised (Meretoja *et al.*, 1972). In the case of late-onset disease, the renal involvement may remain mild even in the 9$^{th}$ decade of life (Makishita *et al.*, 1996).

#### *2.2.4.5. Cardiac manifestations and amyloid angiopathy*

Clinical cardiac manifestations in AGel amyloidosis seem to be rather rare. However, the disease may manifest with cardiac conduction defects, such as atrioventricular blocks, sinus bradycardia and sick sinus syndrome (Meretoja, 1973b; de la Chapelle *et al.*, 1992a; Kiuru, 1992; Kiuru, 1998; Chastan *et al.*, 2006). Clinically detectable cardiomyopathy is not common, at least in middle-aged patients (Kiuru *et al.*, 1994). Valvulopathies have been observed only in occasional patients (Steiner *et al.*, 1995; Kiuru, 1998; Chastan *et al.*, 2006) but they may be more common than previously thought (Laine *et al.*, 2010). AGel amyloidosis patients report more chest pain and consumption of hypertension and cholesterol-lowering drugs than their healthy family members (Laine *et al.*, 2010). One patient with heart transplantation has been reported so far (Fernández *et al.*, 1997).

In the heart, amyloid protein has been detected in the pericardium and endocardium, between the myocardial cells and in the walls of the cardiac arteries (Meretoja &

Teppo, 1971; Maury, 1990; Kiuru *et al.*, 1994). In a study with cardiac samples of three deceased AGel amyloidosis patients, no myocardial cell alteration was detected (Kiuru *et al.*, 1994). In some cases the coronary arteries may be almost totally blocked by amyloid deposits (Meretoja, 1973b), which may increase the risk of ischaemic heart events.

Generalised amyloid angiopathy is a characteristic manifestation of AGel amyloidosis (Kiuru *et al.*, 1999b). It has been recently demonstrated that amyloid angiopathy in AGel amyloidosis leads to significant changes in the arterial walls, and major AGel deposition, collagen derangement and severe elastolysis can be seen. In addition, abnormal accumulation and localisation of collagen types I and III and an increase of collagen type I degradation product is seen in the tunica media (Koskelainen *et al.*, 2016). Interestingly, there is a high level of gelsolin and MT1-MMP expression in the smooth muscle cells (Kwiatkowski *et al.*, 1988b; Su *et al.*, 2004), supposedly amplifying the changes in vessel walls. Amyloid angiopathy involves the arteries of almost every organ, including the peripheral (Makishita *et al.*, 1996; Kiuru-Enari *et al.*, 2002; Tanskanen *et al.*, 2007), autonomic (Kiuru & Seppäläinen, 1994) and, to a lesser extent, central nervous systems (Meretoja & Teppo, 1971; Haltia *et al.*, 1991; Kiuru *et al.*, 1999b). It has been shown that amyloid vessel wall involvement causing vascular wall fragility, together with altered platelet function, may lead to an increased tendency to bruises at an early age and to post-operational haemorrhages more often than in the control group (Kiuru *et al.*, 2000; Koskelainen *et al.*, 2016). Vessel wall fragility may even occasionally lead to severe vascular consequences such as dissections, ruptures and aneurysms. Moreover, arrhythmias that are common for AGel amyloidosis patients often require anticoagulant treatment which, together with the vessel wall fragility and the altered platelet function, may lead to increased risk of cerebral bleeding, a possibly fatal complication of the disease (Meretoja, 1973b), even though cerebrovascular amyloid deposits are usually minor (Kiuru *et al.*, 1999b). AGel angiopathy, together with the AGel deposition in sensory nerve ganglia, may even play a role in the development of the peripheral neuropathy that is a common manifestation of AGel amyloidosis (Kiuru-Enari *et al.*, 2002).

#### *2.2.4.6. Other inner organ manifestations*

In autopsy studies on AGel amyloidosis, amyloid has been detected in the lungs, spleen and thyroid gland but not the liver or pancreas (Meretoja, 1973b). AGel deposition has also been demonstrated in the pituitary and pineal glands (Kiuru *et al.*, 1999b). Further, accumulations associated with the basement membranes of the thyroid follicles, dermal eccrine and sebaceous glands, lacrimal (Meretoja, 1972), parotic (Meretoja & Teppo, 1971) and minor salivary glands (Juusela *et al.*, 2009) have been reported. There are no clinical studies or observations on AGel amyloidosis patients indicating that amyloidosis of these above-mentioned organs has clinical significance despite that Meretoja suggested that patients suffering from AGel amyloidosis, especially women, may have unusually high amounts of thyroid gland related clinical manifestations (Meretoja, 1973b).

#### *2.2.4.7. Amyloid myopathy*

Clinically significant myopathy is not common in AGel amyloidosis but primary muscle involvement has been reported in the facial muscles, contributing to the typical mask-like facial appearance that is mainly caused by bifacial palsy and cutis laxa (Rintala *et al.*, 1988). In elderly patients, macroglossia, dysphagia, muscle weakness in the arms and legs may be at least partially caused by amyloid myopathy (Spuler, Emslie-Smith & Engel, 1998; Kiuru-Enari *et al.*, 2002). In the skeletal muscle, amyloid has been detected in the peri- and endomysium, the basement membranes of muscle fibres and the blood vessels (Darras *et al.*, 1986; Rintala *et al.*, 1988; Kiuru, 1992).

#### *2.2.4.8. Oral manifestations*

AGel amyloidosis patients may suffer from xerostomia, which together with xerophthalmia may even lead to the misdiagnosis of Sjögren's syndrome (Juusela *et al.*, 2009). Hyposalivation with altered salivary composition may compromise the oral health of the patients, and they can be at increased risk of caries and oral candidiasis (Juusela *et al.*, 2013). It has been suggested that, in some individuals, AGel amyloidosis might even be associated with aggressive periodontitis (Juusela *et al.*,

2015). Macroglossia is rather rare in AGel amyloidosis but elderly patients in particular frequently suffer from furrowing and fasciculations of the tongue due to hypoglossal neuropathy (Kiuru, 1998; Conceição *et al.*, 2003; Chastan *et al.*, 2006; Ikeda *et al.*, 2007; Lüttmann *et al.*, 2010). Tissue laxity together with neuropathy may lead to dysarthria, dysphagia and drooling, and sleep apnoea has also been reported (Kiuru *et al.*, 1999a; Kiuru-Enari & Haltia, 2013).

### 2.2.5. Late phases of AGel amyloidosis patients

Meretoja assumed that AGel amyloidosis has possibly a minor effect on the patients' lifespan (Meretoja, 1973b), but so far systematic research on the matter has not been available. Infant mortality of patients is not increased (Meretoja, 1973a).

The causes of death (CoD) of patients are not known. Some of the manifestations of AGel amyloidosis could, however, be fatal. Generalised amyloid angiopathy (Kiuru *et al.*, 1999b) involves the arteries of almost every organ and may cause vascular wall fragility, which may lead to haemorrhages. Renal amyloidosis in homozygous patients may lead to nephrotic syndrome as early as in early adulthood (Meretoja, 1973a; Maury *et al.*, 1992). However, some heterozygotic patients also develop severe renal amyloidosis and even nephrotic symptoms, necessitating dialysis or kidney transplantation (Meretoja, 1973a; Ardalan *et al.*, 2007a; Papathanassiou *et al.*, 2009). The reported cardiac manifestations, such as conduction defects (Chastan *et al.*, 2006) and valvulopathies (Kiuru, 1998), may also be lethal. Moreover, progressive cranial neuropathy, leading to bulbar palsy, can potentially increase the risk of aspiration pneumonia (Laine *et al.*, 2010; Kiuru-Enari & Haltia, 2013). Finally, neurological manifestations, especially severe ataxia, may lead to immobilisation of the patient, and thus increase the risk of venous thrombosis and pulmonary embolism that may have fatal consequences (Tanskanen *et al.*, 2007).

### 2.2.6. Diagnostics and treatment

The clinical diagnosis of AGel amyloidosis is based on the demonstration of the pathognomonic triad of corneal lattice amyloidosis, peripheral facial paresis and cutis laxa (Meretoja, 1973a; Kiuru-Enari & Haltia, 2013). A positive family history

naturally supports the diagnosis. Diagnosis can be also confirmed histologically through skin, muscle or rectum biopsies or abdominal subcutaneous fat aspiration samples (Darras *et al.*, 1986; Kiuru, 1992; Makishita *et al.*, 1996), but nowadays biopsies are not usually necessary as the gene test is available (Paunio *et al.*, 1992). As the disorder is dominantly inherited and its penetration is 100%, detecting the 640G > A or 640G > T mutation confirms the diagnosis.

Currently, specific treatment for AGel amyloidosis is not available but symptomatic treatment may be used to improve the quality of life. Careful ophthalmological care is particularly important, and patients are advised to use artificial tear eye drops and visit an ophthalmologist regularly (Carrwik & Stenevi, 2009). Corneal transplantation has the potential to improve vision in some patients, but since the results of this treatment vary, it may be advisable to limit it to patients with bilaterally compromised vision and otherwise concentrate on improving corneal health in other ways (Starck *et al.*, 1991; Stewart *et al.*, 2000; Carrwik & Stenevi, 2009; Mattila *et al.*, 2015).

In the case of severe renal manifestations, dialysis treatment or kidney transplantation may be needed, especially in the case of homozygous (Maury *et al.*, 1992; Ardalan *et al.*, 2007b) but occasionally also heterozygous patients (Kiuru-Enari & Haltia, 2013). The result of a kidney transplantation was promising based on a case report: for the follow-up period of six years, the patient maintained stable allograft function and did not develop proteinuria (Shoja *et al.*, 2009). So far, only one case of heart transplantation has been reported (Fernández *et al.*, 1997).

The typical facial appearance, due to the bilateral facial paresis and loosening of the skin, may cause severe problems and social disability for patients and can be treated through plastic surgery (Rintala *et al.*, 1988; Starck *et al.*, 1991; Pihlamaa *et al.*, 2011). Some patients are known to have benefited from carpal tunnel syndrome operations (Kiuru-Enari & Haltia, 2013). Further, as the patients can be considered to be in an increased risk for oral problems such as caries, oral candida and xerostomia, possibly greatly affecting their quality of life, patients are likely to profit from careful oral healthcare and preventive action such as dietary advice, extra fluoride lubricants and regular visits to a dentist (Juusela *et al.*, 2013).

Possible ways to pharmacologically target the different stages of amyloid formation have been investigated. It has been noted that the abnormal fragmentation of gelsolin may be reduced by calcium chelators such as EDTA (Paunio *et al.*, 1998; Paunio *et al.*, 1994a), inhibitors of pro-protein convertases (furin) (Kangas, Seidah & Paunio, 2002) and specific tissue inhibitors of metalloproteases (TIMP) (Page *et al.*, 2005). In recent years, anti-amyloidogenic molecules, such as AGel-specific nanobodies, have been shown to protect variant gelsolin from pathological proteolysis by furin and therefore slowing down amyloidogenesis; the results have been promising in mouse models (Van Overbeke *et al.*, 2014; Van Overbeke *et al.*, 2015; Verhelle *et al.*, 2016). A recent study on the topic introduced for the first time a nanobody that protects known amyloidogenic gelsolin variants (c.640G > A, c.640G > T, 633C > A, c.580G > A). The nanobody was proven to be efficient *in vivo* in a c. elegans model (Giorgino *et al.*, 2019). So far, no signs suggesting the involvement of gelsolin protein (GSN) dysfunction in the evolution of AGel amyloidosis have been demonstrated (Juusela, 2016). However, the severity of AGel deposition has been shown to correlate with clinical severity (Pihlamaa *et al.*, 2016b) implying that AGel-genesis and deposition represent the major focus in future specific treatment goals.

# 3. Aims of the study

The current study had five specific aims:

1) to identify Finnish AGel amyloidosis patients and families, and to create a national patient registry for Finnish AGel amyloidosis patients (II)
2) to investigate whether all Finnish AGel amyloidosis patients share the same gelsolin gene variant associated haplotype in order to increase understanding on the disease origin (I)
3) to describe the characteristic natural course of AGel amyloidosis in a representative patient group, and to increase understanding of the disease progression (II, III)
4) to investigate the severity and frequency of pathological inner organ, especially cardiac, involvement in AGel amyloidosis (IV)
5) to recognise the causes of death of AGel amyloidosis patients, compare them with those of the normal Finnish population, and to increase the understanding of the disease's influence on patients' lifespan (V)

# 4. Subjects, materials and methods

## 4.1. FIN-GAR patient registry (II, III)

As part of the present study we founded and updated the National Finnish Gelsolin Amyloidosis Patient Registry (FIN-GAR). Prior to this study, information on Finnish AGel amyloidosis patients was not consolidated in one place. It was known, however, that a significant portion of the patients were members of Suomen Amyloidoosiyhdistys ry (SAMY), the Finnish national organisation for amyloidosis patients founded in 1997. SAMY was contacted, and they kindly agreed to support the research group in contacting their members. SAMY posted their members the research material both in phase I of the study in 2013 (**II**) and in phase II five years later in 2018 (**III**).

### 4.1.1. Founding and updating the FIN-GAR patient registry

The FIN-GAR questionnaires (**II**, **III**) were designed to capture information concerning the first symptoms and their time of onset, the method and time of diagnosis, and known and anticipated clinical manifestations and symptoms of AGel amyloidosis and the year of their appearance. The patients were also asked for genealogical information concerning how they had inherited the disease and their habitual residence. The phase II FIN-GAR questionnaire (**III**) was created based on the questionnaire in phase I, and more signs and symptoms were included in order to obtain more precise picture of the disease course. In phase II, the patients were also asked their subjective view on the disease progression in different organs and organ systems in the past five years. The questionnaires (in Finnish) are available in the Appendices section at the end of this book.

The FIN-GAR phase I questionnaire was sent to all of SAMY's members, at that time 228 patients. The members of SAMY were also asked to act as index patients and invite other affected family members to take part in the study. In addition to the living patients, 16 deceased patients who had been part of previous studies of the research group were included in the registry. Between October 2013 and August 2014, a total

of 227 AGel amyloidosis patients were included in the FIN-GAR registry. The data obtained from the questionnaire were supplemented by data from the patient records of the Hospital District of Helsinki and Uusimaa (HUS). The research group members also contacted most of the patients (87%) in the registry by telephone in order to verify and complete the data given by the patients in the questionnaires. During the phone calls, the patients were also asked for more precise genealogical information, i.e. from which parent and possibly also from which grandparent they inherited the disease, and whether they knew any relatives, including those who had died, who carried the disease.

The FIN-GAR registry was updated five years after it was founded (**III**). Five patients had been added to the registry between 2014 and 2019, and the number of patients in the registry in the beginning of the update was 232. As in phase I, SAMY sent the research material to their members (n = 240). Based on the information supplemented by patients, data in the FIN-GAR registry was updated. New patients (n = 29) who had not been included in the registry in phase I were added. The patient inclusion process in FIN-GAR phase II is presented in Figure 3.

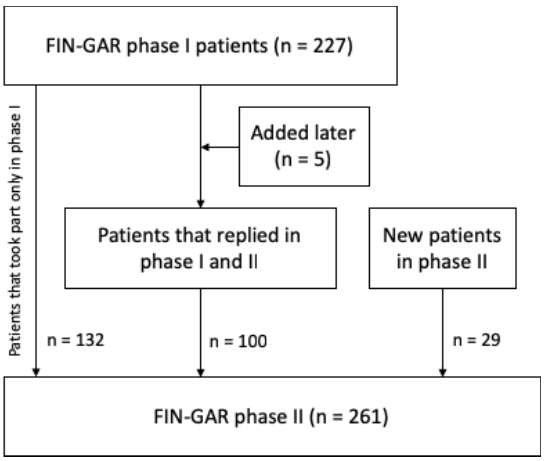



**Figure 3 In the updated FIN-GAR phase II registry the data of 162 patients was obtained from the FIN-GAR phase I registry. Of the patients who were included in the FIN-GAR phase I registry, 100 responded to the phase II questionnaire and their data was updated. In addition, 29 new patients were included in the registry.**

### 4.1.2. Statistical methods

Frequencies and median age of onset of symptoms/signs were calculated. Distributions of the continuous variables were studied and tested for normality. The cumulative frequency of the symptoms (**II**) and operations (**III**) according to the patient's age at the time of their occurrence was outlined. The statistical analyses were done using IBM SPSS Statistics software (SPSS, Inc., New York, USA) versions 19.0 and 25.0.

## 4.2. Genealogical data and haplotype analysis (I)

### 4.2.1. Genealogical data

Genealogical data concerning the Finnish AGel amyloidosis families had been collected over the decades as part of research projects of the research group, and some genealogical data was available from the AGel amyloidosis families. These data were significantly increased during the process of founding the FIN-GAR patient registry. The patients were asked in the FIN-GAR questionnaires (**II**, **III**) from which family and from which city or village they descend. In addition, the vast majority (87%) of the patients included in the FIN-GAR registry in phase I were contacted by telephone and more precise information concerning the gene carrier ancestors and other gene carrier relatives, including those who had already passed away, was asked. The information provided to us was verified and completed using civil and parish registries. Through this process, 79 Finnish families including a total of 707 AGel amyloidosis patients were identified.

### 4.2.2. Subjects

Out of the identified AGel amyloidosis families, those that had at least one member in the FIN-GAR patient registry were enrolled in the study as the registry patients had given consent to contact them for research purposes. One to two index patients were chosen from each family and asked to give a blood sample. The gender distribution resulted in being uneven, because there were 31% male and 69% female patients in the FIN-GAR registry at the time of patient selection. Altogether 71 index patients

representing 64 families, 81% of the identified families, took part in the study. Most of the patients had already been tested to carry the c.640G > A mutation, and for the rest of the patients, mutant gene verification was done as part of the study. All of the study patients signed informed consent. After quality control, 68 patient DNA samples representing 62 families (78%) were available for analysis. The patient selection process is illustrated in Figure 4 and baseline information on the study subjects is presented in Table 3.

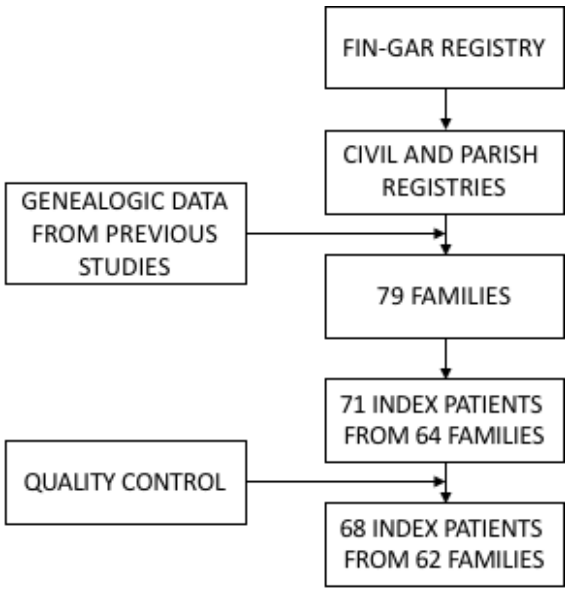



**Figure 4 Patient selection process (I).**

**Table 3 Baseline information on the 68 study subjects.**

| **Parameter** | **N (%)** |
|---|---|
| **Gender distribution** | 22 male (32%), 46 female (68%) |
| **Mean age** | 62 years |
| **Characteristic manifestations of the disease*** | Ophthalmological symptoms: 67 (99%)<br>Neurological symptoms: 56 (82%)<br>Skin symptoms: 59 (87%) |

* Both or one of the most common two symptoms of each category (corneal dystrophy gelsolin type (LCD2) and eye dryness, facial nerve paresis and polyneuropathy, cutis laxa and drooping eyelids).

### 4.2.3. DNA isolation and SNP arrays

Patients' DNA was isolated from the red cells using the standard procedure of the Wizard® Genomic DNA Purification kit. The DNA samples were genotyped using CoreExome single nucleotide polymorphism (SNP) arrays and GenomeStudio v. 2011.1 software (Illumina, Inc., San Diego, CA, USA). The SNP array contains different tag SNPs and detects these markers from samples. Additional quality control for identity by descent and sex was performed using PLINK software, which is a computer program for SNP data handling.

### 4.2.4. Haplotype analysis

Patients' haplotypes were analysed using the homozygosity haplotype method (Miyazawa *et al.*, 2007). Markers with a minor allele frequency of at least 10% within the genotyped patient samples and a genotyping rate of 95% or more were included in the analysis. The 10% minor allele frequency cut-off was chosen to exclude markers with low informativeness. It was still confirmed using two different methods that the identical haplotype at the gelsolin gene locus associated with the mutation c.640G > A was not a random finding, existing in the general Finnish population.

## 4.3. Histological study (IV)

### 4.3.1. Subjects and materials

The histological study (**IV**) was based on 25 AGel amyloidosis patient tissue specimens (heart, kidney, liver, lung, spleen, pancreas and thyroid gland) that were collected at regular autopsies at the time of their death. In addition, the patients' death certificates, autopsy reports and patient records (when available) were used as material. Baseline information on the patients in **Study IV** is presented in Table 4.

**Table 4 Baseline information of the subjects in Study IV.**

| | **Male, N = 20 (80 %)** | **Female, N = 5 (20 %)** |
|---|---|---|
| **Mean age at death, years (range)** | 68.4 (44.4-88.6) | 65.7 (52.0-81.4) |
| **Year of death** | 1983-2012 | 1997-2008 |
| **Forensic autopsy, N (%)** | 11 (55.0%) | 3 (60.0%) |

The study patients were identified based on the death certificates (N = 272) collected for the study concerning the causes of death and life span of Finnish AGel amyloidosis patients (**V**), as death certificates include information on the autopsy hospital and the autopsy identification number. Autopsy had been conducted for 82 patients (30%), and the tissue samples of 25 deceased AGel amyloidosis patients were stored and available for analysis. The autopsy reports and the tissue samples were collected from the provincial archives of the National Archives, the National Institute for Health and Welfare (THL), Helsinki Biobank and the hospitals in which the autopsies had been conducted. The antemortem diagnosis of AGel amyloidosis was based on genetic testing in 10 (40%), clinical diagnosis in 11 (44%) and positive family history with typical clinical manifestations in 4 (16%) of cases.

### 4.3.2. Histological stainings and analysis

Paraffin sections from autopsy specimens were stained with HE, Congo red, and Herovici stains for routine histological evaluation, and with original rabbit antisera against variant gelsolin (Haltia *et al.*, 1990a; Haltia *et al.*, 1990b) and antisera against TTR for immunohistochemical analyses. The antisera against TTR used in the study had been created against ATTRwt but has shown to also be able to detect at least TTR Lys70Glu based amyloid (Raivio *et al.*, 2016). Because there are only a few known cases of ATTR mutation in Finland, in this study we assumed that the detected ATTR based amyloid was wild type TTR protein. The stainings that were conducted are presented in Table 5.

**Table 5 Stainings completed for different tissue samples.**

| | Sample (N) | Staining | | | | |
|---|---|---|---|---|---|---|
| | | Histological | | | IHC | |
| | | HE | Congo Red | Herovici | AGel | ATTRwt |
| **Heart** | | | | | | |
| **Parenchyma** | 25 (100%) | x | x | x | x | x |
| **Blood vessels** | 25 (100%) | x | x | x | x | x |
| **Kidney** | | | | | | |
| **Parenchyma** | 22 (88.0%) | x | x | - | x | - |
| **Blood vessels** | 22 (88.0%) | x | x | - | x | - |
| **Lung** | 24 (96.0%) | x | x | - | x | - |
| **Liver** | 23 (92.0%) | x | x | - | x | - |
| **Pancreas** | 15 (60.0%) | x | x | - | x | - |
| **Spleen** | 14 (56.0%) | x | x | - | x | - |
| **Thyroid gland** | 8 (32.0%) | x | x | - | x | - |

IHC = immunohistochemistry, HE = haematoxylin and eosin, AGel = amyloid protein in hereditary gelsolin amyloidosis, ATTRwt = amyloid protein in wild type transthyretin amyloidosis

The criteria for amyloid positiveness was red to green birefringence in Congo red staining, and immunohistochemical staining was used to verify the amyloid protein type. The amount of amyloid was estimated semi-quantitatively in both the organ parenchyma and the arteries using a 4-step grading (0-3) presented in Table 6. In addition, the amount of fibrosis in the cardiac muscle was graded on a scale of 0-3 (Table 7).

### 4.3.3. Statistical analysis

Parametric correlations of the amount of amyloid in different organs and the age at death were analysed using Pearson′s correlation coefficient. An Independent Samples t- Test was performed to analyse the heart mass under different disease conditions. Finally, Fisher's exact test was performed to study the relationship between severe coronary disease and excess myocardial fibrosis. In all analyses, p values less than 0.05 were considered to be statistically significant. IBM SPSS Statistics software version 25.0 (SPSS, Inc., New York, USA) was used for the analysis.

**Table 6 Grading of amyloid in organs and blood vessels in Congo red.**

| Grade | Organ parenchyma | Blood vessels |
|---|---|---|
| **0** | No amyloid | No amyloid |
| **1** | Small amount of amyloid in one or few locations | Small amount of amyloid in one or few blood vessel walls |
| **2** | Moderate amount of amyloid in several locations | Moderate amount of amyloid in several blood vessels |
| **3** | Large amount of amyloid throughout the specimen | Large amount of amyloid in several blood vessels |

**Table 7 Grading of connective tissue in the myocardium in Herovici stain.**

| Grade | Myocardium |
|---|---|
| **0** | Normal amount of connective tissue |
| **1** | Small amount of excess fibrosis in one or few locations |
| **2** | Moderate amount of excess fibrosis in several locations |
| **3** | Large amount of excess fibrosis throughout the specimen |

## 4.4. Late phases of AGel amyloidosis (III, V)

The study concerning the causes of death (CoD) and life span of AGel amyloidosis patients (**V**) was based on 272 death certificates of deceased AGel amyloidosis patients. The underlying CoD was analysed for 267 patients and the immediate CoD for 203 patients. The underlying cause of death is the disease which started the series of illnesses proceeding directly to death. The immediate cause of death is the disease, failure or injury whose symptoms cause the person to die. The mechanisms of death are not considered to be immediate causes of death (StatFin, 2018).

### 4.4.1. Subjects and materials

The study patients were identified in the process of creating the FIN-GAR patient registry (**II**). While contacting the registry patients by telephone, information about deceased relatives with AGel amyloidosis was requested. Due to the characteristic signs of the disease, namely progressive bilateral facial paresis and cutis laxa, in particular older patients can be recognised based on their clinical symptoms even by laypeople. After that, the Finnish Population Register Centre was searched for patients' identity numbers and their death certificates (n = 272) were collected from the Archive of death certificates at Statistics Finland. Information about the patients

whose death certificates were available is presented in Table 8. Information concerning the underlying and immediate causes of death (CoD) and the age at death was extracted from the death certificates. In addition, information concerning a possible autopsy was recorded and used later in **Study IV**.

**Table 8 Background information on AGel amyloidosis patients with death certificate available.**

| | **Patients n = 272** | |
|---|---|---|
| | ***Underlying* CoD available n = 267** | ***Immediate* CoD available n = 203** |
| Female | 141 (52.8%) | 108 (53.2%) |
| Male | 126 (47.2%) | 95 (46.8%) |
| **Age at death** | | |
| 30- to 59-year-olds | 27 (10.1%) | 15 (7.4%) |
| 60- to 69-year-olds | 49 (18.4%) | 32 (15.8%) |
| 70- to 79-year-olds | 102 (38.2%) | 78 (38.4%) |
| 80- to 99-year-olds | 89 (33.3%) | 78 (38.4%) |
| **Year of death** | | |
| 1969-1974 | 14 (5.2%) | n/a |
| 1975-1984 | 40 (15.0%) | n/a |
| 1985-1994 | 68 (25.5%) | 58 (28.6%) |
| 1995-2004 | 68 (25.5%) | 68 (33.5%) |
| 2005-2014 | 77 (28.8%) | 77 (37.9%) |

CoD = cause of death, n/a = information unavailable

The lifespan of AGel amyloidosis patients was investigated as part of **Study III** and **Study V**. In **Study V**, information concerning the age at death was extracted from the death certificates only (n = 231). In **Study III**, the lifespan was calculated based on survival information of 478 living and deceased AGel amyloidosis patients. Information concerning living patients was extracted from the FIN-GAR phase I patient registry (n = 208) (**II**) and information concerning deceased patients from the death certificates (n = 272) (**V**); two subjects were removed from the data in quality control prior to the analysis.

### 4.4.2. Comparison data and statistical methods

Statistical data on the underlying and immediate causes of death in the general Finnish population were used for comparison; the data was obtained from Statistics Finland. Comparison data for the underlying CoD were available beginning in 1969, and for

the immediate CoD in 1987. Only patients who had died during these years or later were included in the sample. Because all of the patients were over the age of 30 at death, the comparison data included only data on people who died at the age of 30 or older.

The CoDs of the patients were compared to the CoDs of the general population by calculating the proportional mortality ratios (PMR), standardised for sex and age. The PMR shows whether a particular cause of death is more likely for the patient group than for the general population (in which case PMR > 1) or less likely (in which case PMR < 1). If the confidence interval is above 1 for a particular disease, the number of deaths for patients significantly exceeds the number of expected deaths based on the general population (and if the confidence interval is below 1, it is significantly smaller) at the $p < 0.05$ level (Hennekens, Buring & Mayrent, 1987). Assuming that the number of deaths followed a Poisson distribution, chi-square distribution was used to calculate the exact 95% confidence intervals (CI) for the PMRs.

In **Study V,** the patients' lifespan was calculated based on the information extracted from the death certificates. Because comparison data were only available from 1980 onwards, the sample included only patients who died in 1980–2014 (n = 231). Because age at death was skewed, we logarithmically transformed the data to calculate the geometric mean age at death and the associated 95% confidence interval. After that, the results were compared to statistical data on age at death among the general Finnish population, including persons who had died at the age of 30 or older as AGel amyloidosis rarely manifests in persons younger than that.

In **Study III**, the lifespan of the patients was calculated using the relative-survival (RS) framework based on information including both living and deceased patients. Relative survival is the ratio of the all-cause survival of the patients, $S_i(t)$, to the all-cause survival that would be expected $S_i^*(t)$ (Dickman & Coviello, 2015):

$$RS = \frac{\frac{1}{n}\sum_{i=1}^{n} S_i(t)}{\frac{1}{n}\sum_{i=1}^{n} S_i^*(t)}$$

If relative survival is 1, the patient group's survival equals the comparison group's survival. The relative-survival framework does not take into account the cause of death, but is the difference between the total all-cause mortality rate of the patients and the expected mortality rate of a comparable population; in this case, age, gender and calendar year matched the Finnish general population. Information concerning the general Finnish population was obtained from Statistics Finland. For the purpose of running the analysis, disease onset was set to the age of 30 years, and the survival was estimated in five-year time intervals forward until all the patients were deceased. Stata Statistics/Data Analysis program (version 13, StataCorp, College Station, TX) was used for the relative-survival analysis.

## 4.5. Ethical considerations

All of the individual studies presented here were approved by suitable Ethical Committees of HUS (**I** 190/13/03/01/15, **II** HUS 200/13/03/01/13, **III** HUS/1028/2018, **IV** HUS/1835/2016, **V** HUS 200/13/03/01/13). Patients taking part in Studies I, II and III had read patient information material and signed informed consent. In **Study IV**, permissions for collecting autopsy reports and tissue samples were received from the National Institute for Health and Welfare (THL) (THL/1565/5.05.01/2015), the Finnish Ministry of Social Affairs and Health (STM/734/2016) and the Regional State Administrative Agencies (ESAVI/1763/03.02.01/2017). In addition, permission for using tissue samples was obtained from the Helsinki Biobank (HUS/359/2017) and from the hospitals in which the autopsies had been conducted.

# 5. Results

## 5.1. Genetic background of Finnish patients (I)

Based on the genealogical data gathered during previous projects of the research team and from patients during the founding of the FIN-GAR patients registry, and information collected from civil and parish registers, 79 AGel amyloidosis families representing 707 patients could be identified, including deceased patients.

The earliest suspected patients were born in the 1820s. A total of 29 (37%) families originated from Kymenlaakso, which represents only 3% of the Finnish population A second geographical concentration of patients with 10 (13%) families, was found in Lammi, a municipality located in Southern Tavastia. In addition, nine (11%) families originated from areas of the former south-east of Finland, now part of Russia.

In the analysis, blood samples from 68 patients representing 62 families were utilised; only patients belonging to the FIN-GAR patient registry were contacted as they had given consent to contact them for further research projects. All of the studied patients were verified to have the c.640G > A gelsolin gene mutation. A shared 17 SNP long haplotype at the *GSN* locus spanning 361 kb was identified (Figure 5, Table 9). In a genome-wide analysis of the SNP array data, this was the largest haplotype shared by all patients (Figure 6). Among the 62 families, 61 shared a very large haplotype of 1290kb with 55 markers (coordinates 9:123074244–124364349). Thus, 98% of the families have a larger haplotype and a single family reduced the shared haplotype block to 361 kb. A recent recombination event has taken place in this family between markers rs10818516 and rs10760160 (Table 9).

**Figure 5 Haplotype area in chromosome 9 that is shared between all subjects. The gelsolin (*GSN*) c.640G>A variant is marked in red. The 17 array- genotyped SNPs that make up the haplotype are listed under visualizations of genes located in the area.**

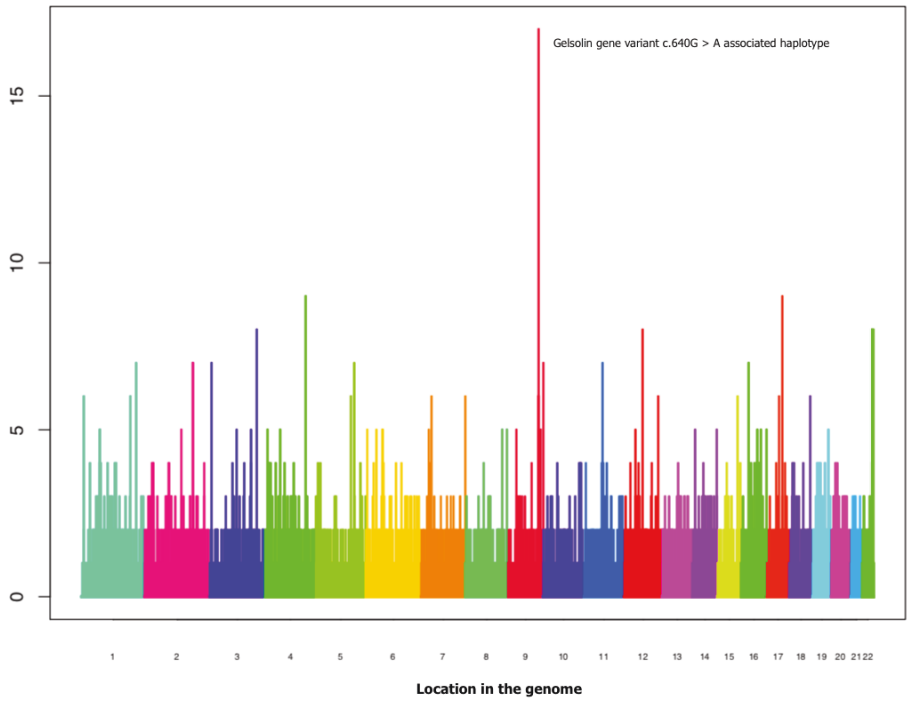



**Figure 6 Genome-wide shared haplotype analysis. Shared haplotypes were detected using the homozygosity haplotype method from SNP array data. The highest peak corresponding to the largest detected shared haplotype between all subjects encompasses the gelsolin gene area and contains the gelsolin c.640G > A mutation.**

By permutating the marker data, it was estimated that the likelihood of a 17-SNP haplotype occurring by chance would be very unlikely, zero occurrence in 10 000 simulated permutations of the data. We also tested whether *GSN* locus haplotype sharing is a common feature of the Finnish population. We empirically tested shared haplotypes in a population-based sample of elderly Finns (Vantaa 85+, n = 300) using the same marker set and methodology. The same number of samples (n = 68) was drawn randomly 10 000 times, and we calculated how many times the *GSN* locus appeared as a shared haplotype. This analysis showed no shared haplotype at the *GSN* locus in any of the 10 000 random samplings. Taken together, the results of the study supported the hypothesis that the Finnish patients have inherited the gene mutation c.640G > A from a common ancestor.

**Table 9 The 361 kb and 1290 kb shared haplotypes.**

| Reference SNP cluster ID | Coordinate (GRCh37) | Genotype | Frequency in general Finnish population |
|---|---|---|---|
| **rs917873** | chr9:123074244 | A | 0,85 |
| **rs2159994** | chr9:123075135 | G | 0,86 |
| **rs1112863** | chr9:123122187 | G | 0,56 |
| **rs1537282** | chr9:123124504 | C | 0,6 |
| **rs7852998** | chr9:123133385 | C | 0,59 |
| **rs7030519** | chr9:123144243 | G | 0,23 |
| **rs914592** | chr9:123170167 | G | 0,15 |
| **rs4837768** | chr9:123170733 | G | 0,79 |
| **rs2297454** | chr9:123171755 | T | 0,74 |
| **rs41296081** | chr9:123239643 | G | 0,02 |
| **rs17343890** | chr9:123349699 | G | 0,46 |
| **rs7861226** | chr9:123351121 | A | 0,22 |
| **rs2416760** | chr9:123374639 | T | 0,27 |
| **rs1324473** | chr9:123404610 | C | 0,75 |
| **rs966396** | chr9:123453281 | C | 0,75 |
| **rs2416799** | chr9:123480866 | T | 0,75 |
| **rs2900177** | chr9:123497645 | T | 0,75 |
| **rs7026635** | chr9:123550027 | A | 0,7 |
| **rs10760117** | chr9:123586737 | G | 0,58 |
| **rs881375** | chr9:123652898 | T | 0,42 |
| **rs10435844** | chr9:123668199 | G | 0,42 |
| **rs1014530** | chr9:123685092 | T | 0,46 |
| **rs3761847** | chr9:123690239 | G | 0,46 |
| **rs7039505** | chr9:123705945 | T | 0,58 |
| **rs4836835** | chr9:123757412 | G | 0,43 |
| **rs17611** | chr9:123769200 | C | 0,5 |
| **rs2300932** | chr9:123770436 | A | 0,36 |
| **rs34552775** | chr9:123785738 | T | 0,02 |
| **rs7031128** | chr9:123791936 | C | 0,28 |
| **rs7029523** | chr9:123817613 | T | 0,28 |
| **rs10985140** | chr9:123822837 | G | 0,42 |
| **rs10818503** | chr9:123850770 | G | 0,37 |
| **rs10733651** | chr9:123858194 | G | 0,37 |
| **rs10818504** | chr9:123860689 | C | 0,53 |
| **rs10156413** | chr9:123867782 | T | 0,35 |
| **rs2146838** | chr9:123876305 | G | 0,45 |
| **rs4836840** | chr9:123914592 | T | 0,37 |
| **rs10818516** | chr9:123955756 | G | 0,53 |
| **rs10760160** | chr9:124003326 | C | 0,55 |
| **rs10760165** | chr9:124021254 | T | 0,58 |
| **rs3827677** | chr9:124060879 | C | 0,75 |
| **rs306761** | chr9:124075544 | G | 0,67 |
| **rs767769** | chr9:124098336 | A | 0,48 |
| **rs17086** | chr9:124125520 | A | 0,68 |
| **rs424829** | chr9:124160616 | C | 0,74 |
| **rs177698** | chr9:124200417 | T | 0,68 |
| **rs4837853** | chr9:124213962 | T | 0,59 |
| **rs3816094** | chr9:124222033 | T | 0,61 |
| **rs10985274** | chr9:124259751 | T | 0,61 |
| **rs7854285** | chr9:124288948 | C | 0,74 |
| **rs4837876** | chr9:124295569 | T | 0,57 |
| **rs556331** | chr9:124336665 | A | 0,3 |
| **rs914388** | chr9:124343306 | G | 0,48 |
| **rs2777319** | chr9:124362502 | C | 0,62 |
| **rs7021714** | chr9:124364349 | T | 0,36 |

## 5.2. Natural course of the disease (II & III)

In phase I in 2014-2015 227 AGel amyloidosis patients were included in the FIN-GAR patient registry. The FIN-GAR phase II questionnaire was returned by 129 patients, 54% of SAMY members. Out of these 129 patients, a total of 100 patients had taken part in the FIN-GAR phase I study in 2013-2014 (47% of the living patients in the FIN-GAR phase I study), and 29 patients were included in the registry as new patients. As the result, the FIN-GAR registry currently includes a total of 261 patients. Baseline information for the patients in FIN-GAR patient registry after the registry update is presented in Table 10.

**Table 10 Baseline information for the FIN-GAR patient registry after the registry update.**

| | |
|---|---|
| Number of patients | 261 |
| Gender distribution | 183 (70%) female, 78 (30%) male |
| Mean age of the patients | 62.7 years (range 26.3-85.8 years) |
| Median age of the patients | 64.7 years (interquartile range 54.9-71.2 years) |
| Mean age at diagnosis | 40.7 years (range 10.4-68.7 years) |
| Genetic testing done | 138 (53%) |
| Follow-up time | 4.92 years (4.06-5.62) |

### 5.2.1. Ophthalmological manifestations

The results of the FIN-GAR phase II study are presented in detail in Table 11. Corneal lattice amyloidosis (previously called lattice corneal dystrophy, type II) was the first ophthalmological sign to appear (median age at diagnosis 42 years) and 231 (89%) of the patients suffered from it. Dry eyes were an even more common problem affecting 242 (93%) patients (median age at onset 45 years). Photophobia and tearing were asked about separately only in the phase II questionnaire and the results are based on those 129 patients. Photophobia was reported by 101 (78%) patients (median age at onset 49 years) and tearing by 34 (26%) patients (median age, 56 years). Cataract was reported by 123 (47%) patients (median age, 62 years), and glaucoma by 60 (23%) patients (median age, 61 years). Three out of four (74%) of the patients told that their ophthalmological symptoms had progressed in the past five years (Figure 9).

Nearly half (46%) of the patients used artificial tears, and 197 (75%) visited an ophthalmologist regularly. The share of the patients receiving symptomatic ophthalmological treatment is presented in Table 12.

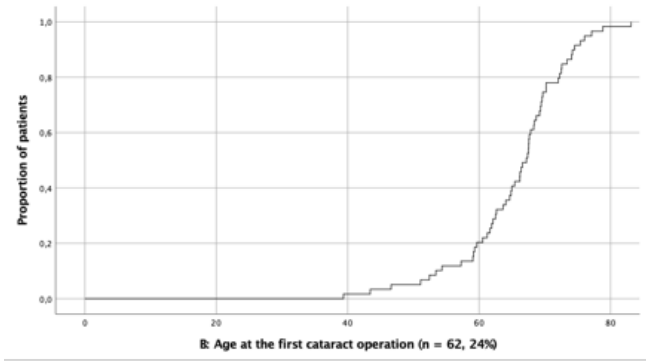
At least one cataract operation had been completed for 62 (24%) patients. The median age at the time of the first cataract operation was 67 years. Cumulative prevalence of selected operations is presented in Figure 7.

### 5.2.2. Cutaneous and soft tissue manifestations

The most prevalent cutaneous symptom was drooping eye lids, caused by cutis laxa and further worsened by facial nerve paresis, reported by 225 (86%) patients (median age at onset 50 years). Cutis laxa, abnormal general loosening of the skin, was reported by 219 (84%) patients (median age, 50 years). Dry skin was reported by 206 (79%) patients (median age, 40 years) and itchiness of the skin by 85 (33%) patients (median age, 49 years). Subsequently, 110 (85%) patients regularly used moisturising skin creams. The skin of the patients appears to be vulnerable: 180 (69%) patients suffered from a tendency to bruising and 82 (64%) patients reported getting wounds easily.

Manifestations of AGel amyloidosis may also include pathological changes in soft tissues other than the skin. Varicose veins were reported by 39 (30%) patients (median age, 36 years) and haemorrhoids by 39 (29%) (median age, 42 years) patients. Hernias of different types were reported by 30 (11%) patients (median age, 49 years), and 21 (8%) patients reported at least one prolapse of any type (median age, 41 years). Of the patients who took part in both studies, 95 (72%) had the perception that their skin and other soft tissue related symptoms had progressed during the follow-up time (Figure 9).

Skin or other soft tissue surgeries had been performed for 170 (65%) patients. On average, patients had gone through 2.4 skin or soft tissue operations (range 1-16, Figure 8). The median age at the time of the first skin operation was 53 years (interquartile range 48-72 years).



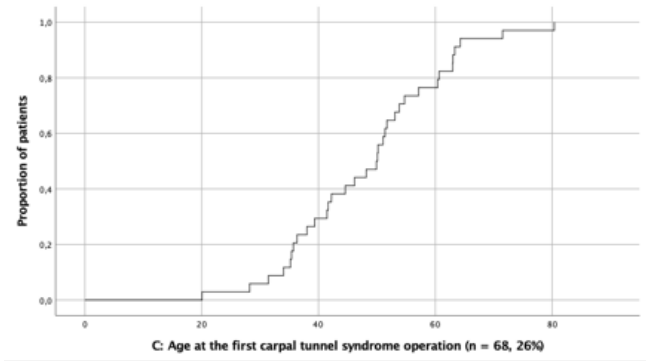




**Figure 7 Cumulative frequency of selected surgeries in 261 patients with AGel amyloidosis in the FIN-GAR phase II registry. A: Skin or other soft tissue surgeries (n=170, 65%). B: Cataract surgeries (n=62, 24%). C: Carpal tunnel syndrome surgeries (n=68, 26%).**

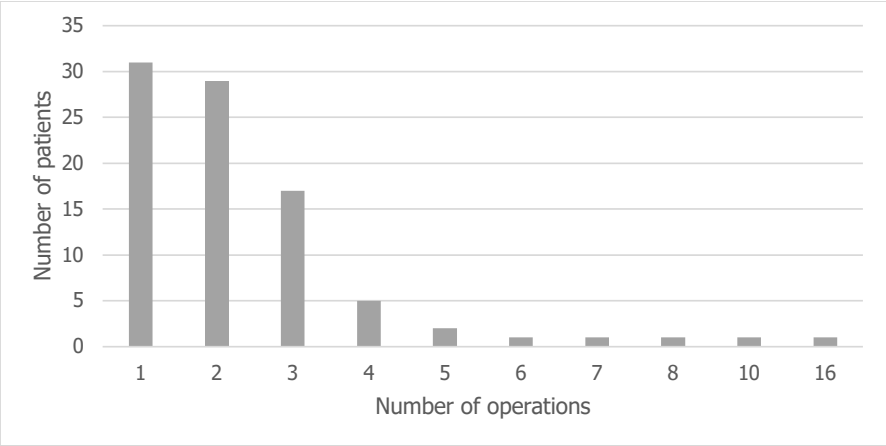



**Figure 8 Number of skin operations per operated patient (n = 89) including eyelid and other operations performed on the face, hernia and prolapse operations and varicose vein operations.**

### 5.2.3. Neurological manifestations

The most prevalent neurological symptoms were numbness, tingling and other paraesthesias, reported by 195 (75%) patients (median age at onset 56 years). Facial paresis in 176 (67%) become symptomatic in the patient at a median age of 50 years. Other frequent neurological symptoms were myokymia (54%, 48 years), carpal tunnel syndrome (42%, 50 years), balance impairment (39%, 64 years) and dysarthria (31%, 61 years). Patients with dysarthria reported to have special difficulties articulating clearly outdoors in cold weather. Approximately half of the patients (53%) stated that they had had progression in the neurological symptoms during the follow-up time (Figure 9). Of 111 (43%) patients with diagnosed carpal tunnel syndrome, 68 (61%) underwent surgery at a median age of 50 years.

### 5.2.4. Cardiovascular manifestations

In the updated questionnaire, we asked separately about cardiac arrhythmias other than atrial fibrillation, whereas in the previous questionnaire there was only a general question concerning arrhythmias. Of the patients who handed in the updated questionnaire 20 (15%) reported cardiac arrhythmias and six (5%) atrial fibrillation.

**Table 11 Frequency of symptoms and median age at their onset.**

| **Eyes** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
|---|---|---|---|---|---|
| Dry eyes | 93% (90-96) | 242 | 261 | 45 | 37-51 |
| Corneal lattice amyloidosis | 89% (85-92) | 231 | 261 | 42 | 34-51 |
| Photophobia | 78% (71-86) | 101 | 129 | 49 | 39-61 |
| Impaired vision | 78% (73-83) | 204 | 261 | 51 | 41-61 |
| Corneal ulcer | 50% (44-56) | 131 | 261 | 49 | 40-60 |
| Cataract | 47% (41-53) | 123 | 261 | 62 | 57-68 |
| Tearing | 26% (19-34) | 34 | 129 | 56 | 46-62 |
| Glaucoma | 23% (18-28) | 60 | 261 | 61 | 51-66 |
| | | | | | |
| **Skin and soft tissues** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
| Drooping eye lids | 86% (82-90) | 225 | 261 | 50 | 42-57 |
| Cutis laxa | 84% (79-88) | 219 | 261 | 50 | 42-57 |
| Dry skin | 79% (74-84) | 206 | 261 | 40 | 17-54 |
| Bruising | 69% (63-75) | 180 | 261 | 39 | 26-47 |
| Wounds, skin vulnerability | 64% (55-72) | 82 | 129 | 34 | 15-49 |
| Itchy skin | 33% (27-38) | 85 | 261 | 49 | 29-60 |
| Varicose veins | 30% (22-38) | 39 | 129 | 36 | 29-47 |
| Haemorrhoids | 29% (21-37) | 38 | 129 | 42 | 30-52 |
| Hernia of any type | 11% (8-15) | 30 | 261 | 49 | 41-63 |
| Prolapse of any type | 8% (5-11) | 21 | 261 | 41 | 32-53 |
| | | | | | |
| **Neurology** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
| Numbness, tingling and other paraesthesias | 75% (69-80) | 195 | 261 | 56 | 44-63 |
| Facial paresis | 67% (62-73) | 176 | 261 | 50 | 44-59 |
| Myokymia | 54% (48-60) | 140 | 261 | 48 | 41-58 |
| Carpal tunnel syndrome | 43% (36-49) | 111 | 261 | 50 | 37-61 |
| Balance impairment | 39% (33-45) | 103 | 261 | 64 | 55-70 |
| Dysarthria | 31% (26-37) | 81 | 261 | 61 | 54-68 |
| | | | | | |
| **Kidneys** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
| Proteinuria | 13% (9-18) | 35 | 261 | 54 | 35-59 |
| Renal failure | 5% (3-8) | 14 | 261 | 63 | 61-71 |
| | | | | | |
| **Heart** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
| Arrhythmias | 16% (9-22) | 20 | 129 | 52 | 30-66 |
| Ischaemic heart disease | 9% (4-14) | 12 | 129 | 54 | 43-73 |
| Cardiomyopathy | 5% (2-8) | 13 | 261 | 58 | 43-65 |
| Valvulopathy | 5% (3-8) | 14 | 261 | 61 | 35-73 |
| Atrial fibrillation | 5% (1-8) | 6 | 129 | 54 | N/A |
| Cardiac failure | 5% (1-8) | 6 | 129 | 61 | 42-74 |
| | | | | | |
| **Oral health** | **Frequency (95% CI)** | **n** | **Total** | **Median age (y)** | **Interquartile range (y)** |
| Dry mouth | 43% (36-49) | 111 | 261 | 55 | 41-63 |
| Caries | 43% (34-51) | 55 | 129 | 24 | 6-39 |
| Gingivitis | 19% (12-26) | 25 | 129 | 54 | 32-59 |
| | | | | | |
| **Other symptoms** | **Frequency (95% CI)** | **n** | **Total** | | |
| Pain | 38% (32-44) | 99 | 261 | | |
| Oedema | 28% (22-33) | 72 | 261 | | |
| Malignancy | 15% (9-21) | 19 | 129 | | |
| Depression | 13% (7-19) | 17 | 129 | | |
| Impaired hearing | 43% (37-49) | 112 | 261 | | |
| Hypothyreosis | 10% (6-13) | 25 | 261 | | |
| Sleep apnoea | 6% (3-9) | 15 | 261 | | |

Altogether six (5%) suffered from cardiac failure and 12 (9%) had ischaemic heart disease. Cardiomyopathy was reported by 13 (5%) (n = 13) patients. A pacemaker was installed for 12 (5%) patients, and eight (6%) patients reported any kind of cardiac surgery of which four were bypass surgery or angioplastias. The majority (61%) of the patients were not aware of any cardiovascular signs or symptoms, excluding hypertension and hypercholesterolemia. However, 12 (9%) patients reported that they had experienced progression in their cardiac symptoms during the follow-up (Figure 9).

### 5.2.5. Renal manifestations

Proteinuria had been detected in 35 (13%) of the patients (median age at onset 54 years). Renal failure was reported by 14 (5%) patients, and two patients (2%) used medication for renal disease. Moreover, three (1%) patients in the FIN-GAR registry received or had previously received dialysis treatment, and kidney transplantation had been performed for five (2%) patients. Conversely, 111 (86%) patients who replied to the updated questionnaire reported no kidney-related signs or symptoms, and only 6 (5%) had the perception that their renal symptoms had progressed during the follow-up (Figure 9).

### 5.2.6. Other manifestations

Regarding oral health, 111 (43%) patients reported dry mouth, 55 (43%) caries and 25 (19%) gingivitis. One fifth of the patients (19%) reported no oral health issues but just over one third (35%) reported progression in them.

Patients also reported other symptoms possibly linked to AGel amyloidosis based on previous studies. Impaired hearing was reported by 112 (43%) patients and 23 (9%) patients had a hearing aid or cochlear implant. Oedema as an unspecific symptom, possibly related to renal problems in some patients, was reported by 72 (28%) patients. Sleep apnoea was reported by 15 (6%), hypothyroidism by 25 (10%), different kinds of pains by 99 (38%), cancer by 19 (15%) and depression by 17 (13%) patients.

**Table 12 Symptomatic treatment of AGel amyloidosis patients.**

| Symptomatic treatment | Frequency | n | Total |
|---|---|---|---|
| Moisturising skin creams | 85% | 110 | 129 |
| Skin and soft tissue operations | 65% | 170 | 261 |
| Moisturising eye drops | 46% | 121 | 261 |
| Carpal tunnel syndrome operation | 26% | 68 | 261 |
| Cataract operation | 24% | 62 | 261 |
| Hearing aid or implant | 9% | 23 | 261 |
| Pacemaker | 5% | 12 | 261 |
| Kidney transplantation | 2% | 5 | 261 |
| Dialysis | 1% | 3 | 261 |

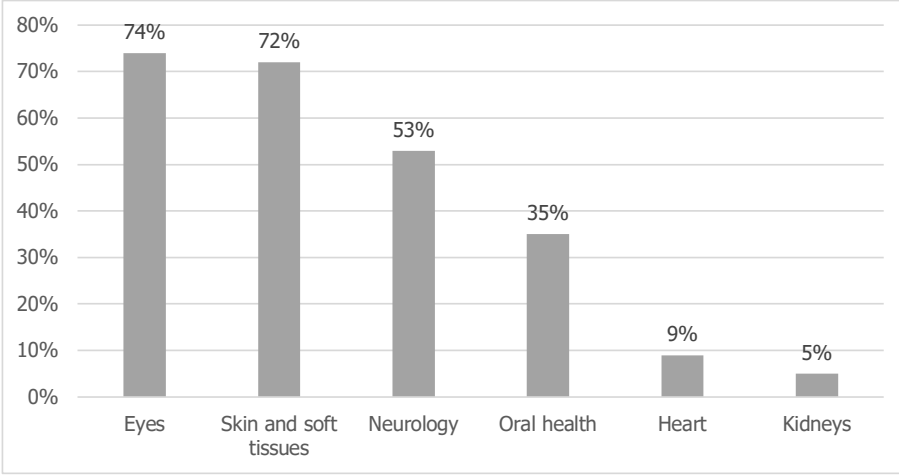



**Figure 9 Share of patients who answered that their symptoms related to different organs/organ systems had progressed during the follow-up time (5 years).**

## 5.3. Pathological disease manifestations in inner organs (IV)

Autopsy specimens and autopsy reports of a total of 25 deceased AGel amyloidosis patients were investigated. Congo red positive amyloid was detected in every subject (100%), and in each case the amyloid protein was based on the low molecular weight gelsolin subunit (AGel) verified by immunohistochemistry. AGel was found on average in four out of the five organs investigated per patient. In five patients with more than one organ available, AGel was detected in every 4-7 organs investigated. In the following, the results are presented per organ. The results are summarised in Table 13.

**Table 13 Frequency and amount of amyloid in the internal organs and blood vessels.**

| | Sample (N) | Amyloid | | | | |
|---|---|---|---|---|---|---|
| | | Present | | | | Not present |
| | | N (%) | G1 N (%) | G2 N (%) | G3 N (%) | |
| **Heart** | | | | | | |
| **Parenchyma** | 25 (100%) | 25 (100%) | 18 (72.0%) | 5 (20.0%) | 2 (8.0%) | 0 (0.0%) |
| **Blood vessels** | 25 (100%) | 25 (100%) | 7 (28.0%) | 17 (68.0%) | 1 (4.0%) | 0 (0.0%) |
| **Kidney** | | | | | | |
| **Parenchyma** | 22 (88.0%) | 13 (59.1%) | 12 (54.5%) | 1 (4.5%) | 0 (0.0%) | 9 (40.9%) |
| **Blood vessels** | 22 (88.0%) | 17 (77.3%) | 11 (50.0%) | 6 (27.3%) | 0 (0.0%) | 5 (22.7%) |
| **Other organs** | | | | | | |
| **Lung** | 24 (96.0%) | 20 (83.3%) | 14 (58.3%) | 5 (20.8%) | 1 (4.2%) | 4 (16.7%) |
| **Liver** | 23 (92.0%) | 15 (65.2%) | 14 (60.9%) | 1 (4.3%) | 0 (0.0%) | 12 (34.8%) |
| **Pancreas** | 15 (60.0%) | 5 (33.4%) | 4 (26.7%) | 1 (6.7%) | 0 (0.0%) | 10 (66.7%) |
| **Spleen** | 14 (56.0%) | 8 (57.1%) | 7 (50.0%) | 0 (0.0%) | 1 (7.1%) | 6 (42.9%) |
| **Thyroid gland** | 8 (32.0%) | 8 (100.0%) | 1 (12.5%) | 5 (62.5%) | 2 (25.0%) | 0 (0.0%) |

G0: no amyloid, G1: small amount of amyloid in one or few locations/blood vessel walls, G2: moderate amount of amyloid in several locations/blood vessels, G3: large amount of amyloid throughout the specimen/in several blood vessels.

### 5.3.1. Findings from autopsy reports

A pacemaker had been installed in three (12%) patients. Valvular disorder was diagnosed in two (8%) and cardiac insufficiency in 10 (40%) patients. Arteriosclerosis of the coronary arteries was reported in 17 (68%), and myocardial infarction, either an old scar or more acute infarction, in 13 (52%) patients.

Heart mass was on average 557 g (N = 19, SD 123 g, range 294-770 g) in males and 445 g (N = 5, SD 134 g, range 315-610 g) in females. There was no statistically significant difference in heart mass in patients with or without coronary heart disease, valvular disorder, cardiac failure or with or without a pacemaker installed.

### 5.3.2. Heart

AGel was detectable in the myocardium in every autopsied subject. In most cases, the amount of amyloid was minor and mostly located as thin streaks between the myocardial cells (Figure 11). In two elderly male patients, who died at the age of 80 and 84 years of cardiac disease, large amounts (grade 3) of amyloid were seen throughout the specimen. Both of them suffered from atrial fibrillation, and one had a pacemaker installed due to bradycardia. However, in one, there was also a large amount of ATTRwt based amyloid in the myocardium that was not co-localised with the AGel amyloid. In addition, ATTRwt was found in small amounts in the myocardial samples in nine (36%) other patients. AGel was detected in the cardiac blood vessel walls in all 25 patients. The amount of amyloid in the myocardium correlated with the age of death ($p = 0.026$) as illustrated in Figure 10. Interestingly, the amount of myocardial amyloid also correlated with the amount of pulmonary ($p = 0.007$) and hepatic ($p = 0.003$) amyloid.

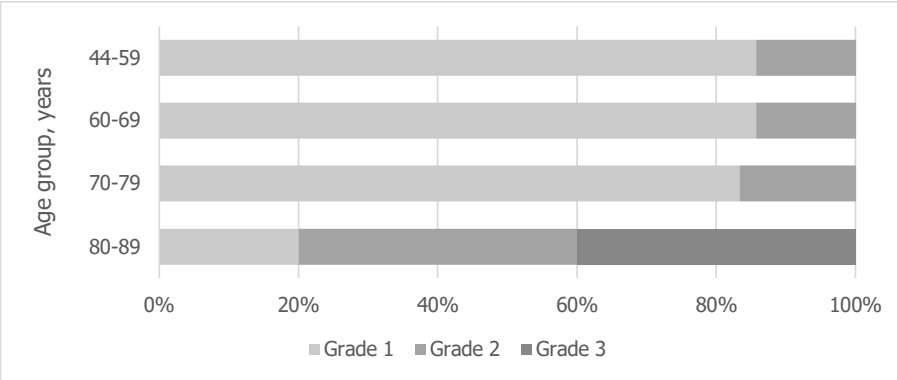



**Figure 10 Proportion of patients in each age group according to amount of amyloid (grade 1-3) in the myocardium in 25 autopsied individuals with AGel amyloidosis.**

Three male patients had encountered sudden cardiac death at the rather young ages of 44, 45 and 59. They all had amyloid in their myocardium and in the cardiac arteries but the role of amyloidosis in these cases remained unclear in the autopsy reports as Congo red staining had not been conducted as part of the histological investigation in any of the cases.

Every subject showed some degree of fibrosis in the myocardium. In 14 (56%) patients, fibrosis was minor (grade 1) and in 11 (44%) patients, the accumulation of fibrosis was moderate to large (grade 2-3). Interestingly, myocardial amyloid deposits co-localised with the fibrotic regions.

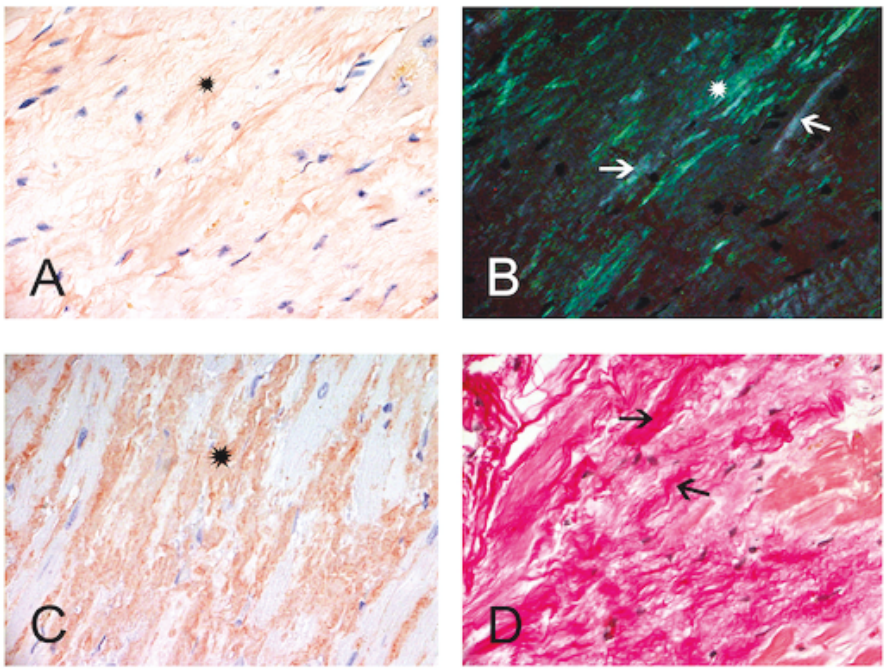



**Figure 11 Amyloid deposits (asterisk) in the myocardium of a patient with AGel amyloidosis (A, B), composed of mutated gelsolin fragments (C), between the collagen fibres (arrows, B, D). Staining methods: Congo red (A) with polarisation (B), IHC against mutated gelsolin fragment (C), Herovichi (D). Original magnification: 400.**

### 5.3.3. Lungs

At least one lung specimen was available for 24 (96%) patients, and in 20 (83%) of them Congo red positive amyloid was present. Immunohistochemistry against the AGel subunit was positive even more often: in 23/24 (96%). Amyloid was seen in both the lung parenchyma and the blood vessels, in small arteries and in capillaries, mainly in small amounts.

### 5.3.4. Kidneys

Kidney specimens were available for 22 (88%) patients and in 13 (59%) of them amyloid was detected in the renal parenchyma. Similarly to the lungs, immunohistochemistry against low molecular weight AGel subunit was even more often positive (20/22, 92%). Amyloid protein in the kidney parenchyma was in all cases concentrated in the glomeruli. As in the case of the cardiac samples, the amount of amyloid in the glomeruli correlated with the age at death ($p = 0.039$). Amyloid was seen more frequently in the renal blood vessels than in the glomeruli (17/22, 77%).

### 5.3.5. Thyroid gland

Few specimens were available from the thyroid gland (n = 8, 32%), which all contained amyloid. In seven patients (87.5%) there was a moderate to large (grade 2-3) amount of amyloid. Amyloid in the thyroid gland accumulated strongly in the blood vessel walls, and in every case also in the basement membranes of the follicles. A minute amount of amyloid was seen in the stroma. Again, all of the specimens showed positivity against the rabbit antisera (Haltia *et al.*, 1990a; Haltia *et al.*, 1990b) against the low molecular weight gelsolin subunit.

### 5.3.6. Liver, pancreas and spleen

Amyloid was found less frequently in the specimens from the liver, pancreas and spleen, and in these organs exclusively in the blood vessels.

## 5.4. Causes of death, retirement and lifespan (III, V)

Proportional mortality ratios (PMR) for underlying and immediate causes of death (CoD) for selected medical conditions in the entire patient group, as well as for male and female patients separately, are presented in Table 14 and Table 15, respectively. The following presents the results with statistically significant difference between the patients and the general population.

### 5.4.1. AGel amyloidosis

AGel amyloidosis was considered the underlying CoD in 53 (19.9%) patients (*PMR* = 114.2; 95% *CI* 85.6-149.4). The difference between the patients and the general population was similar in both male (*PMR* = 156.8; 95% *CI* 102.5-229.8) and female (*PMR* = 91.3; 95% *CI* 60.2-132.8) patients. The control group included various other diagnoses belonging to the disease category "Other endocrine, nutritional and metabolic diseases", whereas the study patients had solely AGel amyloidosis as their underlying CoD. In the three oldest age groups, the portion of patients diagnosed with AGel amyloidosis as their underlying CoD correlated with their age at death, rising as high as 27% in the group aged 80-99 years. The most common immediate CoD of the patients diagnosed with AGel amyloidosis as their underlying CoD were AGel amyloidosis (n = 18) and pneumonia (n = 17).

### 5.4.2. Cancer

Cancer was significantly less common (9.7%) as an underlying CoD in the entire patient group than in the general population (*PMR* = 0.47; 95% *CI* 0.31-0.69) or in either men (*PMR* = 0.46; 95% *CI* 0.25-0.79) or women (*PMR* = 0.44; 95% *CI* 0.23-0.75) separately. We also noted analogous statistically significant differences in age groups 70-79 years (*PMR*=0.29; 95% *CI* 0.12-0.60) and 80-99 years (*PMR*=0.41; 95% *CI* 0.13-0.97) separately. We also studied possible differences in fatal cancer

**Table 14 Proportional mortality ratios (PMR) for selected medical conditions for AGel amyloidosis patients, underlying causes of death.**

| | | All n = 267 | | | Male n = 126 | | | Female n = 141 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Disease category** | **ICD-10** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** |
| Malignant neoplasms | C00-C97 | 26 (9.7%) | 55.4 (20.7%) | 0.47 (0.31-0.69) | 13 (10.3%) | 28.0 (22.2%) | 0.46 (0.25-0.79) | 13 (9.2%) | 29.6 (21.0%) | 0.44 (0.23-0.75) |
| Endocrine, nutritional and metabolic diseases | E00-E90 | 53 (19.9%) | 0.46 (0.2%) | 114.2 (85.6-149.4) | 26 (20.6%) | 0.17 (0.1%) | 156.8 (102.5-229.8) | 27 (19.1%) | 0.30 (0.2%) | 91.3 (60.2-132.8) |
| Ischaemic heart diseases | I20-I25 | 67 (25.1%) | 73.1 (27.4%) | 0.92 (0.71-1.16) | 33 (26.2%) | 38.4 (30.5%) | 0.86 (0.59-1.21) | 34 (24.1%) | 36.0 (25.5%) | 0.94 (0.65-1.31) |
| Other heart diseases excl. rheumatic heart diseases | I30-I425, I427-I52 | 9 (3.4%) | 13.8 (5.2%) | 0.65 (0.30-1.24) | 4 (3.2%) | 5.3 (4.2%) | 0.76 (0.21-1.94) | 5 (3.5%) | 8.4 (6.0%) | 0.59 (0.19-1.38) |
| Cerebrovascular diseases | I60-I69 | 24 (9.0%) | 30.2 (11.3%) | 0.79 (0.51-1.18) | 11 (8.7%) | 11.6 (9.2%) | 0.95 (0.47-1.70) | 13 (9.2%) | 19.3 (13.7%) | 0.67 (0.36-1.15) |
| Other diseases of the circulatory system | I00-I15, I26-I28, I70-I99 | 16 (6.0%) | 12.6 (4.7%) | 1.27 (0.73-2.06) | 6 (4.8%) | 5.2 (4.1%) | 1.16 (0.43-2.53) | 10 (7.1%) | 7.6 (5.4%) | 1.31 (0.63-2.41) |
| Pneumonia | J12-J18, J849 | 9 (3.4%) | 10.1 (3.8%) | 0.89 (0.41-1.69) | 6 (4.8%) | 4.6 (3.6%) | 1.31 (0.48-2.85) | 3 (2.1%) | 5.5 (3.9%) | 0.55 (0.11-1.60) |
| Diseases of the genitourinary system | N00-N99 | 5 (1.9%) | 3.2 (1.2%) | 1.54 (0.50-3.59) | 2 (1.6%) | 1.2 (1.0%) | 1.65 (0.20-5.95) | 3 (2.1%) | 2.1 (1.5%) | 1.43 (0.30-4.18) |
| Other accidents and sequelae of accidents | W20-W64, W75-X39, X50-X59, Y85-Y86 | 2 (0.7%) | 2.0 (0.7%) | 1.01 (0.12-3.64) | 2 (1.6%) | 1.1 (0.9%) | 1.76 (0.21-6.37) | 0 (0.0%) | 0.56 (0.4%) | 0.00 (0.00-6.55) |
| Suicides and sequelae of intentional self-harm | X60-X84, Y870 | 4 (1.5%) | 5.1 (1.9%) | 0.78 (0.21-1.99) | 3 (2.4%) | 2.9 (2.3%) | 1.05 (0.22-3.06) | 1 (0.7%) | 1.1 (0.8%) | 0.91 (0.02-5.08) |

CI = confidence interval, grey colour = statistically significant findings

**Table 15 Proportional mortality ratios (PMR) for selected medical conditions for AGel amyloidosis patients, immediate causes of death.**

| | | All n = 267 | | | Male n = 126 | | | Female n = 141 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Disease category** | **ICD-10** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** | **Observed n (%)** | **Expected n (%)** | **PMR (95% CI)** |
| Malignant neoplasms | C00-C97 | 26 (9.7%) | 55.4 (20.7%) | 0.47 (0.31-0.69) | 13 (10.3%) | 28.0 (22.2%) | 0.46 (0.25-0.79) | 13 (9.2%) | 29.6 (21.0%) | 0.44 (0.23-0.75) |
| Endocrine, nutritional and metabolic diseases | E00-E90 | 53 (19.9%) | 0.46 (0.2%) | 114.2 (85.6-149.4) | 26 (20.6%) | 0.17 (0.1%) | 156.8 (102.5-229.8) | 27 (19.1%) | 0.30 (0.2%) | 91.3 (60.2-132.8) |
| Ischaemic heart diseases | I20-I25 | 67 (25.1%) | 73.1 (27.4%) | 0.92 (0.71-1.16) | 33 (26.2%) | 38.4 (30.5%) | 0.86 (0.59-1.21) | 34 (24.1%) | 36.0 (25.5%) | 0.94 (0.65-1.31) |
| Other heart diseases excl. rheumatic heart diseases | I30-I425, I427-I52 | 9 (3.4%) | 13.8 (5.2%) | 0.65 (0.30-1.24) | 4 (3.2%) | 5.3 (4.2%) | 0.76 (0.21-1.94) | 5 (3.5%) | 8.4 (6.0%) | 0.59 (0.19-1.38) |
| Cerebrovascular diseases | I60-I69 | 24 (9.0%) | 30.2 (11.3%) | 0.79 (0.51-1.18) | 11 (8.7%) | 11.6 (9.2%) | 0.95 (0.47-1.70) | 13 (9.2%) | 19.3 (13.7%) | 0.67 (0.36-1.15) |
| Other diseases of the circulatory system | I00-I15, I26-I28, I70-I99 | 16 (6.0%) | 12.6 (4.7%) | 1.27 (0.73-2.06) | 6 (4.8%) | 5.2 (4.1%) | 1.16 (0.43-2.53) | 10 (7.1%) | 7.6 (5.4%) | 1.31 (0.63-2.41) |
| Pneumonia | J12-J18, J849 | 9 (3.4%) | 10.1 (3.8%) | 0.89 (0.41-1.69) | 6 (4.8%) | 4.6 (3.6%) | 1.31 (0.48-2.85) | 3 (2.1%) | 5.5 (3.9%) | 0.55 (0.11-1.60) |
| Diseases of the genitourinary system | N00-N99 | 5 (1.9%) | 3.2 (1.2%) | 1.54 (0.50-3.59) | 2 (1.6%) | 1.2 (1.0%) | 1.65 (0.20-5.95) | 3 (2.1%) | 2.1 (1.5%) | 1.43 (0.30-4.18) |
| Other accidents and sequelae of accidents | W20-W64, W75-X39, X50-X59, Y85-Y86 | 2 (0.7%) | 2.0 (0.7%) | 1.01 (0.12-3.64) | 2 (1.6%) | 1.1 (0.9%) | 1.76 (0.21-6.37) | 0 (0.0%) | 0.56 (0.4%) | 0.00 (0.00-6.55) |
| Suicides and sequelae of intentional self-harm | X60-X84, Y870 | 4 (1.5%) | 5.1 (1.9%) | 0.78 (0.21-1.99) | 3 (2.4%) | 2.9 (2.3%) | 1.05 (0.22-3.06) | 1 (0.7%) | 1.1 (0.8%) | 0.91 (0.02-5.08) |

CI = confidence interval, grey colour = statistically significant findings

types. Cancers of the larynx, trachea, bronchus and lungs caused death statistically significantly less often in the patient group (*PMR* = 0.18; 95% *CI* 0.02-0.64). Patients also had fewer cases of fatal breast cancers (*PMR* = 0.22; 95% *CI* 0.01-1.23), but the difference did not reach statistical significance. Of all the cancer types studied, only melanoma occurred more often as a CoD in AGel amyloidosis patients than in the general population (*PMR* = 1.31; 95% *CI* 0.03-7.31), but the difference was not statistically significant.

### 5.4.3. Genitourinary diseases

Diseases of the genitourinary system were overrepresented in the female patients as the immediate CoD (*PMR* = 2.82; 95% *CI* 1.13-5.81). Moreover, the entire patient group was 2.29 times more likely to die of a renal complication (95% *CI* 0.99-4.52), but this difference was not statistically significant. All but one of these patients (*n* = 8) died of a renal disease (one died of an unclear haemorrhage). Their *immediate* CoDs were glomerular disease related to amyloidosis, acute tubule-interstitial nephritis and nephrotic syndrome (two cases of each) and acute renal failure (one case).

### 5.4.4. Retirement and lifespan

Of the male and female patients, 42 (54%) and 99 (54%) had retired at mean age of 57.9 and 59.1 years, respectively.

In **Study V**, the age at death of the patients was compared with that of persons that had died at the age of 30 years or older in the normal population (n = 231). The female patients lived on average 78.0 years (95% *CI* 76.4-79.5), which is 1.9 years less than females in the general population (80.1 years), a statistically significant difference. The age at death of the male patients was 73.9 years (95% *CI* 72.0-75.6), which does not statistically differ from the general population (72.1 years).

In **Study III**, the life span was calculated utilising the relative-survival framework (n = 478), and the result of the previous **Study V** was confirmed in that the relative survival in AGel amyloidosis is comparable to that of the general Finnish population: in the age group of 70-74 years, that is 30 to 40 years after the first symptoms, the relative survival is 0.96 for both male and female patients. After that the observed

survival becomes lower than expected survival in both genders, as is seen in Figure 12, Figure 13 and Table 16.

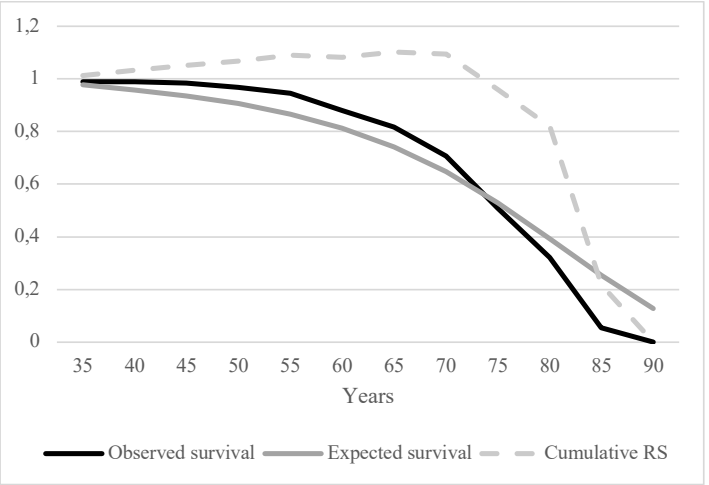



**Figure 12 Relative survival of male AGel amyloidosis patients.**

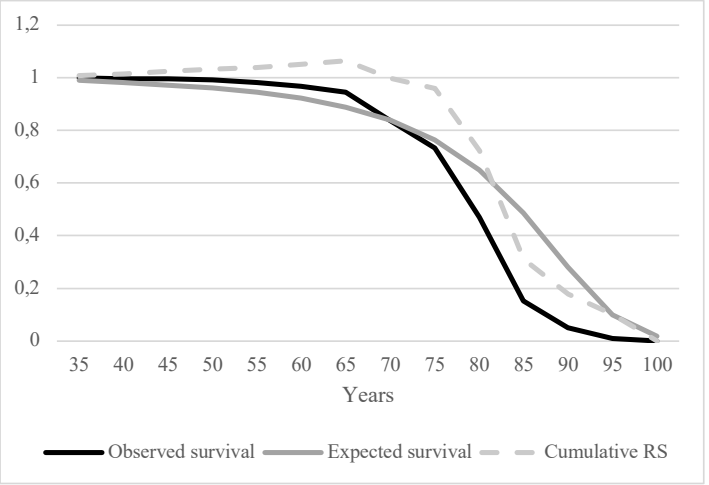



**Figure 13 Relative survival of female AGel amyloidosis patients.**

**Table 16 Relative survival in figures, male and female patients.**

| Male | | | | |
|---|---|---|---|---|
| **Age group** | **n at the beginning** | **Observed survival** | **Expected survival** | **Cumulative relative survival** |
| 30-34 | 188 | 0.9893 | 0.9777 | 1.0119 |
| 35-39 | 185 | 0.9893 | 0.9577 | 1.0330 |
| 40-44 | 183 | 0.9839 | 0.9360 | 1.0511 |
| 45-49 | 180 | 0.9674 | 0.9065 | 1.0671 |
| 50-54 | 174 | 0.9448 | 0.8669 | 1.0899 |
| 55-59 | 165 | 0.8800 | 0.8136 | 1.0817 |
| 60-64 | 145 | 0.8172 | 0.7415 | 1.1021 |
| 65-69 | 125 | 0.7073 | 0.6468 | 1.0936 |
| 70-74 | 97 | 0.5085 | 0.5300 | 0.9594 |
| 75-79 | 62 | 0.3221 | 0.3927 | 0.8200 |
| 80-84 | 36 | 0.0552 | 0.2550 | 0.2165 |
| 85-89 | 5 | 0.0000 | 0.1274 | 0.0000 |

| Female | | | | |
|---|---|---|---|---|
| **Age group** | **n at the beginning** | **Observed survival** | **Expected survival** | **Cumulative relative survival** |
| 30-34 | 290 | 1.0000 | 0.9907 | 1.0094 |
| 35-39 | 287 | 0.9965 | 0.9820 | 1.0147 |
| 40-44 | 284 | 0.9965 | 0.9728 | 1.0244 |
| 45-49 | 280 | 0.9929 | 0.9610 | 1.0332 |
| 50-54 | 267 | 0.9813 | 0.9448 | 1.0387 |
| 55-59 | 246 | 0.9688 | 0.9220 | 1.0507 |
| 60-64 | 222 | 0.9459 | 0.8883 | 1.0648 |
| 65-69 | 195 | 0.8370 | 0.8386 | 0.9982 |
| 70-74 | 149 | 0.7339 | 0.7641 | 0.9605 |
| 75-79 | 110 | 0.4703 | 0.6494 | 0.7241 |
| 80-84 | 59 | 0.1513 | 0.4863 | 0.3112 |
| 85-89 | 17 | 0.0504 | 0.2809 | 0.1796 |
| 90-94 | 5 | 0.0101 | 0.0994 | 0.1015 |
| 95-99 | 1 | 0.0000 | 0.0182 | 0.0000 |

# 6. Discussion

## 6.1. Genetic background

One aim of this study was to identify Finnish AGel amyloidosis patients and their ancestors, and to investigate whether all Finnish patients share a common ancestor or whether the disease is caused by sporadic mutations in the known mutational hot spot in the gelsolin gene. First, we created the FIN-GAR patient registry and then identified 79 AGel amyloidosis families through patient interviews, existing genealogical data and civil and parish registries. Based on the collected information we conducted the largest haplotype analysis in AGel amyloidosis so far, which indicated that all the studied 62 Finnish AGel amyloidosis families are likely to have a common ancestor.

There are different theories on how Finland was initially populated. Archaeological evidence supports the suggestion that there has been a scarce permanent population in Finland since the last glacial period. The southern and western coastal regions have had permanent settlement for at least 2000 years. Who these people were is not exactly known. They were likely to be northern European peoples living nearest to the ice border, but probably also those who, as the arctic climate withdrew, returned to the north (Norio, 2003b). Vast parts of the country were settled permanently only after 1550 (Kere, 2001). At that time the Swedish King Gustavus of Vasa (1523–1560), also governing Finland, gave tax benefits to people moving from Savonia in South-East Finland further north. It has been estimated that the size of the Finnish population had grown to 250 000 by the 1600s (Kere, 2001). Population density in the large country was still very low, and due to geographical isolation, it was likely that the people selected their mating partner within the same group, enriching certain genes locally. Genetic bottlenecks such as the great famines in 1695-1697 and 1866-1868 further accelerated the enrichment of some disease-causing genes and losses of other genes. The Finnish population grew unusually rapidly at the end of the 19$^{th}$ and in the 20$^{th}$ century: in 1751–1760 the Finnish population was on average 457 000 people and by the 1990s there were over 5 million inhabitants (Kere, 2001). Most of the diseases belonging to the Finnish Disease Heritage are autosomal recessive. Because of the founder effect, genetic bottlenecks and geographical isolation, most patients suffering

from these diseases are still found around the areas of late settlement, populated from the 1500s. Internal movement after World War II has, however, somewhat reduced this effect.

Two autosomal dominant diseases belong to the Finnish Disease Heritage, AGel amyloidosis and tibial muscular dystrophy (TMD). Even though autosomal dominant diseases do not require isolation to occur, regional concentration is also seen in them. Interestingly, most of the families identified as part of this study originate from the Kymenlaakso region in south-eastern Finland. In addition, a significant number of families originate from Lammi in Southern Tavastia, located in southern Finland. Meretoja himself speculated that the common ancestor may have been a man called Matti of Oravalae who might have lived in the 14$^{th}$ or 15$^{th}$ century in Lammi. He would have brought the disease to Valkeala, located in Northern Kymenlaakso, later on (Meretoja, 1976). The third most common origin of the AGel amyloidosis families was areas of the former south-east of Finland, now part of Russia. To our knowledge, no cases of AGel amyloidosis have been reported from that area. The age of the disease-causing mutation was not calculated in this study, and the genealogical data did not reach the point where all the stems possibly come together, so we were not able to verify Meretoja's hypothesis. However, the age of the mutation is currently being investigated as a separate project.

In this study, all the investigated Finnish AGel families were shown to share a common haplotype which is associated with the gelsolin gene variant, and thus to share a common ancestor. The alternative hypothesis was that there are several independent mutations causing AGel amyloidosis in Finland, as it has been previously suggested that there is a mutational hot spot in this specific location c.640G, causing AGel amyloidosis in different ethnic groups (de la Chapelle *et al.*, 1992b; Paunio *et al.*, 1995). In Japan at least two independent haplotypes have been reported (Taira *et al.*, 2012). Paunio showed that the haplotype of two Japanese families, similar to each other, is different to that in the Finnish investigated families (Paunio *et al.*, 1995). Interestingly, one family in the United States has been identified to carry the same haplotype as the Finnish AGel amyloidosis families (Steiner *et al.*, 1995). In this case the ancestors originated from Åland, an island belonging to Finland's governance, and

from Stockholm. No other cases of AGel amyloidosis have been reported from these geographical areas. No worldwide haplotype analyses in non-Finnish *GSN* c.640G > A families have so far been performed. The existing research indicates that in addition to the shared Finnish haplotype, sporadic mutations in c.640G in the gelsolin gene have taken place, and that at least in three cases the mutation has spread from Finland through migration (Steiner *et al.*, 1995; Alabdali *et al.*, 2015; de Souza *et al.*, 2017).

## 6.2. Natural course of the disease

As part of this study we founded the FIN-GAR patient registry and updated it five years later. In the first phase, 227 patients were included in the registry, and in the second phase the size of the registry grew to 261 patients. As we estimate the number of patients to be between 600 and 1000, the FIN-GAR registry can be approximated to represent 25-40 % of the Finnish patients, which can be considered to be a representative series of patients suffering from a rare disease.

The patients become symptomatic around their 40$^{th}$ birthday, ophthalmological symptoms being typically the first to appear. In most cases the diagnosis is done by an ophthalmologist based on the finding of corneal lattice amyloidosis. Only in rare cases does the disease manifest with severe signs, but as patients may be symptomatic for decades and the disease is progressive and systemic, older patients in particular typically have a wide variety of symptoms affecting several organ systems. Consequently, the disease burden may be significant, and patients need a variety of symptomatic treatments. Manifold symptoms and signs belonging to the classical triad of ophthalmological, neurological and cutaneous manifestations were shown to be highly prevalent.

Previously it was known that AGel amyloidosis may manifest with cardiac conduction defects, such as atrioventricular blocks, sinus bradycardia and sick sinus syndrome (Kiuru, 1998; Chastan *et al.*, 2006; Meretoja, 1973b; Kiuru, 1992; de la Chapelle *et al.*, 1992a). In a recent study of 51 middle-aged patients, abnormalities in cardiac conduction were found in 43% of the patients, first-degree atrioventricular block being the most common (Mustonen *et al*, manuscript submitted). Signs of amyloid cardiopathy have been reported to be rare at clinical examination and in radiologic-,

echocardio- and electrocardiographic examinations in middle-aged patients (Kiuru *et al.*, 1994). Valvulopathies have been observed in occasional patients (Steiner *et al.*, 1995; Kiuru, 1998; Chastan *et al.*, 2006). Only one case of heart transplantation has been reported (Fernández *et al.*, 1997). This study confirmed the previous assumption that clinical cardiac manifestations in AGel amyloidosis are rare, especially if compared with other systemic amyloidoses such as AL (Kyle & Gertz, 1995; Dubrey *et al.*, 1998) and selected pathological ATTR variants (Thr60Ala, Val122Ile) (Banypersad *et al.*, 2012). However, the study revealed that 4.6% of the patients have a pacemaker, whereas the prevalence of pacemakers in the general adult population is 0.5% (Bradshaw *et al.*, 2014), supporting the assumption that AGel amyloidosis may manifest as cardiac conduction defects. The low frequency of severe cardiac manifestations is likely to contribute to the normal lifespan of the patients.

Clinically significant renal manifestations in AGel amyloidosis have been considered to be rather rare and mild in heterozygous patients (Kiuru, 1992; Steiner *et al.*, 1995; Purcell *et al.*, 1983), which was also confirmed by this study. In a study of 10 AGel amyloidosis patients, $^{123}$I-SAP scintigraphy showed significant amyloid deposits in all studied subjects but only two had any sign of renal dysfunction (proteinuria), and progression to end-stage renal disease was only seen over the follow-up period of 17 years (Rowczenio *et al.*, 2014). This corresponds well to what was observed in the histological study (n = 25, **Study IV**): 59% of the patients had amyloid in the kidney parenchyma and 77% in the renal blood vessels, but only three of them were reported to have clinically significant renal insufficiency, one receiving dialysis treatment. On the contrary, homozygous patients may already be suffering from nephrotic syndrome in their early twenties, proceeding progressively to renal failure, dialysis treatment and death (Meretoja, 1973a; Maury *et al.*, 1992; Ardalan *et al.*, 2007b). However, in some cases the renal manifestations are significant even in heterozygous patients, and in this study five patients (2%) with kidney transplantation were identified, only one of them homozygous.

The prevalence of various ophthalmological manifestations was shown to be high in AGel amyloidosis patients, but comparison data on the prevalence of dry eyes, photophobia and tearing in the general population is not available. In a population-

based survey on the prevalence of major eye diseases in Finland (Laitinen *et al.*, 2010), partly based on self-reported data similarly to this study, the prevalence of cataract and glaucoma for persons aged 30 years and older was reported to be 10% and 5%, and for persons aged 65 years and older 34% and 13%, respectively. In the FIN-GAR registry patients with a median age of 62.7 years, the prevalence of cataract was 47% and that of glaucoma 23%. Cataract and glaucoma have been associated with AGel amyloidosis (Winkelman *et al.*, 1971; Meretoja, 1972; Purcell *et al.*, 1983; Starck *et al.*, 1991; Kiuru, 1992; Akiya *et al.*, 1996; Stewart *et al.*, 2000; Carrwik & Stenevi, 2009; Papathanassiou *et al.*, 2009), but this high prevalence in patients was new information gained through this study.

AGel amyloidosis patients are known to have issues with their oral health, dry mouth being a known clinical manifestation of the disease. Hyposalivation with altered salivary composition has been suggested to increase the risk of caries and oral candidiasis in AGel amyloidosis patients (Juusela *et al.*, 2013). In this study over 40% of the patients reported dry mouth and equally many a tendency to caries, which indicates that oral health care needs to be considered a significant part of the symptomatic treatment of the patients.

The disease burden is well demonstrated also by the number of skin and soft tissue operations performed for patients. As many as 65% of the patients had been operated on at least once, and repeated skin or soft tissue surgery had been completed for 45% of the patients. Repeated plastic surgery is required due to the short-term, even though primarily satisfactory, effects on the skin (Pihlamaa *et al.*, 2011). It has been shown that there is an increasing amount of amyloid in the patients' skin with advancing age and that amyloid spreads even to the deeper dermis and subcutaneous adipose tissue (Pihlamaa *et al.*, 2016b), supposedly leading to the progressive changes in the structure of skin. In addition, the characteristic facial nerve palsy that begins from the frontal branch of the nerve hastens the visible changes in the face. In a series of 35 operated-on patients, none had normal function of the frontal branch of the facial nerve (Pihlamaa *et al.*, 2011). Exceptional tissue laxity is not limited to the skin, and patients also reported varicose veins, haemorrhoids, hernias and prolapses, frequently requiring surgical treatment. However, the prevalence of varicose veins and

haemorrhoids was not demonstrated to be higher than in the general population (Riss *et al.*, 2012; Laurikka *et al.*, 1993); as we did not ask the exact type of hernias and prolapse, it is not possible to find reliable comparison data for them. In addition to skin and other soft tissue operations, a significant share of the patients had had at least one carpal tunnel syndrome (26%) or cataract operation (24%).

AGel amyloidosis does not seem to have a major influence on the age of retirement. The patients (42 males and 99 females) had retired between 1968 and 2019. On average, male AGel amyloidosis patients retired at the age of 57.9 years and female patients at the age of 59.1 years. Statistical data on the average age of retirement in Finland is available only for 1996-2018. In that time the average age of retirement varied between 56.6 and 60.9 years and 57.8 and 60.7 years for men and women, respectively.

## 6.3. Histopathological considerations

The fourth objective of the present study was to investigate the severity and frequency of inner organ, especially cardiac, involvement in AGel amyloidosis. Representative histopathological studies on internal organs have not been available. **Study IV** on 25 autopsied patients with AGel amyloidosis aged 44 years or over showed deposition of AGel based amyloid in every patient, without exception in the heart, in both the parenchyma and cardiac blood vessels and frequently in other inner organs such as the kidney and thyroid gland.

The results confirmed the previous histological findings on frequent cardiac involvement in AGel amyloidosis (Meretoja, 1973b; Meretoja & Teppo, 1971; Kiuru *et al.*, 1994). Moreover, the study demonstrated constant cardiovascular AGel deposition in patients over the age of 44 years. Our finding of the correlation between the amounts of myocardial, renal and hepatic amyloid with age at death may possibly explain the progressive nature of the clinical symptoms and reflects the systemic and progressive nature of the disease, even though clinical cardiac and renal manifestations are considered to be rather rare, and hepatic manifestations by now unknown.

The clinical cardiac manifestations in AGel amyloidosis have been suggested to be caused by disturbance to the conduction system (Meretoja, 1973b; Chastan *et al.*, 2006). Like mentioned earlier, a recent study of 51 genetically verified AGel amyloidosis patients showed cardiac conduction defects of different types in the ECG of 43% of the study subjects (Mustonen *et al.,* manuscript submitted). In this study, information on the exact location of the cardiac tissue specimens was not available, because the tissue samples of this study were collected as part of regular autopsies, and the location of the tissue samples was not systematically recorded. For this reason, we could not investigate the pathways of the conduction system.

There might also be other consequences of the amyloid accumulation in heart. Out of the 25 patients, 17 (68%) were diagnosed with coronary disease, and it is possible that amyloid mass even fills coronary arteries (Meretoja, 1973b). Even though such grave amyloid deposition was not seen in this patient series, amyloid in coronary arteries may have increased the symptoms of coronary disease for some patients. Moreover, amyloid co-localised in the myocardium with fibrosis. It is not known if fibrosis in these subjects is due to the accumulation of amyloid itself or other causes, such as coronary heart disease. Amyloid fibrils increase the stiffness of the myocardium, and amyloid deposition in fibrosis may amplify the sole effect of cardiac fibrosis, increasing the risk of heart failure in AGel amyloidosis patients. Three rather young male patients had encountered sudden cardiac death at the ages of 44, 45 and 59. As the clinical data were incomplete, clinical findings and pathology could not be correlated in them, but three sudden deaths in quite young patients may indicate an increased risk.

Two elderly male patients, both verified by a gene test to carry the 640G > A *GSN* mutation, had large amounts of amyloid in their myocardium. One died at the age of 80 years and had suffered from atrial fibrillation and symptoms of cardiomyopathy while living. Based on the autopsy findings and clinical data, his cause of death was confirmed to be amyloid heart disease. Immunohistochemical staining showed that there was both AGel and ATTR based amyloid in his myocardium. Because there are only a few known cases of hereditary ATTR amyloidosis in Finland, we assume that the detected ATTR based amyloid consisted of wild type TTR protein. However, it is

possible that some of the patients also had ATTRv amyloidosis; genetic testing was not done. The two different amyloid protein types accumulated in different locations: gelsolin amyloid was located between the myocardial cells and in the artery walls, whereas transthyretin amyloid was seen in dense plaque-like collections. The presence of two different types of amyloid has been demonstrated for other amyloidoses, for example, the co-existence of ATTRwt with ATTRv (mutations Val30Met, Val30Leu, Asp38Ala) in the heart has been reported (Yazaki *et al.*, 2000). It is possible that the presence of one type of amyloid may potentiate the accumulation of another type (Bergström *et al.*, 2001; Levy *et al.*, 2001; Bergström *et al.*, 2004). This may possibly be due to the decline of blood flow in the myocardium due to vascular amyloid deposition, as in this particular subject presenting with serious heart disease. The second patient with large amounts of myocardial amyloid died at the age of 84 years, also suffered from clinically significant cardiac symptoms, atrial fibrillation and bradycardia, and had a pacemaker installed. His causes of death were coronary heart disease, cardiac hypertrophy and dilatation and cardiac insufficiency. The large AGel amyloid deposits in the myocardium might have contributed to the pathology of his heart.

The hearts were heavier (557 g in male, 445 g in female patients) than considered normal: in an autopsy study of 355 adult males and 329 adult females the average heart weights were 365 g and 312 g, respectively (de la Grandmaison, Clairand & Durigon, 2001). There was no statistically significant difference in the heart weight between the subjects with and without coronary heart disease, cardiac failure, valvular disorder or pacemaker treatment, and it seems that the presence of AGel amyloidosis as such may be associated with some increase in the heart mass.

This study demonstrated for the first time in a representative patient series that amyloid in AGel amyloidosis may also accumulate in the lungs. Amyloid was seen in both the lung parenchyma and the arteries. Pulmonary amyloidosis is seen in other systemic amyloidoses and is usually asymptomatic. Nevertheless, the pulmonary interstitial form in particular may contribute, in association with cardiac amyloidosis, to cardiopulmonary failure (Milani *et al.*, 2017). Pulmonary artery amyloidosis, even though usually clinically irrelevant, may give rise to pulmonary hypertension

(Cordier, Loire & Brune, 1986; Shiue & McNally, 1988; Milani *et al.*, 2017). There is no information on the clinical relevance of pulmonary amyloidosis in AGel amyloidosis. Previous studies do not suggest the patients have problems with the respiratory tract, but the topic requires more thorough investigation.

Regarding thyroid gland specimens, moderate to large amyloid deposits were found in the majority of them. We found that amyloid is visible in the basement membranes of the thyroid follicle similarly to the sweat glands of the skin (Pihlamaa *et al.*, 2016b), confirming the previous findings (Meretoja, 1972). In ATTRv amyloidosis, amyloid frequently accumulates in the thyroid gland (Takahashi *et al.*, 1991), presumably having the consequence that the active form of thyroid hormone inversely correlates with the disease duration (Huang *et al.*, 2017). It has been suggested that patients suffering from AGel amyloidosis, especially women, may have an unusual amount of abnormalities in their thyroid glands (Meretoja, 1973b). Further investigations are needed in order to understand the possible clinical significance of the finding.

Hepatic involvement is common in AL and AA amyloidoses (Wechalekar, Gillmore & Hawkins, 2016) but does not usually lead to clinical manifestations (Gertz & Kyle, 1988). However, hepatomegaly and abnormal liver function laboratory results may be seen (Park *et al.*, 2003; Lachmann *et al.*, 2007; Sarsik *et al.*, 2012). In this study, small amyloid deposits in the blood vessels of the liver and pancreas were demonstrated for the first time. The minor amyloid deposits in the pancreas, spleen and liver arteries may be interpreted as a manifestation of generalised amyloid angiopathy that is known to be characteristic of AGel amyloidosis (Kiuru-Enari & Haltia, 2013). This may be without clinical significance, and previous studies have not reported symptoms that could be associated with the amyloid angiopathy of these organs. The vascular amyloid deposits in the heart, kidney and thyroid gland were larger than in the organ parenchyma. In a few patients there was positivity in the immunohistochemistry against gelsolin without amyloid in Congo red in some organs (lung, kidney), also shown earlier in other histological studies on AGel amyloidosis (Kiuru *et al.*, 1994) and in other amyloid diseases as well (Tanskanen *et al.*, 2012). The possible reason is that during the deposition of amyloid protein, part of the amyloid-forming precursor protein is not in fibrillar form and thus not visible in Congo red staining.

The first histopathological study on AGel amyloidosis with a representative patient series shows that that the patients typically have wide but mild amyloid accumulation in their internal organs, which confirms the findings of previous studies. Some patients may, however, develop serious amyloid heart disease that may even be fatal.

## 6.4. Causes of death and lifespan

As part of this study we investigated the causes of death of AGel amyloidosis patients. We collected 272 death certificates, which, to our knowledge, is the largest systematic collection of material on this subject to date. Previous studies have reported sporadic observations of possible causes of death in AGel amyloidosis (Meretoja & Teppo, 1971; Kiuru *et al.*, 1999b; Maury *et al.*, 1992; Makishita *et al.*, 1996).

The study (**V**) demonstrated that the death of a significant portion of the patients was attributed to AGel amyloidosis, as 20% of the patients had AGel amyloidosis as their underlying cause of death. The underlying cause of death is defined to be the disease which has initiated the series of illnesses leading directly to death. The treating physicians have interpreted that the death of these patients was essentially caused by the amyloidosis disease.

A highly interesting preliminary finding of the study was that AGel amyloidosis patients die significantly less frequently of cancer than the general population. The frequency of fatal respiratory tract cancers was especially low, followed by that of breast cancer, while of all the different malignant neoplasms, only fatal melanoma was slightly more common in our patients than in the general population. Previously no observation on a possible association between malignancies and AGel amyloidosis has been presented, but gelsolin protein has been associated with cancer as either a protective or promoting factor. Gelsolin expression is downregulated in various types of human cancers: gastric, bladder, colon and lung (Kuzumaki *et al.*, 1998) as well as breast (Chaponnier & Gabbiani, 1989; Asch *et al.*, 1996), endometrial and ovarian (Afify & Werness, 1998) and prostate carcinomas (Lee *et al.*, 1999). In melanoma, gelsolin inhibits metastatic processes by suppressing cell migration (Fujita *et al.*, 2001). Moreover, there is evidence that the long arm of chromosome 9 is frequently deleted in non-small cell lung cancers (Merlo *et al.*, 1994) and that gelsolin is found

only in reduced or undetectable levels in these cancer cells compared with normal bronchial epithelial cells (Dosaka-Akita *et al.*, 1998). Gelsolin overexpressing urinary bladder cells have been reported to result in tumour growth inhibition in nude mice (Tanaka *et al.*, 1999). However, the results are somewhat controversial, as there is also evidence that gelsolin overexpression can promote tumour cell motility and invasion (Chen, Murphy-Ullrich & Wells, 1996; Lader *et al.*, 2005). In AGel amyloidosis, the gelsolin metabolism is altered intracellularly, and the disease results in elevated gelsolin serum levels (Paunio *et al.*, 1994b). Whether such an altered gelsolin metabolism could offer some protective effect remains unknown.

The effect of the disease on patients' lifespan was calculated using two independent methods. In **Study V** the age at death of AGel amyloidosis patients, extracted from their death certificates, was compared with that of the general Finnish population. The calculation did not take into account the surviving, that is the living patients, and is because of that was prone to bias. The result was confirmed utilising the relative-survival method in **Study III**: the relative survival of the patients was comparable to that of the general Finnish population. The 478 subjects of the **Study III** consisted of both deceased and living patients.

AGel amyloidosis seems to be an exception among other systemic amyloidoses in respect to the life span. Of the hereditary systemic amyloidoses, transthyretin (ATTRv) amyloidosis has been estimated to lead to death on average within 10 years after the disease onset (Planté-Bordeneuve & Said, 2011); this may be prolonged by Tafamidis treatment (Maurer *et al.*, 2018). Cystatin C (ACys) amyloidosis leads to death before the age of 40 years (Palsdottir *et al.*, 2008), fibrinogen (AFib) amyloidosis in a median time of 15.2 years after disease manifestation (Gillmore *et al.*, 2009), and in lysozyme (ALys) amyloidosis the survival time varies between 1 to 20 years after the diagnosis and none of the known patients has lived over the age of 60 years (Gillmore *et al.*, 1999). This study shows that even though AGel amyloidosis causes a significant disease burden, the lifespan is not shorter compared with that of the general Finnish population.

The relatively low frequency of severe clinical cardiac and renal manifestations may particularly contribute to the finding that AGel amyloidosis does not affect the patients' lifespan. The finding of **Study V** that the frequency of cancer as a cause of death in AGel amyloidosis patients is lower than in the general population may also have an influence on the result.

## 6.5. Future implications

The basis for this thesis was the FIN-GAR patient registry. In the future it is important to keep the registry up to date, to include new Finnish patients and to possibly widen the registry to include patients from other countries. Through a more comprehensive national patient registry it would be possible to investigate whether the phenotype varies between families and kindred groups. So far it is not known whether the AGel amyloidosis phenotype varies between different countries, as is the case with some ATTRv gene variants. Moreover, an international patient registry would enable us to conduct an international haplotype analysis to find out if some of the foreign patients and kindred groups, in addition to the identified American family with Scandinavian ancestors (Steiner *et al.*, 1995), share the same haplotype, and a common ancestor, with the Finnish patients. The age of the Finnish AGel amyloidosis haplotype, potentially giving interesting information on when and how the gelsolin gene variant came to Finland, is currently being investigated as a separate project.

This study increased the understanding of the disease pathogenesis in different inner organs. Clinically significant manifestations in the heart and kidneys were previously known but more investigation is needed to understand the possible clinical significance of amyloidosis in the lungs and thyroid gland in particular. Understanding thoroughly how the systemic disease influences the inner organs is likely to be valuable in the future when developing a medicine for AGel amyloidosis. Moreover, better understanding the disease pathogenesis helps in understanding the mechanisms that make AGel amyloidosis lethal for 20% of patients.

Lastly, the preliminary result of this study that AGel amyloidosis patients die more seldom from cancer than does the normal Finnish population needs to be confirmed and the possible mechanisms leading to this understood.

## 6.6. Strengths and limitations of the study

This study was based on FIN-GAR patient registry including 261 patients, blood samples and DNA from 68 patients representing 62 nuclear families, autopsy reports and tissue samples of 25 deceased patients, and death certificates of 272 deceased patients. Each of the material sets was collected for the first time for this study. Taking into consideration the small overall number of AGel amyloidosis patients, the material of each individual study can be considered representative. There are some limitations to this study.

Even though **Study I** included an index patient of the large majority (78%) of the known Finnish AGel amyloidosis families, its coverage is not 100%. Getting complete coverage is not feasible as there still might be some unidentified families and not all patients wish to take part in studies. For that reason, it cannot totally be ruled out that there are possibly Finnish patients who do not share the common ancestor.

For **Studies II** and **III,** the data was collected through the patient survey and in the majority of the cases also by telephone interview. The data were self-reported and collected through a patient survey and complemented by telephone interviews in the phase I study, and not all the reported signs and symptoms were verified by a physician, which may artificially increase their prevalence. However, it is unlikely that patients would report signs like cataract or glaucoma without having a diagnosis from an ophthalmologist. As the symptoms begin gradually, over many years, and many patients have a spectrum of different symptoms, memorising the exact starting point of each symptom is subject to recall error. For this reason, the given years of onset must be taken as rough estimates in most cases. Secondly, the patients were recruited through a patient organisation. Patients who belong to patient organisations might be different, for example have better health, to patients who do not want to take part in patient organisation activities. Finally, the gender distribution in this study was uneven, which is likely to be due to the fact that females are more likely to answer

questionnaires and surveys (Curtin, Presser & Singer, 2000; Singer, Van Hoewyk & Maher, 2000; Smith, 2008).

In **Study IV**, due to the retrospective nature of the study, the specimens were not as representative as they could have been in a prospective study and, for example, whole hearts could not be examined. The locations from which the tissue specimens were taken varied between the patients and the range of specimens was incomplete. Second, as over half of the autopsies were forensic, the range of specimens was incomplete, as in forensic autopsies the focus is not on medical disease. Further, postmortem delay might have influenced the quality and interpretation of the IHC to a degree.

The death certificates used as material in **Study V** possibly included patients who did not carry the *GSN* c.640G > A mutation, because the identification of the patients was based on genealogical data and patient interviews and no medical records were used. However, the risk can be estimated to be small because AGel amyloidosis significantly influences the facial appearance, and patients generally have an in-depth knowledge of their ancestors and other family members with AGel amyloidosis. Secondly, instead of investigating the deceased patients by ourselves, the study was based on death certificates, usually completed by the treating physician. The comparison data, however, are based on information collected in a similar way (death certificates of the general population) and are therefore subject to similar flaws.

# 7. Conclusions

Based on the study, I make the following conclusions.

1. The 62 studied Finnish AGel amyloidosis families share a gelsolin gene variant c.640G > A associated haplotype that is not otherwise common in the Finnish population. The Finnish AGel amyloidosis patients are likely to descend from a common ancestor.
2. AGel amyloidosis patients experience their first symptoms at their forties, and a variety of symptoms belonging to the classical triad of ophthalmological (dry eyes in 93%; corneal lattice amyloidosis in 89%), neurological (numbness, tingling and other paraesthesias in 75%; facial paresis in 67%), and dermatological (drooping eyelids in 86%; cutis laxa in 84%) manifestations were highly prevalent.
3. A significant number of patients need repeated plastic surgery as symptomatic treatment, and the incidence of cataract and carpal tunnel syndrome operations is also high.
4. Amyloid deposits in the myocardium and cardiac blood vessels are common in middle-aged and elderly patients, possibly leading to clinical cardiac manifestations. The disease is systemic, and amyloid deposits are frequently found also in the kidneys, lungs, thyroid gland, liver, spleen and pancreas.
5. AGel amyloidosis is the underlying cause of death in 20% of the patients, underlining the severity of the disease. Even though renal symptoms are usually mild, renal manifestations may prove lethal even in heterozygotes. Interestingly, for an unknown reason, malignancies are significantly less common as causes of death in AGel amyloidosis patients than in the general Finnish population.
6. Even though the disease burden is significant, the lifespan is comparable with that of the normal population, and the patients do not seem to retire earlier than the general population.

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# Appendix 1: FIN-GAR I questionnaire (Study II)

**KYSELYLOMAKE MERETOJAN TAUTIA SAIRASTAVILLE**

Kyllä/Ei-kohdissa ympyröikää oikea vaihtoehto

_____

Nimi, sosiaaliturvatunnus

_____

Osoite, puhelinnumero

_____

Sairastumisvuosi, milloin diagnoosi on tehty ja sairaala sekä klinikka, missä diagnoosi tehtiin

Onko Meretojan taudin geenitutkimus tehty? Kyllä/Ei

Jos on, niin minä vuonna ja missä sairaalassa?_____

_____

Jos ei, mihin diagnoosi perustui? Silmätutkimus, jokin muu tutkimus?

_____

Ensioireet: minkälaisia, minä vuonna alkoivat?

_____

Esiintyykö suvussanne Meretojan tautia? Kuinka monella? Mitä sukua he ovat Teille?

_____

Minkä niminen suku on kyseessä?_____

Miltä paikkakunnalta sairaan vanhemman/isovanhemman suku on kotoisin (jostiedetään)?-

_____

Onko Teillä silmäoireita? Kyllä/Ei

_____

Minkälaisia, minä vuonna oireet alkoivat?

Onko todettu glaukoomaa (kohonnut silmänpaine)? Kyllä/Ei

Jos on, milloin?_____

Onko todettu katarakta (harmaakaihi)? Kyllä/Ei

Jos on, milloin? _____

Onko näkönne heikentynyt? Kyllä/Ei

Jos on, milloin huomasitte sen heikentyneeksi?_____

Onko Teillä todettu sarveiskalvon rappeumaa? Kyllä/Ei

Jos on, milloin?_____

Onko Teillä muita silmäoireita (esim. sarveiskalvohaavaumaa)? Kyllä/Ei

_____

Jos on, millaisia ja minä vuonna ne alkoivat?

Onko tehty silmiin kohdistuvia toimenpiteitä/leikkauksia? Kyllä/Ei

_____

Jos on, niin minkälaisia toimenpiteitä/leikkauksia? Minä vuonna?

Käytättekö silmälääkkeitä? Kyllä/Ei

Jos käytätte, niin mitä? _____

Käyttekö säännöllisesti silmälääkärissä? Kyllä/Ei

Onko ilmaantunut tai todettu neurologisia oireita? Kyllä/ei

_____

Jos on, niin millaisia oireita ja minä vuosina?

Onko näihin käytössä lääkkeitä? Kyllä/ei

_____

Jos on, niin mitä lääkkeitä?

Onko kuulonne heikentynyt? Kyllä/Ei

Jos on, milloin huomasitte sen?_____

Esiintyykö Teillä usein elohiiriä? Kyllä/Ei

Jos on, missä ja milloin ne alkoivat?_____

Onko puhuminen vaikeutunut? Kyllä/Ei

Jos on, milloin huomasitte sen?_____

Esiintyykö Teillä muita hermostoperäisiä oireita, kuten ilmeettömyyttä (esim. otsa ei rypisty), raajojen tunnottomuutta, käsien kömpelyyttä,

tasapainovaikeutta, nielemisvaikeuksia? Kyllä/Ei

_____

Jos on, niin millaisia ja milloin ne alkoivat?

Onko tehty leikkauksia (esim. rannetunnelisyndrooman leikkaus)? Kyllä/ei

_____

Jos on, niin mitä? Minä vuonna?

Onko ilmaantunut tai todettu iho-oireita? Kyllä/ei

_____

Jos on, niin millaisia oireita ja minä vuosina?

Jos, mitä ihovoiteita käytätte? Kuinka usein?_____

Onko tehty ihoon kohdistuvia toimenpiteitä/leikkauksia? Kyllä/Ei

_____

Jos on, niin minkälaisia toimenpiteitä/leikkauksia? Minä vuonna?

Onko todettu uniapneaa? Kyllä/Ei

_____

Jos on, milloin ja miten sitä hoidetaan/on hoidettu?

Onko todettu lisääntynyt verenvuototaipumusta? Kyllä/Ei

Tuleeko mustelmia herkästi? Kyllä/Ei

Jos tulee, milloin tämä taipumus alkoi (vuosi)?_____

Esiintyykö rytmihäiriöitä? Kyllä/Ei

Jos esiintyy, milloin alkoivat (vuosi)?_____

Onko käytössä rytmihäiriölääke? Kyllä/Ei

Onko sydämen tahdistinta? Kyllä/Ei

Onko esiintynyt munuaistoiminnan häiriöitä? Kyllä/Ei

_____

Jos on niin millainen häiriö, milloin se alkoi ja miten sitä hoidetaan?

Esiintyykö turvotuksia? Kyllä/Ei

Jos, missä ja milloin alkoivat?_____

Onko suu- tai hammasongelmia? Kyllä/Ei

_____

Jos on niin millaisia, milloin ne alkoivat ja miten niitä hoidetaan?

_____

Jos on, onko käytössä lääkitystä tai muuta hoitoa niihin, millaista?

Onko esiintynyt kipuja? Kyllä/Ei

_____

Jos on, missä kivut ovat ja koska ne alkoivat?

_____

Jos on, onko käytössä kipulääkitystä? Millaista kipulääkitystä?

Onko toteutettu kuntoutusta Meretojan taudin vuoksi? Kyllä/Ei

Jos on, minkälaista?_____

Onko muita sairauksia Meretojan taudin lisäksi? Kyllä/Ei

_____

Jos on, niin mitä muita sairauksia?_____

_____

_____

Mahdolliset pysyvät lääkehoidot muihin sairauksiin

Mahdolliset leikkaushoidot muihin sairauksiin (esim. suonikohjut, laskeumat

(virtsarakko, peräsuoli, kohtu) ?_____

_____

Oletteko opiskelija/työelämässä/eläkkeellä/ työkyvyttömyyseläkkeellä Meretojan taudin (mikä oire?)/muun syyn takia (minkä?)?_____

Jos jouduitte jäämään työkyvyttömyyseläkkeelle Meretojan taudin vuoksi, niin

mikä oli pääoire, minkä vuoksi eläke myönnettiin, ja minä vuonna jäitte eläkkeelle?_____

_____

Asutteko yksin/puolison kanssa?_____

Kiitos vastauksista!

Sari Kiuru-Enari, neurologi, HYKS Sari Atula, neurologi, HYKS

# Appendix 2: FIN-GAR I questionnaire (Study III)

## KYSELY MERETOJAN TAUTIA SAIRASTAVILLE

Päivämäärä ___ / ___ 20___

| 1. HENKILÖTIEDOT | |
|---|---|
| Nimi | |
| Henkilötunnus | |
| Katuosoite | |
| Postinumero ja –toimipaikka | |
| Puhelinnumero | |

| 2. OIREIDEN ALKAMINEN JA MERETOJAN TAUDIN TOTEAMINEN | |
|---|---|
| Oireiden alkamisvuosi | |
| Diagnoosivuosi | |
| Onko Meretojan taudin geenitutkimus tehty? | Kyllä ☐ Ei ☐<br>Sairaala:<br>Vuosi: |
| Jos geenitutkimusta ei ole tehty, mihin tutkimukseen diagnoosi perustui (esim. silmälääkärin tekemä tutkimus)? | |

| 3. SUKUTAUSTA | |
|---|---|
| Esiintyykö suvussanne Meretojan tautia? | Kyllä ☐ Ei ☐ |
| Kummalta vanhemmalta olette perineet sairauden? | Äidiltä ☐ Isältä ☐<br>Molemmilta vanhemmilta ☐ |
| Jos kyllä, mitä sukua tuntemanne potilaat ovat teille? | |
| Miltä paikkakunnalta sairaan vanhemman/isovanhemman suku on kotoisin, jos asia on tiedossa? | |

Seuraavaksi kysymme oireistanne, diagnooseistanne ja sairauksienne hoidosta. Valitkaa kultakin riviltä sopiva vastausvaihtoehto (kyllä/ei). Merkitkää myös oireen alkamisvuosi tai toimenpidevuosi taulukon mukaisesti, jos mahdollista.

| 4. SILMÄT | | |
|---|---|---|
| Sarveiskalvon rappeuma | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Sarveiskalvon haavauma | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Näön heikkeneminen | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Glaukooma (kohonnut silmänpaine) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Katarakta (kaihi) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kuivasilmäisyys (kyynelvajaus) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kyynelehtiminen | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Silmien valonarkuus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Käytättekö kostuttavia silmätippoja? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Käytättekö silmänpainetta alentavia silmätippoja? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Oletteko säännöllisessä silmälääkärin seurannassa? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Onko teillä muita silmäoireita? | Kyllä ☐ Ei ☐ | |
| Jos teillä on muita silmäoireita, mitä ja mistä vuodesta alkaen? | | |
| Onko teille tehty silmäleikkauksia? | Kyllä ☐ Ei ☐ | |
| Jos teille on tehty silmäleikkauksia, mitä ja minä vuonna? | | |
| Kuinka silmäoireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐<br>Halutessanne voitte antaa lisätietoja. | | |

| 5. NEUROLOGIA | | |
|---|---|---|
| Pistely ja puutuminen, muu tunto-oire | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Elohiiri (myokymia) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kasvohermohalvaus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Tasapainovaikeus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kuulon heikkeneminen | Kyllä ☐ Ei ☐ | Alkamisvuosi |

| | | |
|---|---|---|
| Onko teillä kuulolaite tai sisäkorvan implantti? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Puheen tuoton ongelma | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kielen surkastuminen tai nykinä | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Rannekanavaoireyhtymä | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Rannekanavaoireyhtymän leikkaus | Kyllä ☐ Ei ☐ | Leikkausvuosi |
| Onko teillä muita neurologisia oireita? | Kyllä ☐ Ei ☐ | |
| Jos teillä on muita neurologisia oireita, mitä ja minä vuonna oireet ovat alkaneet? | | |
| Käytättekö lääkkeitä neurologisiin vaivoihin? | Kyllä ☐ Ei ☐ | |
| Jos käytätte lääkkeitä neurologisiin vaivoihin, mitä lääkkeitä ja mistä vuodesta alkaen? | | |
| Kuinka neurologiset oireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐<br>Halutessanne voitte antaa lisätietoja. | | |

| **6. IHO JA PEHMYTKUDOKSET** | | |
|---|---|---|
| Ihon löystyminen | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Silmäluomioireita (esim. luomien roikkuminen) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kutina | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Tuleeko teille helposti haavoja? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Laskeuma (esim. kohtu) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Tyrä | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Suonikohjut | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Peräpukamat | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Mustelmataipumus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Ihon kuivuus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Käytättekö ihovoiteita? | Kyllä ☐ Ei ☐ | |
| Jos käytätte ihovoiteita, mitä valmisteita ja kuinka usein? | | |

| | | |
|---|---|---|
| Onko teille tehty ihoon kohdistuvia leikkauksia? | Kyllä ☐ Ei ☐ | |
| Jos teille on tehty ihoon kohdistuvia leikkauksia (esim. silmäluomet tai otsan kohotus), mitä ja minä vuonna? | | |
| Kuinka iho- ja pehmytkudosoireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐<br>Halutessanne voitte antaa lisätietoja. | | |

| **7. SYDÄN- JA VERENKIERTOELIMISTÖ** | | |
|---|---|---|
| Rytmihäiriö (muu kuin eteisvärinä) | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Eteisvärinä | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Sydämen tahdistin | Kyllä ☐ Ei ☐ | Asennusvuosi |
| Läppävika | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Sydämen vajaatoiminta | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Sepelvaltimotauti | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kardiomyopatia | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Verenpainetauti | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Koholla oleva kolesteroli | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Jos käytätte lääkkeitä edellä mainittuihin vaivoihin tai muihin sydän- ja verenkiertoelimistön vaivoihin, mitä lääkkeitä ja mistä vuodesta alkaen? | | |
| Onko teille tehty sydämeen kohdistuvia leikkauksia? | Kyllä ☐ Ei ☐ | |
| Jos teille on tehty sydämeen kohdistuvia leikkauksia, mitä ja minä vuonna? | | |
| Kuinka sydänoireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐ | | |

| |
|---|
| Halutessanne voitte antaa lisätietoja. |

| **8. MUNUAISET** | | |
|---|---|---|
| Proteiinia eli valkuaista virtsassa | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Munuaisten vajaatoiminta | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Dialyysi | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Munuaissiirto | Kyllä ☐ Ei ☐ | Leikkausvuosi |
| Käytättekö lääkkeitä munuaisoireisiin? | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Jos käytätte lääkkeitä munuaisoireisiin, mitä lääkkeitä ja mistä vuodesta alkaen? | | |
| Kuinka munuaisoireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐<br>Halutessanne voitte antaa lisätietoja. | | |

| **9. SUU JA HAMPAAT** | | |
|---|---|---|
| Kuiva suu | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Hampaiden reikiintymistaipumus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Ientulehdus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Käytättekö suun kostuttamiseen tarkoitettuja valmisteita? | Kyllä ☐ Ei ☐ | |
| Onko teillä muita suun tai hampaiden oireita? Jos kyllä, mitä ja mistä vuodesta alkaen? | | |
| Kuinka suu- ja hammasoireenne ovat kehittyneet viimeisen 5 vuoden aikana?<br>Vähentyneet ☐ pysyneet samanlaisina ☐ lisääntyneet ☐<br>Halutessanne voitte antaa lisätietoja. | | |

| 10. MUUT OIREET | | |
|---|---|---|
| Kipuja | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Jos teillä on kipuja, minkälaisia kipuja ja mitä kipulääkkeitä käytätte? | | |
| Uniapnea | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Turvotuksia | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Masennus | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Kilpirauhasen vajaatoiminta | Kyllä ☐ Ei ☐ | Alkamisvuosi |
| Jokin syöpäsairaus | Kyllä ☐ Ei ☐ | |
| Jos teillä on todettu jokin syöpäsairaus, mikä syöpä ja minä vuonna? | | |
| Muita oireita | Kyllä ☐ Ei ☐ | |
| Jos teillä on muita oireita, mitä ja mistä vuodesta alkaen? | | |
| Onko teillä käytössänne muita lääkityksiä kuin mitä olette jo edellä ilmoittanut? | Kyllä ☐ Ei ☐ | |
| Jos käytätte muita lääkkeitä, mitä lääkkeitä, mihin vaivaan ja mistä vuodesta alkaen? | | |
| Onko teille tehty muita leikkauksia kuin mitä olette jo edellä ilmoittanut? | Kyllä ☐ Ei ☐ | |
| Jos teille on tehty muita leikkauksia, mitä ja minä vuonna? | | |

| 11. KOKONAISTILANNE | |
|---|---|
| Kun vertaatte nykytilannettanne 5 vuoden takaiseen tilanteeseen, ovatko oireenne nyt | vähentyneet ☐<br>pysyneet samanlaisina ☐<br>lisääntyneet ☐ |
| Mahdollisia lisätietoja (esim. millaisia uusia vaivoja on ilmennyt viimeisen 5 vuoden aikana aiemmin mainittujen lisäksi?) | |

| 12. ELÄMÄNTILANNE | |
|---|---|
| Oletteko tällä hetkellä | työelämässä ☐<br>vanhuuseläkkeellä ☐<br>sairaseläkkeellä ☐<br>työtön ☐ |
| Eläköitymisvuosi, jos eläkkeellä | |
| Jos olette sairaseläkkeellä, minkä sairauden vuoksi eläköidyitte? | |

Kiitos vastauksistanne!

# ORIGINAL PUBLICATIONS

## Studies I-V