

Gastrointestinal disturbances in hereditary transthyretin amyloidosis

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Every day, once a day, give yourself a present. Don't plan it. Don't wait for it. Just let it happen. It could be a new shirt at the men's store, a catnap in your office chair, or two cups of good, hot, black coffee.

Special Agent Dale Cooper, 1990

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Abstract

Background Transthyretin amyloid (ATTR) amyloidosis is a systemic disorder caused by amyloid deposits formed by misfolded transthyretin (TTR) monomers. Two main forms exist – wild-type and hereditary ATTR amyloidosis, the latter associated with TTR gene mutations.

Wild-type ATTR amyloidosis has a late onset and primarily cardiac manifestations, whereas hereditary ATTR amyloidosis is a rare autosomal dominant condition with a considerable phenotypic diversity. Both disorders are present all over the world, but endemic areas of the hereditary form are found in Sweden, Portugal, Brazil and Japan.

Gastrointestinal (GI) complications are common in hereditary ATTR amyloidosis and play an important role in the patients' morbidity and mortality. Malfunction of the autonomic and enteric nervous systems has been proposed to contribute to the GI disturbances, but the underlying mechanisms have not been fully elucidated.

The aims of this thesis were to assess the prevalence of GI disturbances for different subtypes of ATTR amyloidosis, to further explore the mechanisms behind these disturbances, and to evaluate the outcome of the patients' GI function after liver transplantation, which currently is the standard treatment for hereditary ATTR amyloidosis.

Methods The Transthyretin Amyloidosis Outcomes Survey (THAOS) is the first global, multicenter, longitudinal, observational survey that collects data on patients with ATTR amyloidosis. THAOS enrollment data were used to assess the prevalence of GI symptoms and to evaluate their impact on nutritional status (mBMI) and health-related quality of life (EQ-5D Index Score).

Data from routine investigations of heart-rate variability and cardiovascular response to tilt tests were utilized to evaluate the impact of autonomic neuropathy on the scintigraphically measured gastric emptying half-times in Swedish patients with hereditary ATTR amyloidosis.

Gastric wall autopsy specimens from Japanese patients with hereditary ATTR amyloidosis and Japanese non-amyloidosis controls were analyzed with immunohistochemistry and computerized image analysis to assess the densities of interstitial cells of Cajal (ICC) and nervous tissue.

Data from gastric emptying scintigraphies and validated questionnaires were used to evaluate the outcome of Swedish patients' GI function after liver transplantation for hereditary ATTR amyloidosis.

Results Sixty-three percent of the patients with TTR mutations and 15 % of those with wild-type ATTR amyloidosis reported GI symptoms at enrollment

into THAOS. Subsequent analyses focused on patients with TTR mutations and, among them, unintentional weight loss was the most frequent symptom (32 %) followed by early satiety (26 %). Early-onset patients (<50 years of age) reported GI symptoms more frequently than late-onset cases (70 % vs. 50 %, $p < 0.01$), and GI symptoms were more common in patients with the V30M mutation than in those with non-V30M mutations (69 % vs. 56 %, $p < 0.01$). Both upper and lower GI symptoms were significant negative predictors of nutritional status and health-related quality of life ($p < 0.01$ for both).

Weak but significant correlations were found between gastric emptying half-times and the function of both the sympathetic ($r_s = -0.4$, $p < 0.01$) and parasympathetic ($r_s = -0.3$, $p < 0.01$) nervous systems.

The densities of c-Kit-immunoreactive ICC were significantly lower in the circular (median density 0.0 vs. 2.6, $p < 0.01$) and longitudinal (median density 0.0 vs. 1.8, $p < 0.01$) muscle layers of the gastric wall in patients compared to controls. Yet, no significant differences in protein gene product 9.5-immunoreactive nervous cells were found between patients and controls either in the circular (median density 3.0 vs. 6.8, $p = 0.17$) or longitudinal (median density 1.4 vs. 2.5, $p = 0.10$) muscle layers.

Lastly, the patients' GI symptoms scores had increased slightly from before liver transplantation to the follow-ups performed in median two and nine years after transplantation (median score 7 vs. 10 vs. 13, $p < 0.01$). However, their gastric emptying half-times (median half-time 137 vs. 132 vs. 125 min, $p = 0.52$) and nutritional statuses (median mBMI 975 vs. 991 vs. 973, $p = 0.75$) were maintained at follow-ups in median two and five years after transplantation.

Conclusion GI disturbances are common in hereditary ATTR amyloidosis and have a negative impact on the patients' nutritional status and health-related quality of life. Fortunately, a liver transplantation appears to halt the progressive GI involvement of the disease, although the patients' GI symptoms tend to increase after transplantation. An autonomic neuropathy and a depletion of gastrointestinal ICC seem to contribute to the GI disturbances, but additional factors must be involved.

Original articles

This thesis is based on the following articles, which will be referred to in the text by the corresponding Roman numerals (**I-IV**):

I. Wixner J, Mundayat R, Karayal O, Karling P, Anan I and Suhr OB. THAOS: Gastrointestinal manifestations of transthyretin amyloidosis – common complications of a rare disease. *Orphanet J Rare Dis.* 2014 Apr;9(1):61.

II. Wixner J, Karling P, Rydh A, Hörnsten R, Wiklund U, Anan I and Suhr OB. Gastric emptying in hereditary transthyretin amyloidosis: The impact of autonomic neuropathy. *Neurogastroenterol Motil.* 2012 Dec;24(12):1111-e568.

III. Wixner J, Obayashi K, Ando Y, Karling P and Anan I. Loss of gastric interstitial cells of Cajal in patients with hereditary transthyretin amyloidosis. *Amyloid.* 2013 Jun;20(2):99-106.

IV. Wixner J, Sundström T, Karling P, Anan I and Suhr OB. The outcome of gastric emptying and gastrointestinal symptoms after liver transplantation for hereditary transthyretin amyloidosis. Manuscript.

Abbreviations

AA	Amyloid A
A β ₂ M	Amyloid β ₂ -Microglobulin
AL	Amyloid Light Chain
ANS	Autonomic Nervous System
ATTR	Transthyretin Amyloid
CI	Confidence Interval
CNS	Central Nervous System
ELISA	Enzyme-Linked Immunosorbent Assay
ENS	Enteric Nervous System
EQ-5D	EuroQol Five Dimensions
FAC	Familial Amyloid Cardiomyopathy
FAP	Familial Amyloid Polyneuropathy
GI	Gastrointestinal
HF	High-frequency
HRV	Heart Rate Variability
ICC	Interstitial Cells of Cajal
ICC-CM	Interstitial Cells of Cajal in the Circular Muscle Layer
ICC-LM	Interstitial Cells of Cajal in the Longitudinal Muscle Layer
Ig	Immunoglobulin
IR	Immunoreactive
LF	Low-frequency
ln	Natural Logarithm
mBMI	Modified Body Mass Index
OR	Odds Ratio
RBP	Retinol Binding Protein
SCA	Senile Cardiac Amyloidosis
SD	Standard Deviation
SE	Standard Error
SSA	Senile Systemic Amyloidosis
THAOS	The Transthyretin Amyloidosis Outcomes Survey
TMEM16A	Transmembrane Protein 16A
TTR	Transthyretin

Populärvetenskaplig sammanfattning

Ärftlig transthyretinamyloidos eller familjär amyloid polyneuropati (FAP) är en sjukdom som beror på en förändring (mutation) i arvsanlaget (genen) för proteinet transthyretin (TTR). FAP förekommer över hela världen men är vanligare i vissa avgränsade områden (endemiska områden) som finns i norra Sverige, Portugal, Brasilien och Japan. I Sverige kallas sjukdomen också för Skelleftesjukan eftersom trakterna kring Skellefteå ingår i det endemiska området. Det finns över 100 kända mutationer i TTR-genen men den s.k. V30M-mutationen är den variant som förekommer i de endemiska områdena.

Muterat TTR är instabilt och splittras lättare upp i mindre beståndsdelar som i sin tur binds ihop till svårlösliga proteintrådar (amyloid). Amyloid lagras in i kroppen och orsakar efter hand diverse organskador. De olika TTR-mutationerna ger upphov till lite skilda symptom men nervstörningar med domningar och smärta i främst fötter och underben, hjärtrytmrubbningar, störningar i mag-tarmfunktionen, synnedsättning, impotens, svårigheter att tömma urinblåsan och blodtrycksfall är vanliga problem.

FAP är en dödlig sjukdom med en genomsnittlig överlevnad utan behandling på 9-13 år efter sjukdomsdebuten. Studier har visat att en levertransplantation stoppar bildningen av muterat TTR vilket, i många fall, förhindrar att sjukdomen fortskrider. Levertransplantation är den för tillfället effektivaste behandlingen av FAP och överlevnaden för dem som genomgått levertransplantation är i de flesta fall god. På senare år har studier också visat att vissa läkemedel kan minska försämringstakten i sjukdomen genom att stabilisera muterat TTR. Det pågår också studier där man med s.k. genterapi slår ut produktionen av TTR i kroppen.

Störningar i mag-tarmfunktionen är betydelsefulla vid FAP eftersom de påverkar patienternas livskvalitet och överlevnad, dock är mekanismerna bakom dessa störningar inte helt kända. Syftet med denna avhandling var att närmare undersöka förekomsten av mag-tarmstörningar vid olika typer av transthyretinamyloidos, att utreda mekanismerna bakom dessa störningar samt att utvärdera effekten av levertransplantation på patienternas mag-tarmfunktion.

Arbete I är baserat på data från en stor internationell databas (THAOS) som innehåller information om bl.a. symptom, kön, ålder, livskvalitet och näringsstatus hos patienter med transthyretinamyloidos. Resultaten från detta arbete visade att mag-tarmsymptom är vanligt hos patienter med ärftlig transthyretinamyloidos, även tidigt i sjukdomsförloppet, och att dessa symptom var kopplade till sämre näringsstatus och lägre livskvalitet. Mag-tarmsymptom var vanligare hos patienter med tidig sjukdomsdebut (<50 års ålder) och ökade i frekvens med sjukdomens varaktighet. Däremot hade

patienter med mutationer som företrädesvis ger hjärtkomplikationer inte mer symptom från mag-tarmkanalen än normalbefolkningen. Inga tydliga skillnader mellan könen kunde påvisas.

I **arbete II** undersökte vi kopplingen mellan störningar i det icke-viljestyrda (autonoma) nervsystemet och mag-tarmfunktionen hos 188 svenska FAP-patienter. Vi fann en viss koppling mellan en nedsatt autonom funktion och långsam magsäckstömning, men vissa patienter med en långsam magsäckstömning hade en normal autonom funktion och omvänt såg vi att patienter med en normal magsäckstömning kunde ha en nedsatt autonom funktion. Patienter med långsam magsäckstömning hade sämre näringsstatus än dem med normal tömning och patienter med diarré och/eller förstoppning hade sämre magsäckstömning än dem utan mag-tarmsymptom. Inga tydliga skillnader mellan könen kunde påvisas.

I **arbete III** undersökte vi antalet Cajal-celler (pacemakerceller som startar magsäckens rörelser) och mängden nervvävnad i magsäcken hos elva avlidna japanska FAP-patienter samt hos tio avlidna japanska patienter utan FAP. FAP-patienterna hade färre Cajal-celler men väsentligen lika mycket nervvävnad som dem utan FAP. Vi kunde dock inte påvisa någon tydlig koppling mellan mängden amyloidinlagringar och antalet Cajal-celler eller nervceller i magsäcken hos FAP-patienterna.

Arbete IV är baserat på data från 115 svenska FAP-patienter som genomgått levertransplantation mellan 1990 och 2011. Vi jämförde patienternas mag-tarmsymptom, magsäckstömning och näringsstatus före och efter levertransplantation och undersökningarna visade att patienternas symptom hade ökat något i snitt nio år efter transplantationen, men att deras magsäckstömning och näringsstatus var bevarade under en uppföljningstid på i genomsnitt fem år efter ingreppet.

Sammantaget blir slutsatserna från dessa arbeten att störningar i mag-tarmfunktionen är vanliga hos patienter med ärftlig transthyretinamyloidos och att de har en negativ inverkan på deras livskvalitet och näringsstatus. En nedsatt funktion i det autonoma nervsystemet och en minskad mängd Cajal-celler i mag-tarmkanalen bidrar till dessa störningar, men sannolikt finns även andra bakomliggande orsaker. Levertransplantation, som i dagsläget är den effektivaste behandlingen av sjukdomen, stabiliserar patienternas magsäckstömning och näringsstatus trots att deras besvär från mag-tarmkanalen snarast ökar efter ingreppet.

Aims

The general aims of the thesis were to closely investigate the prevalence and distribution of gastrointestinal manifestations in patients with transthyretin amyloid (ATTR) amyloidosis, and to further explore the mechanisms behind these complications.

Specific aims

I: To explore the prevalence of gastrointestinal symptoms for different subtypes of ATTR amyloidosis, and to evaluate their impact on the patients' nutritional status and health-related quality of life.

II: To investigate the impact of autonomic neuropathy on gastric emptying and gastrointestinal symptoms in patients with hereditary ATTR amyloidosis.

III: To assess the densities of gastric interstitial cells of Cajal and gastric nerves in patients with hereditary ATTR amyloidosis compared to non-amyloidosis controls.

IV: To evaluate the outcome of gastric emptying, gastrointestinal symptoms and nutritional status after liver transplantation for hereditary ATTR amyloidosis.

Introduction

Transthyretin

Transthyretin (TTR), formerly known as pre-albumin, is a tetrameric protein of four identical sub-units each consisting of 127 amino acids. TTR is mainly produced by the liver, but small amounts are also produced in the choroid plexus, the eyes and in the islets of Langerhans in the pancreas [1,2]. TTR functions as a transporter of thyroxine (T₄) and retinol binding protein (RBP), which binds retinol (vitamin A). However, most of the plasma thyroxine is transported by thyroxine binding globulins [3,4] and only about 30 % of the TTR in plasma is bound to RBP indicating that most of the plasma TTR is free of ligand and without apparent function [5]. There are, however, data suggesting that TTR acts as a detoxifying agent in plasma [5] and that it might also be important for neuronal regeneration and axonal growth [6,7]. Furthermore, pancreatic TTR seems to play a role in glucagon expression and glucose homeostasis [8].

Amyloidosis

Amyloidosis is a rare condition caused by an extracellular deposition of insoluble fibrillar protein (amyloid) [9,10]. Amyloid fibrils are formed by misfolded proteins that assemble into beta-pleated sheets, which, in turn, form thin filaments that build up the fibrils. The amyloid formation and deposition negatively affect the function of the targeted tissues, either mechanically or by toxic effects [10].

The diagnosis of amyloidosis is based on light microscopy of tissue samples, e.g. abdominal fat pad biopsies, where the amyloid protein exhibits affinity for Congo red and displays a typical apple green birefringence in polarized light microscopy (Figure 1) [11].

Several types of amyloidosis have been described, however, two main forms exist – localized and systemic amyloidosis. The systemic amyloidoses can, in turn, be classified as either hereditary or non-hereditary. Immunohistochemistry, ELISA, mass spectrometry and Western blot can be used to identify the protein component of the amyloid deposits [12] and thereby help to establish the type of amyloidosis.

Amyloid light chain (AL) amyloidosis and Alzheimer's disease (A β amyloid) are probably the most common forms of localized amyloidosis. However, the association between amyloid deposits and organ dysfunction in Alzheimer's disease, as well as in other diseases in which amyloid locally occurs (i.e. prion diseases and type II diabetes mellitus), is not well established [10,13,14].

The four main types of non-hereditary systemic amyloidosis are systemic AL amyloidosis, amyloid A (AA) amyloidosis, amyloid β_2 -microglobulin

(A β ₂M) amyloidosis and senile systemic amyloidosis (SSA) [13]. AL amyloidosis is caused by a monoclonal gammopathy, with or without myelomatosis, whereas AA amyloidosis is secondary to chronic inflammation, A β ₂M amyloidosis is dialysis-related, and SSA is caused by amyloid fibril formation from wild-type TTR.

Hereditary transthyretin amyloid (ATTR) amyloidosis is the most common form of hereditary systemic amyloidosis. Other types of hereditary systemic amyloidosis are the Apolipoprotein AI and AII, Gelsolin, Lysozyme, Cystatin C and the Fibrinogen A α -chain amyloidoses [15], all of which are autosomal dominant [9].

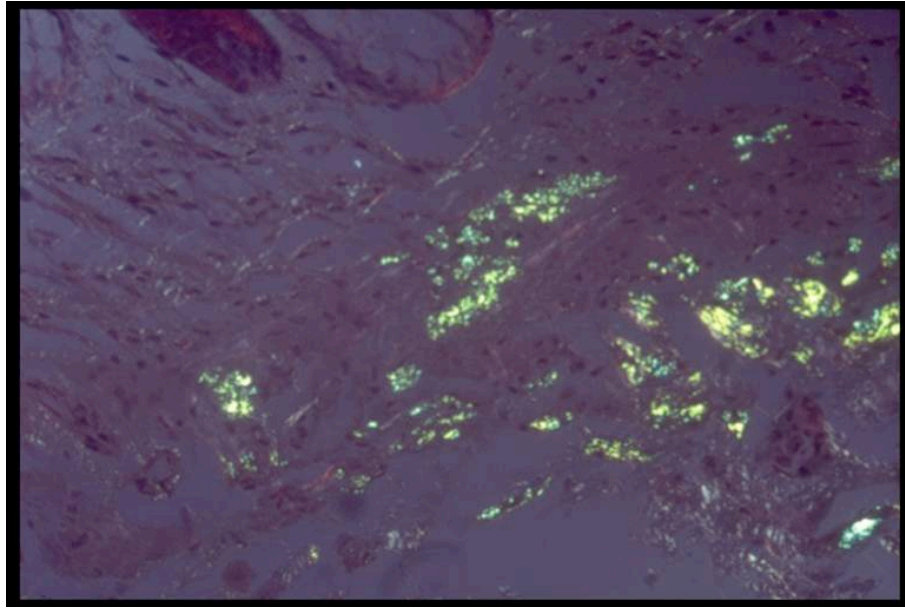


Figure 1: Polarized light microscopy of amyloid deposits (green) in abdominal fat after Congo red staining. Photo by: Rune Andersson.

ATTR amyloidosis

In ATTR amyloidosis, amyloid is formed by misfolded TTR monomers (Figure 2). Increasing age and amyloidogenic TTR gene mutations both decrease the stability of the TTR tetramer, making it more prone to dissociate into monomers [16]. A decreased protein aggregate clearance with aging might also contribute to age-associated nature of the disease [17].

During its formation and deposition the TTR amyloid appears to disturb the function of the targeted tissues both through toxic and structural effects [18–21]. The amyloid fibril type has also been shown to be important for the phenotypic appearance of the disease [22,23], however, the exact pathogenesis is not fully understood [24].

ATTR amyloidosis can be divided into various subtypes depending on the phenotypic appearance, however, wild-type and hereditary ATTR amyloidosis are the two main forms of the disease.

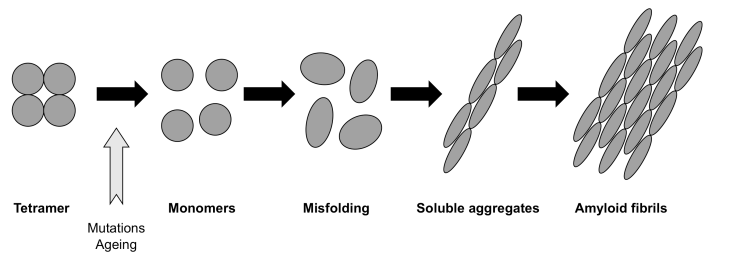


Figure 2: Schematic model of the amyloid formation process from TTR tetramer to amyloid fibril.

Wild-type ATTR amyloidosis

Wild-type ATTR amyloidosis is also called senile systemic amyloidosis (SSA), or senile cardiac amyloidosis (SCA) due to its late onset and preferably cardiac manifestations.

The disease is caused by amyloid fibril formation from normal (wild-type) TTR and usually affects males aged 70 years or more [25]. A slowly progressing restrictive cardiomyopathy is a typical finding in these patients, and a history of carpal tunnel syndrome is also common [26].

Hereditary ATTR amyloidosis

Hereditary ATTR amyloidosis, also known as familial amyloid polyneuropathy (FAP) or familial amyloid cardiomyopathy (FAC) depending on the phenotype, is a rare but fatal disease caused by TTR gene mutations. The disease is present all over the world, but endemic areas are found in Sweden, Portugal, Brazil and Japan. The Portuguese neurologist Andrade first described the disease in 1952 and it was subsequently described in Sweden and Japan in the late 1960s [27–29].

To date, more than 100 amyloidogenic TTR mutations have been described, all of which cause systemic amyloidoses [30]. The V30M mutation, in which Methionine is substituted for Valine at position 30, is found in endemic areas [30], but the V122I mutation is probably the most common variant globally with a prevalence of about 4 % in African-Americans [31].

A considerable phenotypic diversity has been observed in patients with hereditary ATTR amyloidosis and, apart from the genotype, environmental and other genetic factors seem to be important for the disease presentation [32–35]. Homozygosity for the TTR V30M mutation does, however, not implicate a more severe form of the disease [36].

The age at disease onset has also been shown to play a role in the disease phenotype [37–39] and patients with an onset before the age of 50 years are usually defined as early-onset cases. Autonomic neuropathy is more common and usually more severe in early-onset cases, whereas cardiomyopathy is more frequent in late-onset cases [40–42], especially in late-onset males [43]. The mechanisms behind these differences are not fully elucidated, but differences in amyloid fibril composition seem to be involved [22].

Despite the phenotypic diversity of the disease, several common features can be noted – a length dependent sensorimotor polyneuropathy (Figure 3), autonomic neuropathy, cardiac arrhythmias, restrictive cardiomyopathy, gastrointestinal (GI) disturbances, kidney failure, carpal tunnel syndrome, vitreous opacities and glaucoma are all frequently reported complications [30,44]. Central nervous system (CNS) complications, such as dementia, headache, ataxia and subarachnoid artery bleedings, are dominating the clinical presentation in a few genotypes [45–49].



Figure 3: Advanced peripheral polyneuropathy in the lower extremities of a Swedish patient with end-stage ATTR V30M amyloidosis (FAP). Photo by: Rune Andersson.

Depending on the most prominent symptom, the disease can be classified as of polyneuropathic, cardiac, leptomeningeal, oculoleptomeningeal or mixed type. The TTR V30M mutation, for instance, is linked to a mainly polyneuropathic phenotype, whereas the V122I mutation is associated with a cardiac phenotype, and the E89Q mutation with a more mixed phenotype [50].

Symptomatic treatments are available to relieve the patients' symptoms and, in addition, a liver transplantation has been shown to halt the progress of the disease in a majority of the patients by ceasing the production of mutated TTR [51]. Furthermore, the TTR stabilizing drugs tafamidis and diflunisal have recently been proven to reduce disease progression [52–54], and gene therapy is currently under evaluation, aiming to diminish TTR production [55,56].

GI manifestations of hereditary ATTR amyloidosis

GI complications are common in hereditary ATTR amyloidosis [44,50,57] and can occur early in the disease [58,59]. Constipation and/or nausea and vomiting are often the presenting symptoms, although diarrhea can be the first GI symptom in some cases [32]. In later stages of the disease the diarrhea usually becomes continuous, often coupled with fecal incontinence and severe malnutrition [60]. Moreover, the GI disturbances have been shown to play an important role in the patients' morbidity and mortality, both before and after liver transplantation [61,62].

The mechanisms behind these complications are not fully elucidated, however, malfunction of the autonomic and enteric nervous systems seem to be of importance [63–71]. This malfunction, in turn, causes GI motility disturbances that can lead to bacterial overgrowth and malabsorption in the small intestine [59,60].

The autonomic nervous system

The visceral, or autonomic, nervous system (ANS) is involved in the control of involuntary functions of, for example, the cardiovascular system, the gut and the urinary bladder. The ANS is commonly divided into two major divisions – the sympathetic and parasympathetic nervous systems (Figure 4), however, the enteric nervous system is often regarded as a third division of the ANS [72].

Neurons that make up the sympathetic nervous system originate from the thoraco-lumbar parts of the spinal cord, whereas neurons in the parasympathetic nervous system emanate from the cranio-sacral spinal cord. In both the sympathetic and parasympathetic subdivisions, the neurons project to the innervated tissues via peripheral ganglia located pre- or paravertebrally or near the target organs. The sympathetic neurons in connection with the GI tract emanate from the lower thoracic spinal cord and innervate

the gut through the celiac and mesenteric ganglia. Parasympathetic neurons innervating the gut, on the other hand, are derived from the dorsal nucleus of the vagus nerve and from the sacral spinal cord and project to the end organs via vagal and pelvic pathways [72,73].

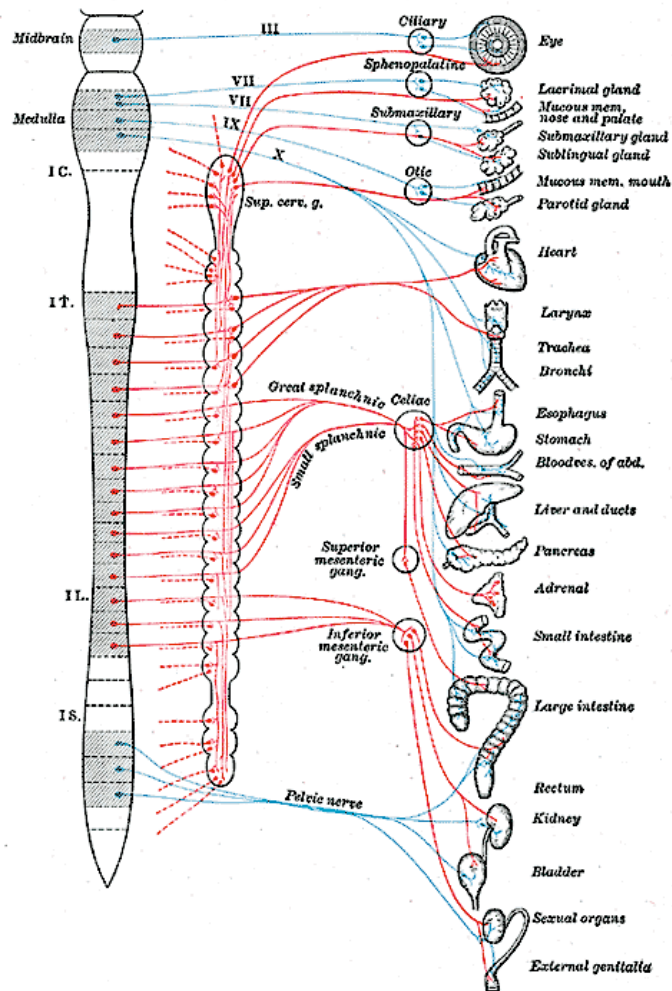


Figure 4: Overview of the autonomic nervous system with its sympathetic (red) and parasympathetic nerves (blue) and the targeted organs. Originally published in Gray's Anatomy of the Human Body, 20th edition, 1918.

The sympathetic and parasympathetic nervous systems are constantly active at some level and contribute to bodily homeostasis. However, the CNS predominantly activates the sympathetic system in stressful “fight or flight” situations, whereas the parasympathetic system activity predominates under more peaceful “rest and digest” conditions [72].

The enteric nervous system

A vast number of neurons are located in and around the human gut and together they make up the enteric nervous system (ENS). The ENS contains local sensory neurons, interneurons and motor neurons that are organized into the myenteric (Auerbach's) plexus, located between the circular and longitudinal muscle layers, and the submucosal (Meissner's) plexus, located in the submucosa of the GI tract. The myenteric plexus primarily regulates smooth muscle activity of the gut, whereas the submucosal plexus mainly is concerned with the glandular secretion of digestive enzymes, mucus, etc. [72].

The ENS has extensive connections with the CNS and the sympathetic and parasympathetic nervous systems, and the organization of the ENS is generally more complex than that of the ANS. The CNS and the ANS are involved in the regulation of the ENS, but the ENS is also capable of a certain autonomous function to control the digestive system [74,75].

Gut motility

Motility, or the ability to move, is a central property of the digestive system. The tunica muscularis (i.e. the external circular and longitudinal muscle layers, Figure 5) of the gut is responsible for the mixing and transportation of food and waste through the digestive tract.

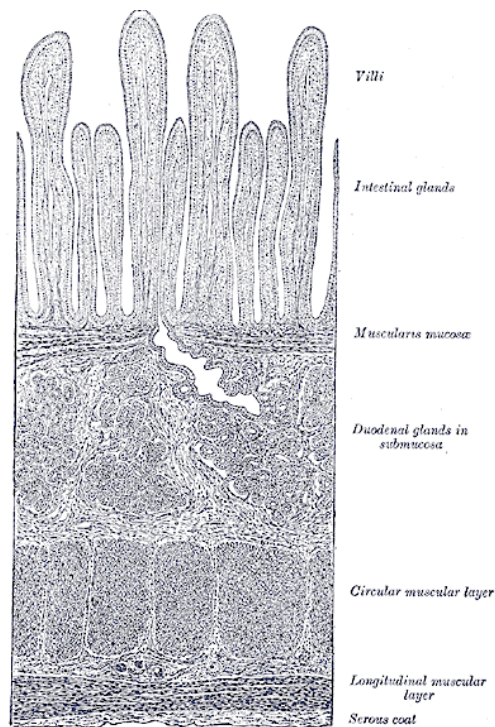


Figure 5: Section of the duodenum showing its different layers. Originally published in Gray's Anatomy of the Human Body, 20th edition, 1918.

All muscles of the tunica muscularis, except for the striated muscles of the proximal esophagus and the external anal sphincter, are smooth muscles that are able to generate spontaneous contractions. Two main fashions of contraction exist – tonic and phasic contractions, the former associated with the so-called gastrointestinal tone and the latter with the mixing and propulsive movements of the gut [76,77].

Throughout the GI tract there is a basic electrical rhythm characterized by slow waves that regulate the frequency and direction of propagation of the smooth muscle contractions. The electrical rhythm is generated by the interstitial cells of Cajal (ICC), which are described more closely in a separate paragraph. The frequency of the slow waves varies in different parts of the gut and ranges from 3 cycles/min in the gastric antrum to 12 cycles/min in the duodenum. Phasic contractions can also occur in more complex patterns, like the migrating motor complex of the stomach and small bowel and the giant migrating contractions of the colon [76,78,79].

Besides the neural control, there is an extensive paracrine/endocrine regulatory system associated with the digestive system and, in fact, the gut is the largest endocrine organ in the body producing more than 100 hormonally active peptides. The peptides are released in an endocrine, neurocrine, paracrine or autocrine fashion and some of the GI peptides can function as hormones, growth factors and neurotransmitters [76,80]. This combined neural and endocrine control system is also referred to as the neuroendocrine system of the gut.

GI motility is also closely associated with the gut microbiota in a two-way fashion – the microbiota has been shown to be altered in disorders associated with changes in GI motility and, conversely, gut microbes seem to be able to influence the GI motility [81].

Gastric emptying

Gastric emptying is the result of a coordinated activity of the proximal stomach, antrum, pylorus and duodenum (Figure 6). The proximal stomach is mainly involved in gastric accommodation, whereas the distal part of the stomach is predominantly concerned with gastric peristalsis. Gastric ICC generate the slow waves that determine the contractile activity of the stomach and the main pacemaker region is located in the greater curvature. The number of ICC, however, increases toward the gastric antrum [82,83].

The pattern of gastric emptying differs depending on the composition of the ingested food. Solids empty at a slower rate than liquids and usually display a lag phase of 20-40 min during which the solids move from the proximal to the distal part of the stomach. Liquids generally do not exhibit a lag phase; however, high-nutrient liquids have a slower emptying rate and, like solids, a more linear emptying pattern [83].

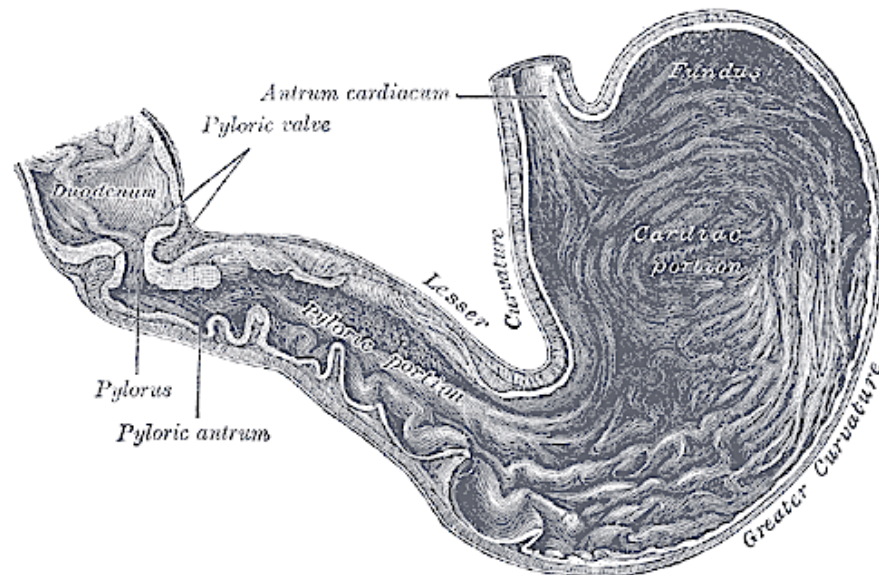


Figure 6: Interior of the stomach and its sections. Originally published in Gray's Anatomy of the Human Body, 20th edition, 1918.

A delayed gastric emptying can be idiopathic, but it can also occur as a long-term result of diabetes mellitus and after upper abdominal surgery [84]. In addition, gastroparesis is a relatively frequent complication of hereditary ATTR amyloidosis [85]. The pathogenesis of gastroparesis is multifactorial, yet vagus nerve degeneration, reduction of enteric nerves and ICC, smooth muscle fibrosis and increased inflammatory cells have been observed in histopathological studies on idiopathic and diabetic gastroparesis [84].

Classic symptoms associated with a delayed gastric emptying are nausea, bloating, vomiting, abdominal pain and early satiety, however, the correlation between upper GI symptoms and gastric emptying rates are generally weak [85–88].

Interstitial cells of Cajal

Santiago Ramón y Cajal, a Spanish pathologist and neuroscientist, first described in 1911 the interstitial cells located between nerve endings and smooth muscle cells of the GI tract [89].

The ICC have today been demonstrated throughout the digestive tract and can be divided into several subtypes depending on their anatomical localization. One example is the intramuscular portion of ICC, i.e. the ICC in the circular (ICC-CM) and longitudinal muscle layers (ICC-LM), which can be found all the way from the esophagus to the distal colon [90,91].

There is increasing evidence that the ICC are crucial for a normal GI motility by generating slow waves, but the ICC also appear to be important for neurotransmission [92], for the membrane potential of smooth muscle cells [93], and for mechanosensation [94] in the GI tract. Moreover, changes in the density and/or structure of ICC have been observed in numerous GI motility disorders, such as diabetic gastroenteropathy, slow-transit constipation and Hirschsprung's disease [95].

Liver transplantation

The world's first successful liver transplantation was performed in the United States in 1967, and the first Swedish liver transplantation was performed in 1984. Today, around 150 patients undergo liver transplantation in Sweden every year and the majority of the transplantations are carried out with whole liver grafts from deceased donors. Prior to transplantation the potential candidates undergo a thorough evaluation of their physical and mental status, as well as their social conditions. Following transplantation, long-term immunosuppressive treatment is required to prevent graft rejection [96].

The first liver transplantation for hereditary ATTR amyloidosis was performed in Stockholm, Sweden in 1990 and the early results were promising [97,98]. Since then, more than 2000 liver transplantations have been performed with this indication worldwide and, overall, the outcome has been beneficial. However, for patients with non-V30M mutations, late-onset cases (≥ 50 years) and for those transplanted late in the course of the disease, the outcome has been less favorable [99].

Patients and Methods

Study cohorts (I-IV)

Three different cohorts of patients participated in the studies included in the thesis. Article I was based on data from patients enrolled into a large international registry (THAOS, see separate paragraph), whereas articles II and IV were based on data from Swedish patients with hereditary ATTR amyloidosis who had been examined at the Department of Medicine, Norrlands University Hospital, Umeå, Sweden. Article III was written in collaboration with the Department of Diagnostic Medicine, Kumamoto University, Kumamoto, Japan and was based on samples from deceased Japanese patients with hereditary ATTR amyloidosis and deceased non-amyloidosis controls.

It should be noted that some of the Swedish patients who were enrolled into THAOS (article I) had also participated in the studies on the impact of autonomic neuropathy on gastric emptying (article II) and on the outcome of gastric emptying and GI symptoms after liver transplantation (article IV). In addition, most of the patients in article IV had also been included in article II.

Diagnosis of ATTR amyloidosis (I-IV)

In the Swedish and Japanese patient material (articles II-IV), the diagnosis of hereditary ATTR amyloidosis was based on the presence of typical symptoms together with a TTR mutation determined by DNA sequencing and histopathologically proven amyloid deposits. Punch biopsies from abdominal skin and fat were primarily used for the diagnosis.

In the THAOS registry (article I), only 56 % of the symptomatic patients had a biopsy proven diagnosis of hereditary ATTR amyloidosis, which is due to the fact that a histopathological diagnosis is not routinely sought for in endemic areas with a high incidence of the disease.

Since 2005, typing of the amyloid protein has regularly been performed in Swedish patients (articles II, IV) in order to determine that the amyloid deposits truly consist of TTR and, if possible, the amyloid fibril type has also been established.

THAOS (I)

The Transthyretin Amyloidosis Outcomes Survey (THAOS) is an international, longitudinal, observational registry that collects data on the progression of ATTR amyloidosis and it is sponsored by Pfizer Inc. THAOS was established in December 2007 and is open to adults (≥ 18 years of age) with hereditary or wild-type ATTR amyloidosis and to asymptomatic TTR-variant carriers. Eligible patients were consecutively enrolled into THAOS by

each of the participating sites. De-identified data obtained during routine clinical practice were entered into THAOS using an internet-based application [100].

Only baseline data from the time of inclusion into THAOS were used for the analyses in the thesis.

GI symptoms (I-IV)

The GI symptoms reported in THAOS (article I) were based on a checklist including the items early satiety, nausea, vomiting, constipation, alternating diarrhea/constipation, diarrhea, fecal incontinence and unintentional weight loss. Early satiety, nausea and vomiting were regarded as upper GI symptoms, whereas constipation, alternating diarrhea/constipation, diarrhea and fecal incontinence were regarded as lower GI symptoms.

Clinical data from routine investigations performed for evaluation of the disease were used in articles II and III and the items included were nausea, vomiting, constipation, alternating diarrhea/constipation and diarrhea.

In article IV, two different questionnaires were used for GI symptom assessment and both are described more closely in a separate paragraph.

Nutritional status (I-IV)

The modified body mass index (mBMI), in which BMI (kg/m^2) was multiplied by s-albumin (g/L) to compensate for edema, was used to assess the patients' nutritional status. Values below $750 \text{ kg}/\text{m}^2 \cdot \text{g}/\text{L}$ were regarded as consistent with underweight and values below $600 \text{ kg}/\text{m}^2 \cdot \text{g}/\text{L}$ were regarded as consistent with severe malnutrition [61,62].

mBMI has been used as a measure of nutritional status in several studies on patients with hereditary ATTR amyloidosis and is considered to be a valid marker in these patients [52].

Questionnaires (I, IV)

Three different questionnaires were used in the thesis (Appendix).

In article I, the patients' health-related quality of life (HRQoL) was evaluated with the validated EuroQoL Five Dimensions (EQ-5D) questionnaire, which includes a descriptive and visual analogue scale assessment of the present health status. The EQ-5D Index Score was calculated using a predefined scoring algorithm and full health was represented by a score of 1 [101,102].

Two questionnaires were used to evaluate the outcome of GI symptoms after liver transplantation (article IV). The first questionnaire contained seven items on GI symptoms, which were combined into two symptom clusters – upper and lower GI symptoms. A ten-point rating scale (0-10) was used for symptom assessment, where 0 represents no symptoms and 10 represents unbearable symptoms. This questionnaire, together with another

54 questions regarding other somatic symptoms, mental symptoms, information, social aspects, confidence and satisfaction, has been validated and used in previous studies on patients with hereditary ATTR amyloidosis [103,104], and was utilized at all three time-points in article IV. The second questionnaire contained supplementary questions regarding concomitant diseases, current medication and consent to access the patients' medical records for reviewing other factors with possible impact on their GI function. Polypharmacy was defined as five concomitant drugs or more in the analyses of the relationship between the patients' medication and their GI symptoms [105,106].

Gastric emptying scintigraphy (II, IV)

Gastric emptying was investigated with gastric emptying scintigraphies (GES) according to the method employed in the Swedish multi-center study of gastric emptying [107]. The test meal was ingested after an overnight fast and consisted of a Tc^{99m}-labelled omelet and a low-calorie drink. Scintigraphic acquisitions were performed using the STARCAM and Millenium MPR gamma cameras (General Electric, Milwaukee, WI).

Two variables, the total half-time (T_{50}) and retention at 90 minutes, were used for the analyses of gastric emptying (Figure 7). Since the settings of the scintigraphic software had changed during the study period, the T_{50} values were manually measured by a single reviewer for consistency over time. In article IV, the retention at 90 minutes was added to the analyses for verification of the results.

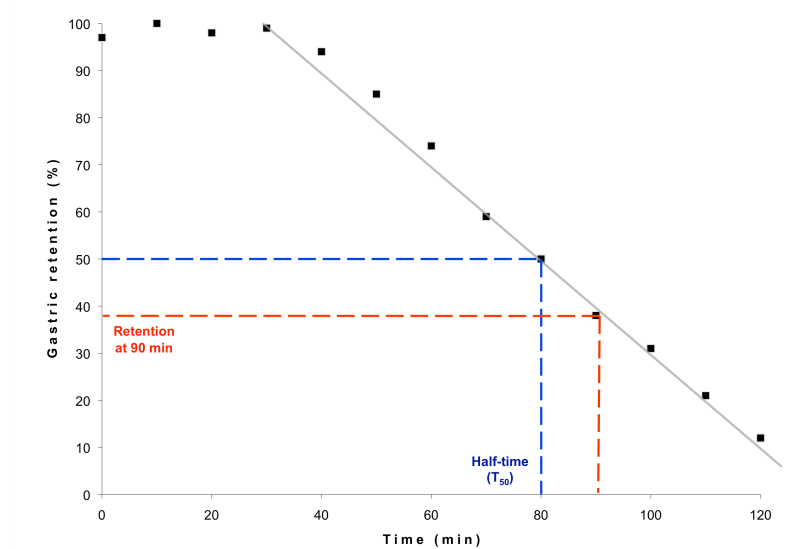


Figure 7: Graph showing the result of a normal gastric emptying scintigraphy and the variables used in the studies. Lag phase from 0 to 30 min, a T_{50} of 80 min and a retention at 90 min around 38 %.

Gastric retention was defined as T_{50} above 133 minutes (mean + 2 SD) or a retention of more than 76 % (mean + 2 SD) at 90 minutes, according to the reference values obtained by the Swedish multi-center study [107]. T_{50} values over 350 minutes were entered as 350 minutes.

Autonomic testing (II)

The ANS function was assessed by analyses of the heart rate variability (HRV) and the cardiovascular response to tilt tests.

Power spectrum analysis was performed on heart rate data from two-minutes sequences in the supine and upright positions using autoregressive modeling [108]. Patients with pacemaker treatment and frequent non-sinus beats were excluded from the analysis. The spectral powers in two frequency bands were used in the study, i.e. the high-frequency component recorded in a supine position and the low-frequency component recorded in an upright position (Figure 8). The respiration-related high-frequency component of HRV represents an estimate of parasympathetic cardiac control and the low-frequency component, recorded after a postural change from a supine to an upright position, is considered a useful marker of sympathetic activity [109,110].

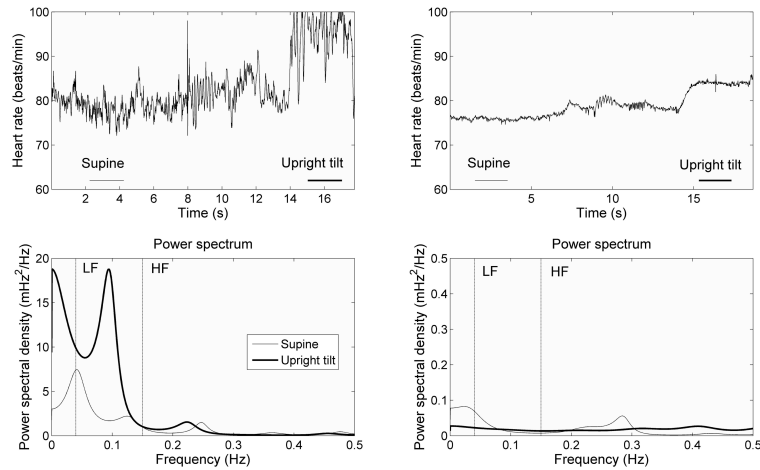


Figure 8: Heart rates and spectral analyses of the heart rate variability (HRV) in two patients with hereditary ATTR amyloidosis. To the left a case with a virtually normal HRV and to the right a case with a low HRV. The high-frequency component in the supine position was used as an indirect estimate of parasympathetic function and the low-frequency component in an upright position as an estimate of sympathetic function.

In connection with the HRV recordings, the patients' blood pressures were measured after three minutes in the supine resting position and the tilted (70°) upright position, respectively. The mean heart rate was calculated from the two-minute ECG recordings adjacent to the blood pressure

measurements. Changes in systolic blood pressure and mean heart rates after tilting were then calculated, and a decrease in blood pressure of 20 mm Hg or more was regarded as consistent with orthostatic hypotension [111], which was used as a marker of sympathetic dysfunction.

Staining and immunohistochemistry (III)

Full-thickness gastric antrum wall samples were collected during autopsies performed by a pathologist on call, and the samples were then formalin fixed, paraffin embedded and cross-sectionally cut into 10 µm thick sections.

After Congo red staining of the sections, amyloid deposits were visualized in polarized light microscopy.

The sections were immunostained by the avidin-biotin complex (ABC) method [69,112] after microwave antigen retrieval. C-Kit and Transmembrane Protein 16A (TMEM16A) antibodies were used as primary antibodies to detect ICC, whereas Protein Gene Product (PGP) 9.5 antibodies were used to detect nervous tissue and TTR antibodies were used to detect TTR. Biotinylated rabbit anti-goat IgG were used as secondary antibodies for c-Kit and rabbit anti-mouse IgG were used for TMEM16A, PGP 9.5 and TTR. The primary antibody was omitted on sections serving as negative controls.

Computerized image analysis (III)

To quantify the densities of ICC, nervous tissue and TTR computerized image analysis was performed using an Olympus microscope type BX50 with a x20 objective and the QWIN and QUIPS softwares from Leica. The classic stereological point-counting method [113–115] was used, which is based on a mathematic model where the relative volume density of a structure (A) that lies within another structure (B) is calculated. Both structures occupy a certain volume and by numbering points covering the different structures a ratio, the relative volume density, between points covering structures A and B can be calculated. Twenty fields from the circular and longitudinal muscle layers, respectively, were randomly chosen from each individual.

Statistics (I-IV)

Comparisons between cohorts and subgroups in THAOS (article I) were carried out using the one-way ANOVA for continuous variables and the chi-square test for categorical variables. In articles II, III and IV, the Mann-Whitney U test, Kruskal-Wallis test and the chi-square test were used for comparisons between groups, whereas Spearman's Rank Order test was used to analyze correlations. Multiple regression analyses were carried out to identify potential predictors of GI symptoms, mBMI and the EQ-5D Index Score (article I), as well as of gastric retention (article II). The outcome of different variables after liver transplantation was analyzed with the Wilcoxon Signed Rank test, Friedman's Two-Way Analysis of Variance by Ranks and

the Cochran's Q test (article IV). P-values below 0.05 were regarded as statistically significant. SAS 9.1.3 (article I) and IBM SPSS Statistics 18-20 for Macintosh (articles II-IV) were used for the analyses.

Ethics (I-IV)

Prior to the enrollment of patients into THAOS (article I), each of the participating sites obtained approval from their local ethical or institutional review board. All patients provided written informed consent and research was conducted in accordance with the Declaration of Helsinki.

The study on autonomic neuropathy and gastric emptying (article II) was part of a larger project that had been approved by the Regional ethics board in Umeå, Sweden (reference number 06-084M). Approval from the Regional ethics board in Umeå had also been provided for article IV (reference number 2011-365-31M), whereas a Japanese ethical committee had approved the analyses in article III (reference number Kumamoto University No. 17-86).

Results and Discussion

GI disturbances are known to be common in hereditary ATTR amyloidosis [50], however, the prevalence of these complications in different genotypic and phenotypic subgroups has not been studied in detail. The mechanisms behind the GI disturbances have also remained largely unexplained, although malfunction of the autonomic and enteric nervous systems has been demonstrated [63–71]. The results in this thesis will fill at least some of the knowledge gaps in the distribution and underlying mechanisms of the GI manifestations of the disease.

Patients (I-IV)

The main characteristics of the patients included in the different study cohorts are outlined in Table 1.

Table 1: Characteristics of the different study cohorts.

	I	II	III Patients	Controls	IV
n	1744	188	11	10	115
Median age	45	60	43	62	59
Males	55 %	62 %	45 %	70 %	59 %
V30M	74 %	97 %	100 %	0 %	97 %
Origin	Multinational	Swedish	Japanese	Japanese	Swedish

A total of 1744 subjects had been enrolled into THAOS as of June 2013 and were available for the study on GI manifestations of ATTR amyloidosis (article I). Of those, 160 subjects had been diagnosed with wild-type ATTR amyloidosis and 1219 were symptomatic patients with TTR mutations. Most subjects (46 %) were of Portuguese origin (Figure 9) and a majority (74 %) carried the V30M mutation. The V122I mutation was the second most frequent variant in the registry, carried by 5 % of the subjects.

One hundred and eighty-eight patients who had been examined at our centre between 1990 and 2009 were included in a study on gastric emptying and autonomic neuropathy (article II). All patients had been diagnosed with hereditary ATTR amyloidosis and the vast majority (97 %) carried the V30M mutation. The examinations performed were part of the routine investigation of their disease and, for a majority of the patients, also part of the evaluation for liver transplantation.

Eleven deceased Japanese patients with hereditary ATTR V30M amyloidosis and ten deceased Japanese controls were included in a case-control study on gastric ICC and nervous tissue (article III). All patients had died from complications of their amyloidosis, whereas, in the control group, five had died of colon cancer, two of acute myocardial infarction, one of lymphoma, one of lung cancer and one of adult T-cell leukemia. Both

patients and controls had been examined at the Kumamoto University Hospital and all autopsies except one had been performed within five hours after death.

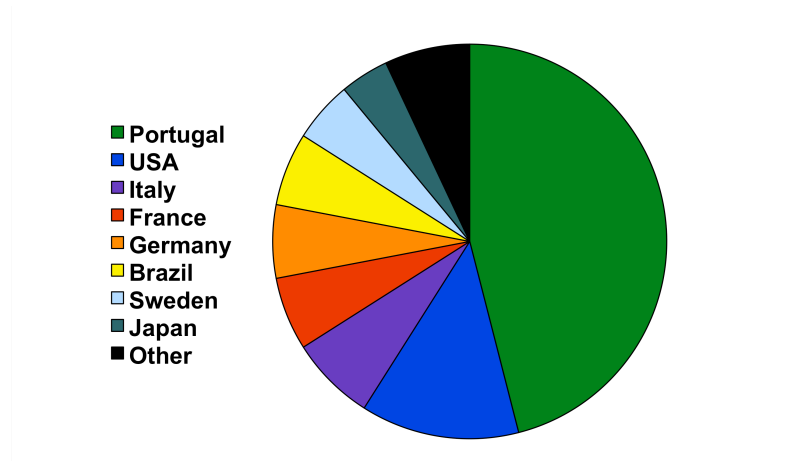


Figure 9: Distribution of the countries of origin for the patients enrolled into THAOS as of June 2013.

One hundred and fifteen patients who had been evaluated at our centre and undergone liver transplantation between November 1990 and September 2011 participated in the study on the outcome of gastric emptying and GI symptoms after liver transplantation (article IV). The study questionnaires were sent to the 92 patients who were still alive and Swedish residents at the time of the study. Of those, 77 (84 %) had responded to the questionnaires by June 2012. Apart from the study questionnaires, all examinations were part of the routine clinical evaluation of the patients.

Prevalence of GI symptoms (I, II, IV)

Fifty-nine percent of all patients in THAOS (article I) reported at least one GI symptom, which is well above the prevalence of functional gastrointestinal disorders of about 10-25 % reported by the general population [116]. The distribution of the individual GI symptoms in patients with wild-type and hereditary ATTR amyloidosis, respectively, is presented in Table 2. Since patients with wild-type ATTR amyloidosis reported GI symptoms at a frequency equal to that of the general population, subsequent analyses were focused on those with the hereditary form of the disease.

Unintentional weight loss was the most frequently reported GI symptom for patients with TTR mutations enrolled into THAOS, and one may argue that weight loss is not merely a symptom of GI dysfunction. Nevertheless, unintentional weight loss was recorded as a GI symptom in the registry and a marked loss of weight has been observed in patients with hereditary ATTR

amyloidosis even before the onset of other symptoms [58]. The mechanisms behind this weight loss remain unexplained, yet early satiety could be a contributing factor as it often occurs early in the disease (Figure 10) and may affect the patients' energy intake negatively before they notice it.

Table 2: GI symptoms in patients with ATTR amyloidosis at enrollment into THAOS.

GI Symptom	Wild-type (n = 140)	TTR mutation (n = 1114)
Any GI symptom	21 (15 %)	696 (63 %)
Early satiety	5 (4 %)	291 (26 %)
Nausea	3 (2 %)	189 (17 %)
Vomiting	0 (0 %)	147 (13 %)
Constipation	5 (4 %)	230 (21 %)
Diarrhea/constipation	2 (2 %)	267 (24 %)
Diarrhea	5 (4 %)	218 (20 %)
Fecal incontinence	0 (0 %)	68 (6 %)
Unintentional weight loss	4 (3 %)	346 (32 %)

Early satiety was the second most common symptom in THAOS followed by alternating diarrhea/constipation. Expectedly, fecal incontinence was the least frequent symptom as it occurs in late stages of the disease [60] and as the median duration of disease at inclusion in THAOS was merely 4.9 years.

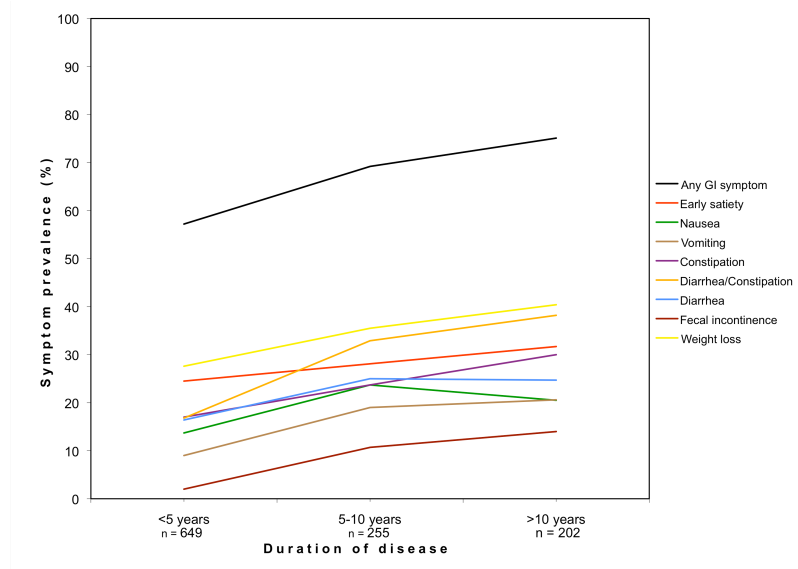


Figure 10: Prevalence of GI symptoms in patients with TTR mutations and disease duration of <5 years, 5-10 years, and >10 years, respectively. A majority of the patients suffered from GI symptoms even at early stages of the disease, whereas the reported prevalence of GI symptoms in the general population usually ranges from 10 to 25%. Unintentional weight loss, early satiety and alternating diarrhea/constipation were the most common symptoms in all disease stages. The symptom prevalence increased significantly with disease duration for all symptoms ($p < 0.01$, for all), except for early satiety ($p = 0.11$).

As expected, the prevalence of GI symptoms was higher for patients enrolled in later stages of their disease; however, as much as 57 % of the patients with disease duration of less than five years suffered from GI symptoms (Figure 10).

All of the deceased Japanese patients had suffered from diarrhea and four of them (36 %) also had periods of constipation, and another two patients (18 %) had also suffered from nausea.

In the Swedish patient material, 59 % of the patients reported GI symptoms after a median disease duration of 3.0 years (article II) and, of those, a majority (74 %) reported lower GI symptoms (constipation or diarrhea). Furthermore, 84 % of the patients reported GI symptoms shortly before liver transplantation (article IV), which was performed in median 3.3 years after disease onset, and virtually all patients (97 %) had GI complaints at follow-up nearly nine years after transplantation.

Genotypic differences (I)

GI symptoms were more prevalent in patients with the V30M mutation than in those with non-V30M mutations (69 % vs. 56 %, $p < 0.01$), which is consistent with previous reports of a less frequent autonomic neuropathy in non-V30M patients [30].

Table 3: TTR mutations with ten or more symptomatic individuals enrolled into THAOS (in descending order) and some of the associated clinical manifestations.

TTR mutation	Sensory neuropathy	Motor neuropathy	GI symptoms	Cardiac complications
V30M	707 (90 %)	305 (39 %)	547 (69 %)	212 (27 %)
V122I	35 (60 %)	11 (19 %)	16 (27 %)	57 (97 %)
S50R	26 (90 %)	16 (55 %)	19 (66 %)	13 (45 %)
E89Q	21 (96 %)	10 (46 %)	13 (68 %)	13 (65 %)
T60A	16 (80 %)	5 (25 %)	8 (40 %)	19 (91 %)
F64L	18 (90 %)	11 (55 %)	10 (50 %)	7 (35 %)
S77Y	16 (94 %)	8 (47 %)	12 (71 %)	9 (53 %)
I68L	7 (47 %)	6 (40 %)	2 (13 %)	13 (87 %)
I107V	10 (83 %)	9 (75 %)	7 (58 %)	8 (67 %)
G47A	8 (73 %)	2 (18 %)	2 (18 %)	1 (9 %)
L111M	1 (10 %)	0 (0 %)	1 (10 %)	7 (70 %)

Patients carrying mutations predominantly associated with cardiac manifestation (i.e. the V122I, I68L and L111M variants) reported GI symptoms with a prevalence similar to that of the general population (Table 3) and were therefore excluded from GI symptom analyses in the non-V30M group.

Geographical differences (I)

Phenotypic variations have been observed between patients with hereditary ATTR V30M amyloidosis of different geographic origins and one example is

the difference in age at onset and penetrance between the Swedish and Portuguese V30M populations [117–119].

A multiple regression analysis on factors associated with GI symptoms in V30M patients enrolled into THAOS showed that Swedish patients were less likely to suffer from GI symptoms than non-Swedish patients (OR 0.3, 95 % CI 0.17-0.57). Unexpectedly, no significant association was found for early disease onset (OR 1.5, 95 % CI 0.99-2.10), indicating that age at onset is not the major factor behind the different prevalence of GI symptoms between Swedish and Portuguese patients.

Further studies on possible genetic and environmental mechanisms behind these phenotypic differences between the countries would be of great interest.

Nutritional status (I-IV)

The nutritional status (mBMI) has been shown to be important for both the treated and untreated survival of patients with hereditary ATTR amyloidosis [61,62,120] and, consequently, mBMI was included as a variable in all of the studies.

Simple regression analyses revealed that each of the individual GI symptoms registered in THAOS (article I) was negatively associated with mBMI and both upper and lower GI symptoms remained significant predictors of the patients' nutritional status in a multiple regression analysis (Table 4).

Table 4: Factors associated with the nutritional status of patients with TTR mutations in THAOS.

Predictor variable	Beta	SE	t value	p value
Gender (male vs. female)	-11.1	17.4	-0.6	0.52
Age at onset (early vs. late)	-34.5	19.0	-1.8	0.07
Disease duration (years)	-3.8	1.3	-2.8	<0.01
Upper GI symptoms	-81.4	20.0	-4.1	<0.01
Lower GI symptoms	-98.4	18.9	-5.2	<0.01
Liver transplant	-48.5	26.0	-1.9	0.06

The duration of the disease also showed a significant negative association with mBMI, which probably reflects the development of multiple GI complications together with a marked peripheral polyneuropathy.

A weak but significant negative correlation was found between the gastric emptying (T_{50}) and mBMI ($r_s = -0.2$, $p < 0.01$) of the Swedish patients included in article II, and the 13 patients with a severely delayed gastric emptying ($T_{50} \geq 350$ min) had significantly poorer nutritional status than those with shorter gastric emptying half-times (median mBMI 809 vs. 961, $p < 0.01$).

The median mBMI of the Japanese V30M patients who had died from their amyloidosis (article III) was merely 579 kg/m²•g/L and six patients

(55 %) had an mBMI below 600 kg/m²•g/L (severe malnutrition). However, no significant difference in mBMI was found between patients and controls (median mBMI 579 vs. 555 kg/m²•g/L, $p = 0.47$) and no significant correlation was found between the densities of gastric c-Kit immunoreactive (IR) ICC and mBMI in the circular ($r_s = -0.3$, $p = 0.33$) or longitudinal muscle layers ($r_s = -0.4$, $p = 0.18$) of the patients.

The outcome of Swedish patients' nutritional status after liver transplantation (article IV) is presented separately.

Health-related quality of life (I)

As for the nutritional status, a significant negative association was found between each of the individual GI symptoms and the EQ-5D Index Score. Both upper and lower GI symptoms, as well as early disease onset and duration of disease, remained significant negative predictors of the patients' HRQoL in a multiple regression analysis (Table 5). Expectedly, liver transplantation prior to enrollment into THAOS exhibited a significant positive association with the EQ-5D Index Score.

Table 5: Factors associated with the HRQoL of patients with TTR mutations enrolled into THAOS.

Predictor variable	Beta	SE	t value	p value
Gender (male vs. female)	0.00	0.01	-0.1	0.91
Age at onset (early vs. late)	-0.09	0.01	-6.2	<0.01
Disease duration (years)	-0.01	0.00	-6.9	<0.01
Upper GI symptoms	-0.07	0.01	-4.8	<0.01
Lower GI symptoms	-0.13	0.01	-9.3	<0.01
Liver transplant	0.06	0.02	3.2	<0.01

Gastric emptying (II, IV)

A delayed gastric emptying was demonstrated in 63 (39 %) out of the 162 patients who had performed a GES (article II), and 13 patients (8 %) had a severely delayed gastric emptying with T_{50} values above 350 min.

Patients with lower GI symptoms had a significantly slower gastric emptying than those with no GI symptoms (median T_{50} 123 vs. 113 min, $p = 0.04$) and an even larger difference was found for those with both upper and lower GI symptoms (median T_{50} 192 vs. 113 min, $p < 0.01$). Unexpectedly, no significant difference was found between the patients with only upper GI symptoms and those without GI symptoms (median T_{50} 119 vs. 113 min, $p = 1.0$).

From this we can conclude that gastric retention is common in patients with hereditary ATTR amyloidosis and that it, as for other conditions [86–88], appears to be difficult to predict the patients' gastric emptying from their GI symptoms alone.

The outcome of gastric emptying after liver transplantation (article IV) is described in a separate section.

Impact of autonomic neuropathy (II)

Weak but significant negative correlations were found between the patients' gastric emptying half-times (T_{50}) and the supine high-frequency component (HF_{sup}) of HRV ($r_s = -0.3$, $p < 0.01$), as well as the tilted low-frequency component (LF_{tilt}) of HRV ($r_s = -0.4$, $p < 0.01$).

A weak but significant correlation was also found between mBMI and LF_{tilt} ($r_s = 0.3$, $p < 0.01$), whereas no significant correlation was found for HF_{sup} ($r_s = 0.2$, $p = 0.09$).

Patients with gastric retention showed a greater decrease in systolic blood pressure after tilting than those without retention (median change -10 vs. -5 mm Hg, $p = 0.04$) and, moreover, gastric retention was more common in patients with orthostatic hypotension ($\chi^2 = 6.7$, $p = 0.01$).

In a multiple regression analysis on factors associated with gastric retention, age at onset and sympathetic function (LF_{tilt}) were the only significant predictors (Table 6).

Table 6: Factors associated with gastric retention in Swedish patients with hereditary ATTR amyloidosis.

Predictor variable	Univariate		Multivariate	
	Crude OR	95 % CI	Adjusted OR	95 % CI
Gender (female vs. male)	1.40	0.74-2.67	0.76	0.31-1.84
Age at onset (ln years)	0.74	0.25-2.22	0.10	0.02-0.52
Parasympathetic function (HF_{sup})	0.58	0.32-1.06	1.81	0.72-4.56
Sympathetic function (LF_{tilt})	0.45	0.27-0.74	0.23	0.10-0.51

Altogether, only weak correlations were found between the measures of ANS function and gastric emptying, as well as nutritional status. Sympathetic activity seemed more strongly related to the GI function, which probably reflects a more advanced disease in patients with sympathetic dysfunction since a reduced parasympathetic function often precedes a sympathetic dysfunction in these patients [121].

Gastric ICC and nervous tissue (III)

The relative volume densities of gastric c-Kit-IR ICC were significantly lower in patients with hereditary ATTR V30M amyloidosis than in non-amyloidosis controls (Figure 11) and the results were equivalent for TMEM-16A-IR ICC, which supports the loss of ICC in the patients.

No significant differences were found in relative volume densities of PGP 9.5-IR gastric nervous tissue between patients and controls either in the circular (median density 3.0 vs. 6.8, $p = 0.17$) or longitudinal muscle layers (median density 1.4 vs. 2.5, $p = 0.10$). In addition, no difference in the number of enteric neurons in myenteric ganglia was found between patients and controls (median count 19.0 vs. 14.5, $p = 0.43$).

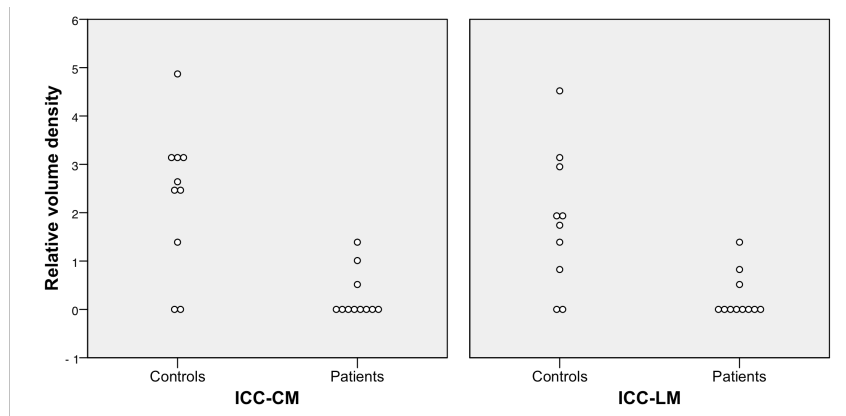


Figure 11: Relative volume densities of c-Kit immunoreactive gastric interstitial cells of Cajal (ICC) in patients with hereditary ATTR V30M amyloidosis and non-amyloidosis controls. Significant differences between patients and controls were found in both the circular (median density 0.0 vs. 2.6, $p < 0.01$) and the longitudinal muscle layers (median density 0.0 vs. 1.8, $p < 0.01$). CM: circular muscle layer, LM: longitudinal muscle layer.

Amyloid deposits were detected in all sections from the patients, but the correlations between the densities of amyloid deposits and c-Kit-IR ICC did not reach statistical significance either in the circular or longitudinal muscle layers ($r_s = -0.6$, $p = 0.07$ for both). Further, no significant correlations were found between the amyloid deposits and the PGP 9.5-IR nervous tissue in the circular ($r_s = 0.1$, $p = 0.75$) or longitudinal muscle layers ($r_s = 0.1$, $p = 0.83$).

Double staining showed that the amyloid was located outside the c-Kit- and TMEM16A-IR areas, indicating that the amyloid deposits induce toxic rather than structural effects on the ICC. Vascular changes might also contribute to the loss of ICC since most of the amyloid was found in the blood vessel walls. An enteric neuropathy, however, does not seem to be a major factor for the loss of ICC, or for the patients' GI symptoms, since no convincing destruction of enteric neurons could be found.

Impact of liver transplantation (I, IV)

The overall outcomes of gastric emptying, GI symptom scores and nutritional status after liver transplantation (article IV) are displayed in Table 7, and no significant change in the patients' gastric emptying or nutritional status was found over time. Their total GI symptom scores had, however, increased significantly after transplantation.

Ninety-nine patients had undergone GES (article IV) in median 0.7 years prior to liver transplantation, whereas 71 patients had completed a second GES in median 1.9 years after transplantation, and 31 patients had completed a third GES in median 5.0 years after the procedure. No significant change in T_{50} was found either between the evaluation before liver

transplantation and the first follow-up after transplantation ($p = 0.24$), or between the two post-transplant evaluations ($p = 0.96$).

Table 7: Outcome of Swedish patients' gastric emptying, total GI symptom scores and nutritional status after liver transplantation (LTx) for hereditary ATTR amyloidosis.

Variable	Pre LTx	1 st post LTx	2 nd post LTx	p value
<i>Gastric emptying</i>				
T ₅₀ (min)	137	132	125	0.52
Retention at 90 min (%)	78.1	77.8	78.6	0.61
<i>GI symptoms</i>				
Total score	7	10	13	<0.01
<i>Nutritional status</i>				
mBMI (kg/m ² •g/L)	975	991	973	0.75

Fifty-nine patients had completed all three sets of study questionnaires (article IV) and their GI symptom scores were assessed in median 0.7 years before liver transplantation and 2.0 and 8.7 years after transplantation. A significant increase in total symptom scores was found both between the pre-transplant evaluation and the first assessment after transplantation ($p < 0.01$) and between the two post-transplant evaluations ($p = 0.02$). However, no significant increase in upper GI symptom scores was found over time (Figure 12).

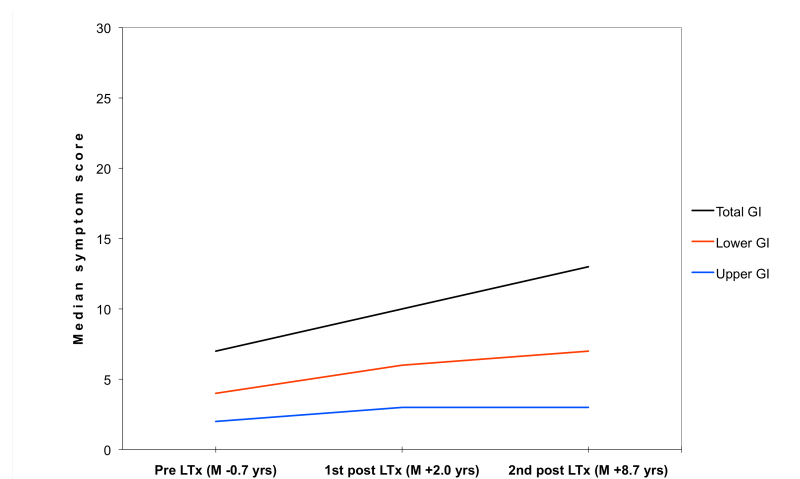


Figure 12: Outcome of gastrointestinal symptoms after liver transplantation (LTx). The total and lower gastrointestinal (GI) symptom scores had both increased significantly over time ($p < 0.01$, for both), whereas no significant change was found for upper GI symptom scores ($p = 0.09$). Nausea, vomiting and loss of appetite were regarded as upper GI symptoms, whereas constipation, diarrhea and fecal incontinence were regarded as lower GI symptoms. Unintentional weight loss was included in the total GI symptoms category. Maximum possible score was 30 for upper and lower GI symptoms, respectively, and 70 for total GI symptoms. M: median.

As for the symptom scores, the prevalence of GI complaints had increased significantly from before transplantation to the assessments performed after transplantation (84.2 % vs. 91.2 % vs. 96.5 %, $p = 0.03$) and, in addition, liver transplantation prior to enrollment in THAOS (article I) was a significant predictor of GI symptoms (OR 4.4, 95 % CI 2.46-7.72) in symptomatic patients carrying the TTR V30M mutation.

Data on nutritional status (article IV) was available for 114 patients prior to liver transplantation (median 0.7 years), and at the two follow-ups after transplantation for 79 patients (median 1.9 years) and 41 patients (median 4.7 years), respectively. No significant change in mBMI was found either between the pre-transplant evaluation and the first assessment after transplantation ($p = 0.38$), or between the post-transplant evaluations ($p = 0.88$).

Taken together, the patients' gastric emptying and nutritional status appeared to be maintained after liver transplantation, although their GI symptoms had increased over time. It is difficult to determine whether the increased GI symptoms were caused by the ATTR amyloidosis itself, since GI complications frequently occur after liver transplantation [122,123] and since GI symptoms are common side effects of several drugs. The GI symptom scores were, however, generally low and since the patients' overall GI function seemed preserved the slight increase in GI symptoms was probably caused by a combination of post-operative complications and medications rather than a progression of the amyloidosis.

Impact of gender and age at onset (I-IV)

Since hereditary ATTR amyloidosis is an autosomal dominant condition, both sexes are at equal risks of inheriting a TTR mutation from their parents and, apart from a certain maternal anticipation [124], no major gender related differences have been described in the disease. As regards to age at onset, however, a considerable phenotypic diversity has been observed between early and late-onset cases [41–43,57]. Both gender and age at onset were accounted for in all of the studies, except for article III, in which only gender related differences were analyzed.

No significant difference in the prevalence of GI symptoms was found between male and female patients with TTR mutations (63 % vs. 63 %, $p = 0.97$) enrolled into THAOS (article I). Furthermore, gender was not a significant predictor of either nutritional status (Table 4) or HRQoL (Table 5). Early-onset patients, on the other hand, reported GI symptoms more frequently than late-onset cases (70.3% vs. 49.6%, $p < 0.01$) and an early disease onset was also negatively associated with the HRQoL (Table 5).

Moreover, age at onset but not gender was shown to be a significant predictor of gastric retention in article II (Table 6), and the higher the age at onset, the lower the odds of gastric retention.

No significant gender related differences were found in the densities of gastric c-Kit-IR ICC (article III) in the circular ($z = 1.1$, $p = 0.43$) or longitudinal muscle layers ($z = 0.6$, $p = 0.66$) of the Japanese patients with ATTR V30M amyloidosis. As regards PGP 9.5-IR nervous tissue, male patients displayed higher densities than females in the longitudinal muscle layer ($z = 2.2$, $p = 0.03$), but no significant gender related difference was found in the circular muscle layer ($z = 0.6$, $p = 0.66$).

Lastly, the outcome of gastric emptying, GI symptoms and nutritional status (article IV) was similar for early- and late-onset cases, as well as for males and females after liver transplantation (Table 8), suggesting that the treatment is equally effective in stabilizing GI function in these subgroups of patients, although GI complications generally are more frequent in early-onset cases.

Table 8: Outcome of gastric emptying (T_{50}), total GI symptom scores and nutritional status (mBMI) after liver transplantation (LTx) for hereditary ATTR amyloidosis in relation to age at onset and gender.

Subgroup	Variable	n	Pre LTx	1 st post LTx	2 nd post LTx	p value
<i>Early-onset</i>	T_{50} (min)	20	138	145	116	0.20
	Symptom score	35	11	11	15	0.07
	mBMI (kg/m ² •g/L)	25	949	948	941	0.73
<i>Late-onset</i>	T_{50} (min)	6	130	106	163	0.57
	Symptom score	22	4	6.5	9.5	<0.01
	mBMI (kg/m ² •g/L)	8	1006	1054	978	0.20
<i>Male</i>	T_{50} (min)	13	145	150	131	0.16
	Symptom score	26	4	10.5	11.5	0.02
	mBMI (kg/m ² •g/L)	21	1028	1013	941	0.12
<i>Female</i>	T_{50} (min)	13	129	126	109	0.69
	Symptom score	31	9	9	15	0.02
	mBMI (kg/m ² •g/L)	12	955	970	978	0.34

Conclusions

GI disturbances are common in hereditary ATTR amyloidosis, even early on after disease onset, and have a negative impact on the patients' nutritional status and health-related quality of life. However, patients with wild-type ATTR amyloidosis and TTR mutations mainly associated with cardiac manifestations do not appear to have an increased prevalence of GI symptoms.

An autonomic neuropathy and a depletion of gastrointestinal ICC seem to contribute to the GI disturbances, but additional factors are probably involved since the correlations with the measures of autonomic neuropathy were weak and since others have demonstrated malfunction of the neuroendocrine and enteric nervous systems of the gut. We were, however, not able to detect a destruction of enteric nerves in our material.

The patients' gastric emptying half-times and nutritional statuses were largely maintained almost five years after liver transplantation, although their GI symptom scores had increased slightly over time, indicating that liver transplantation halts the progressive GI involvement of the disease. Post-operative complications and medications are possibly responsible for the increased GI symptoms after transplantation.

Altogether, these findings underscore the importance of a thorough evaluation of the GI function in patients with hereditary ATTR amyloidosis and should encourage further studies on the mechanisms behind the GI disturbances and the phenotypic differences related to genotype, age at onset and geographic origin.

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Appendix

1. EQ-5D form



Health Questionnaire

*English version for the UK
(validated for Ireland)*

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By placing a tick in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

- I have no problems in walking about ☐
- I have some problems in walking about ☐
- I am confined to bed ☐

Self-Care

- I have no problems with self-care ☐
- I have some problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

Usual Activities (e.g. work, study, housework, family or leisure activities)

- I have no problems with performing my usual activities ☐
- I have some problems with performing my usual activities ☐
- I am unable to perform my usual activities ☐

Pain/Discomfort

- I have no pain or discomfort ☐
- I have moderate pain or discomfort ☐
- I have extreme pain or discomfort ☐

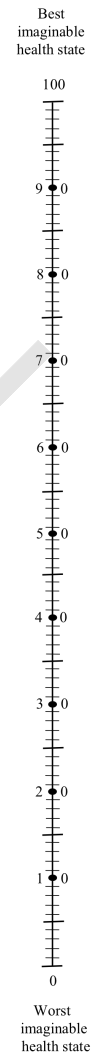
Anxiety/Depression

- I am not anxious or depressed ☐
- I am moderately anxious or depressed ☐
- I am extremely anxious or depressed ☐

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own
health state
today**



2. Symptom score questionnaire

Datum _____

FRÅGOR OM SKELLEFTESJUKAN (FAP)

Markera genom att fylla i den siffra Du tycker motsvarar graden av Dina besvär just nu.

0	1, 2, 3	4, 5, 6	7, 8, 9	10
Inte alls	Ganska ringa Lite	Betydande	Stark Påtaglig Mycket	Outhärdlig

Kroppssymptom

Illamående _____
 Kräkningar _____
 Diarré _____
 Förstoppning _____
 Problem att hålla avföringen _____
 Matleda _____
 Viktminskning _____
 Yrsel _____
 Svinningsbesvär _____
 Allmän trötthets- eller matthetskänsla _____
 Blåstömningsbesvär _____
 Impotens _____
 Synbesvär/ögonbesvär _____
 Smärta i armar _____
 Smärta i ben _____
 Känselbortfall i armar _____
 Känselbortfall i ben _____
 Nedsatt muskelkraft i armar _____
 Nedsatt muskelkraft i ben _____
 Gångsvårigheter _____
 Gripsvårigheter _____

Är det något som fattas i symptombilden som Du besvärar av?
 Ange i så fall vad och i vilken grad: _____

Markera genom att fylla i den siffra Du tycker motsvarar graden av Dina besvär just nu.

0	1, 2, 3	4, 5, 6	7, 8, 9	10
Inte alls	Ganska ringa Lite	Betydande	Stark Påtaglig Mycket	Outhärdlig

Psykiska symptom

Oro/ångest _____

Stress/spänning _____

Rastlöshet _____

Irritation _____

Oföretagsamhet _____

Nedstämdhet _____

Likgiltighet _____

Livsleda _____

Sömnsvårigheter _____

Försämrad uppmärksamhet _____

Försämrad koncentration _____

Försämrat minne _____

Försämrad bedömningsförmåga _____

Kommunikationsbesvär _____

Är det något som fattas i symptombilden som Du besväras av?

Ange i så fall vad och i vilken grad: _____

3. Supplementary questionnaire

Datum _____

ALLMÄNNA FRÅGOR

1. Har Du några andra sjukdomar än Skelleftesjukan (FAP) och i så fall vilka?

2. Vilka läkemedel (inkl. naturläkemedel och p-piller) tar Du för närvarande?

3. Är du opererad i magen någon gång?

☐ Ja ☐ Nej

4. Har vi tillåtelse att läsa Din journal för att kunna utvärdera eventuella andra tillstånd som kan ge mag-tarmsymptom samt bedöma utfallet av tidigare utförda undersökningar av mag-tarmkanalen?

☐ Ja ☐ Nej

Tack för din medverkan!