Chapter 21
Familial Amyloidotic Polyneuropathy and Transthyretin

Takamura Nagasaka

Abstract There has been much progress in our understanding of transthyretin (TTR)-related amyloidosis including familial amyloidotic polyneuropathy (FAP), senile systemic amyloidosis and its related disorders from many clinical and experimental aspects. FAP is an inherited severe systemic amyloidosis caused by mutated TTR, and characterized by amyloid deposition mainly in the peripheral nervous system and the heart. Liver transplantation is the only available treatment for the disease. FAP is now recognized not to be a rare disease, and to have many variations based on genetical and biochemical variations of TTR. This chapter covers the recent advances in the clinical and pathological aspects of, and therapeutic approaches to FAP, and the trend as to the molecular pathogenesis of TTR.

Keywords Familial amyloidotic polyneuropathy · Senile systemic amyloidosis · Transthyretin · Amyloidosis

21.1 Introduction

The representative clinical syndromes associated with transthyretin (TTR) aggregation are the familial amyloidotic polyneuropathies (FAP), cardiomyopathies (FAC), and senile systemic amyloidosis (SSA). FAP is an autosomal dominant inherited disease characterized by amyloid deposition in various organs. FAP, Portuguese type variant I (FAP type I), was first described by Corino de Andrade in 1952 (Andrade 1952). The disease originated in northern Portugal, but is now found all over the world as a result of the Portuguese expeditions of the fifteenth century, by Magellan. Most patient clusters are now observed in Portugal, Sweden, and Japan (Saraiva et al. 1983). The genetic defect in the kindreds from northern Portugal, i.e. heterozygosity for a valine-to-methionine single amino acid substitution at residue 30 of TTR (Val30Met), has been reported (Saraiva et al. 1984). TTRVal30Met is the most common variant of TTR, and more than 100 other amino acid substitutions of variant TTR, including ones in cases without pathological findings, have been reported

T. Nagasaka (✉)
Department of Neurology, University of Yamanashi,
1110 Shimokato, Chuou-city, Yamanashi 409-3898, Japan
e-mail: nagat@yamanashi.ac.jp

elsewhere (Benson and Kincaid 2007; Connors et al. 2003; Saraiva 2001; Sekijima et al. 2001). Genetic differences lead to heterogeneity of clinical manifestations due to the variant deposition in amyloid fibrils. Recent studies focused on the TTR gene and protein have provided insights into the pathogenesis of TTR amyloidosis. Based on these several lines of research, a new approach has been tried to establish a fundamental therapy. This chapter provides a brief overview of TTR-related amyloidosis, especially FAP.

21.2   Structure, Function and Genetics of TTR

21.2.1   Structure and Function of TTR

TTR was previously known as prealbumin because of its detection as a component that migrated ahead of albumin in an electrical field in cerebrospinal fluid (CSF) and serum. In 1981, the International Union of Biochemists adopted the name 'transthyretin' instead of prealbumin. The name 'transthyretin' comes from the transport properties of the protein, which binds both thyroxine (T4) and retinol-binding protein (RBP) (Raz and Goodman 1969). Its expression occurs during embryonic development and throughout life, but TTR evidently is not essential for life because mice that have had the TTR gene inactivated show no abnormalities in development, although the levels of serum retinol, RBP, and thyroid hormone are significantly depressed (Episkopou et al. 1993). In a member of an FAP lineage, the amyloid is not formed until adulthood, although a variant TTR is present in the serum from the time of birth (Harats et al. 1989). TTR is also known as a negative acute-phase protein. The serum TTR concentration is reduced in acute and choronic inflammation or during malnutrition as a result of decreased transcription in response to the inflammatory cytokines interleukin 6, interleukin 1 and TNFα, and mediation by hepatocyte nuclear factor 3α (Ingenbleek and Young 1994). In recent studies have focused on the relationship between TTR and β-amyloid (Aβ) as a pathogenesis of Alzheimer's disease (AD). Reduced TTR levels in the CSF of AD patients were reported (Serot et al. 1997). Several *in vitro* evidences that TTR binds to Aβ resulting in reducing Aβ concentration, aggregation and neurotoxication, were demonstrated (Buxbaum et al. 2008; Du and Murphy 2010; Li et al. 2011; Liu and Murphy 2006; Schwarzman et al. 2004).

TTR is mainly synthesized in the liver, choroid plexus of the brain, retinal pigment epithelium, pancreas and visceral yolk sac endoderm (Cavallaro et al. 1990; Herbert et al. 1986; Jacobsson et al. 1989). The TTR protein normally circulates in the plasma as a soluble protein having a tetrameric structure (Blake et al. 1974). The liver appears to be responsible for almost all the circulating TTR tetramers that aggregate in tissues other than the brain and eyes, since TTR does not cross the blood-brain barrier. As for the brain, the choroid plexus is able to secrete highly destabilized TTR tetramers into the CSF due to the preventional mechanism comprising that the high concentration of T4 in this tissue can bind and stabilize the TTR variants close to the site of secretion

into the extracellular space (Gambetti and Russo 1998; Hammarström et al. 2003b; Hurxhman Babbes et al. 2008; Sekijima et al. 2003, 2005). TTR in CSF is also secreted into the eyes by the infiltrating retinal pigment epithelium, a monolayer of cells with tight junctions that serves as a blood barrier for the retina (Pfeffer et al. 2004). TTR mRNA was detected by RT-PCR in the dorsal root ganglia (DRG) and cauda equina of humans and rodents, and not in the sural nerve, lumbar plexus or sympathetic ganglia in humans, or in the sciatic nerve in rodents (Murakami et al. 2008). However, whether TTR gene is expressed in DRG or not is unclear (Sousa and Saraiva 2008; Murakami et al. 2010). The most prominent organ of amyloid deposition is not necessarily a site for degradation. The liver, kidney, muscle and skin are thought to be the major sites of TTR degradation in the rat (Makover et al. 1988), although the site of catabolic processing has not been well defined in the human.

Native TTR, a tetrameric protein of four identical subunits of 14 kDa (Costa et al. 1978), is composed of 127-amino acid residues (Hamilton and Benson 2001; Pras et al. 1983) after cleavage of a 20-amino acid signal peptide (Mita et al. 1984; Whitehead et al. 1984), and is a very prominent plasma protein (20–40 mg/dl) that has a plasma residence time of only 1–2 days (Benson et al. 1996). The TTR tetramer exhibits two different dimer-dimer interfaces (Blake et al. 1974, 1978), and contains two identical T4-binding sites located in a channel at the center of the molecule. Each T4 binding site is characterized by an inner binding cavity and a larger outer binding one (Hörnberg et al. 2000; Klabunde et al. 2000). The outer binding cavity, the interface between the outer and inner binding sites, and the inner cavity include pairs of symmetric hydrophobic concave subsites referred to as halogen-binding pockets, wherein the iodine atoms of T4 exist (Wojtczak et al. 1996). The two binding sites exhibit negative cooperativity, which is due to an allosteric effect resulting from the occupancy of the first binding site. TTR in the plasma transports about 15 % of the total T4 (Schreiber et al. 1990; Kassem et al. 2006). In the CSF, TTR is the main T4 transporter, and may contribute to the transport of serum T4 across the blood-brain and blood-choroid plexus CSF barriers. Meanwhile, the four binding sites for RBP are located on the surface of a TTR molecule. The ratio RBP:TTR in plasma is around 0.3 in healthy individuals. The secondary structure of TTR predominantly comprises β-sheet, with each monomer consisting of eight β-strands (A-H), and a short α-helix between strands E and F (Blake et al. 1978). The β-strands are organized into a wedge-shaped β-barrel, which is formed by two antiparallel four stranded β-sheets containing the DAGH strands (inner sheets; wrapped around a central channel) and CBEF strands (outer sheets; forming the external surface of the tetramer), respectively. Two monomeric units are related by a non-crystallographic 2-fold axis and join edge-to-edge to form a dimer stabilized by extensive anitiparallel hydrogen-bonding between the two adjacent H (residues Ser115-Thr123) strands and F (residues Ala91-Ala97) strands of each monomer. There are five main-chain-main-chain hydrogen bonds between the adjacent H strands, while the two F strands more widely are separated, as a result, only two hydrogen bonds (between Glu89 and Val94) are formed directly through the main-chain interactions. Two dimers assemble into a tetramer through their opposing inner β-sheets with the hydrophobic interactions between the

A–B and G–H loops (Yang et al. 2003). The inner sheets that wrap around a central channel are about 8 Å in diameter and 50 Å in length and contain the two binding sites for T4 (Blake and Oatley 1977; Hamilton and Benson 2001). The channel can be divided into three pairs of regions along it, starting from the center: (1) the hydrophilic center formed by the hydroxyl groups of Ser112, Ser115, Ser117, and Thr119, (2) the hydrophobic patch comprising the side chains of Leu17, Thr106, Ala108, Leu110, and Val121, and (3) the charged entrance including Lys15, Glu54, and His56. The side-chains of Leu110, Ser115, and Ser117 cause a constriction, narrowing the channel around the middle (Lei et al. 2004). The overall three-dimensional struture of TTR has been maintained throughout vertebrate evolution, and the T4-binding sites are preserved in all species as to the amino-acid sequences.

21.2.2   Genetics of TTR

The complete sequence of TTR was determined by direct amino acid sequence analysis (Kanda et al. 1974), and TTR-specific cDNA clones were isolated from an adult human liver cDNA library (Mita et al. 1984; Wallace et al. 1985). The human TTR gene has been shown to be localized to chromosome 18 (Wallace et al. 1985; Whitehead et al. 1984), and identified at 18p11.1-q12.3 (Jinno et al. 1986). It spans approximately 7 kb and has four exons; exon 1 encodes a single peptide of 20 amino acid residues and only the first three residues of the mature protein, and exon 2 codes for amino acid residues 4–47, exon 3 for residues 48–92, and exon 4 for residues 93–127 (Sasaki et al. 1985; Tsuzuki et al. 1985). No mutation has been detected for exon 1 and most abundantly for exon 3 (Ando et al. 2005). Although the majority of the mutations of TTR are single, a trinucleotide deletion in exon 4, resulting in the loss of one of two valines at position 121 or 122, has been reported (Uemichi et al. 1997). TTR Asp99Asn is predicted to be a non-pathogenic benign mutation with WT properties (Groenning et al. 2011). The most affected individuals are heterozygous for a pathogenic mutation, and express both normal and variant TTR. A few cases of homozygosity as to the Val30Met mutation have been reported, and it has been demonstrated that the age of onset and the progression of symptoms are the same as in heterozygous patients (Holmgren et al. 1988, 1992, 1994; Skare et al. 1990; Yoshinaga et al. 1992). Polymorphisms have been also investigated, it being found that one polymorphic site in C1QA (rs172378) and one in C1QC (rs9434) as well as the ε2 allele are correlated with the age of onset (Dardiotis et al. 2009).

21.2.3   Animal Models

While no reproduction model of the neuropathology seen in FAP has been reported, a few transgenic models involving variant human TTR have been used for elucidating the elementary pathogenesis (Buxbaum 2009). Recently, these models are

also being utilized to investigate the therapeutic effects. Initially, transgenic mice carrying and expressing the human WT TTR (Yamamura et al. 1987) and the human mutant amyloidgenic TTR Val30Met were developed (Wakasugi et al. 1987). It was demonstrated in the mice that amyloid deposition, the deposits containing TTR and mouse serum amyloid P-component, started in the gastrointestinal tract, cardiovascular system, and kidneys 6 months after birth, and extended to various other organs and tissues with advancing age similar to observed in human autopsy cases of FAP, except for the absence of amyloid in the choroids plexus, and peripheral and autonomic nervous systems (Yi et al. 1991). The significant roles in TTR-derived amyloid deposition of the serum amyloid P-component level and the intestinal flora have been demonstrated in a transgenic mouse model of FAP (Murakami et al. 1992; Noguchi et al. 2002). In comparison, the differences in the effects of different genetic regions or length have been examined. There is evidence that 3 kbp of the upstream sequence of the TTR gene contains positive elements required for choroid plexus expression that are distinct from those utilized by hepatocytes, and that it may also contain negative elements that function to suppress transcription in other brain cell types (Yan et al. 1990). An experiment involving a transgenic mouse model of homozygous FAP showed that the introduction of the human TTR mutant gene (6.0-hMet30) carrying a null mutation at the endogenous ttr locus (ttr-/-) significantly depressed the serum levels of T4 and RBP (Kohno et al. 1997). Transgenic mouse lines carrying the human mutant TTR gene containing 6.0 kb (6.0-hMet30) of the upstream region have been expressed in the yolk sac, liver, and choroid plexus, where the mouse endogenous TTR gene is also expressed, in contrast, expression of the 0.6-hMet30 gene is restricted to the yolk sac and liver. These results suggest that the 6-kb upstream sequence contains the cis-elements required for developmental, tissue-specific, and quantitatively normal expression (Nagata et al. 1995). In the same transgenic mouse lines, the expression levels of the 6.0-hMet30 gene in the liver and serum were the same as in man and about 10 times higher than those of the 0.6-hMet30 gene. The amyloid deposition started earlier and was more extensive in 6.0-hMet30 than 0.6-hMet30 mice, suggesting that the serum levels of human mutant TTR are correlated with the occurrence and degree of amyloid deposition, to some extent (Takaoka et al. 1997). It was demonstrated that heterotetramers composed of murine and human TTR subunits imposes kinetic stability, preventing dissociation and subsequent amyloidogenesis (Reixach et al. 2008). A report has suggested the importance of environmental factors at onset such that the specific pathogen-free conditions under maintaining the transgenic mice at 30 °C can completely prevent amyloid deposition (Inoue et al. 2008). An examination concerning WT TTR has been reported, that is, TTR knock mice have been described as having a defect in neuronal regeneration in response to injury, and an abnormal ratio of proliferating to apoptotic cells in the subventricular region of the adult brain (Fleming et al. 2007; Richardson et al. 2007). Recently, Val30 tetramer instability was observed in a transgenic model developed to 'knock in' human TTR into the murine TTR locus, that is the expression levels of human TTR mRNA and protein in the livers of homozygous human TTR mice were about twice those in heterozygous mouse/human TTR mice, however, the serum human TTR levels in the homozugous mice were much lower

than those in the heterozygous mice due to unstable Val30 tetramers (Zhao et al. 2008). Other than murine models, *Drosophila* is also useful for studies of TTR-associated amyloid diseases (Berg et al. 2009). While, these findings are elementary, the transgenic models of the TTR amyloidoses have provided interesting insights into the pathogenesis of TTR FAP.

21.3   Molecular Pathogenesis of TTR

21.3.1   *Mechanism of TTR Amyloidogenesis*

Although *in vitro* data cannot reflect the *in vivo* dynamics of fibrillogenesis precisely, because amyloid fibrils are influenced by universal components such as proteoglycans (Snow et al. 1987) and the P component (Prelli et al. 1985) that affect *in vivo* fibril turnover and dyanamics, several recent experimental approaches to explain the mechanism uderlying fibril formation for TTR will more than compensate for this shortcome. Formerly, extensive β-sheet structure was proved to have amyloidgenic potential. On X-ray analysis, a slight conformational change of the crystal structure of variant TTR was demonstrated (Terry et al. 1993), including distortion of the T4-binding cavity, resulting in a decreased affinity for T4 (Hamilton et al. 1993). Regarding the mechanism underlying initiation of the aggregation of WT and several variants of TTR, amyloid fibril formation might be triggered by tetramer dissociation into a compact non-native monomer with low conformational stability, which results in non-fibrillar TTR aggregates or partially unfolded monomeric species that can subsequently self-assemble into profibrils and mature amyloid fibrils (Colon and Kelly 1992; Lai et al. 1996, 1997; Hammarström et al. 2003a; Jiang et al. 2001; Quintas et al. 1997, 1999, 2001; Schneider et al. 2001). The folded monomer that results from the process of dissociation must subsequently undergo partial denaturation to misassemble into aggregate structures, including amyloid fibrils. The monomeric amyloidogenic intermediate undergoes self-assembly into a ladder of quaternary structural intermediates for the formation of WT, Val30Met, and Leu55Pro TTR amyloid fibrils (Lashuel et al. 1998). TTR most likely disassembles and forms amyloid when its ligands are released to the target organs (White and Kelly 2001). It was proposed that monomers are the building block of fibrils, *in vitro,* composed of multiple elementary protofilaments, which contain two twisted β-sheets and comprise a single vertical stack of structurally modifed TTR monomers (Cardoso et al. 2002; Inouye et al. 1998; Quintas et al. 2001). *In vivo* studies have revealed that TTR might be deposited in a non-fibrillar or pre-fibrillar form in the nerves prior to major nerve fiber degeneration in the early stages of FAP (Sousa et al. 2001a). N-terminally truncated dimers can also form amyloid fibrils after limited proteolysis (Schormann et al. 1998).

A correlation between amyloidogenesis and the thermodynamic stability of TTR variants (Sekijima et al. 2003; Shnyrov et al. 2000; Quintas et al. 2001), or high susceptibility to water infiltration (Ferrao-Gonzales et al. 2003) has been demonstrated.

In vitro studies under acidic conditions demonstrated that TTR amyloidogenesis is accelerated (Colon and Kelly 1992; Gustavsson et al. 1991), variant TTR has been found to form fibrils faster than WT TTR (Jiang et al. 2001; McCutchen et al. 1993), and the kinetics of denaturation are much faster for monomeric TTR than for tetrameric TTR. Synchrotron diffraction studies suggested a model of the protofilament of the FAP amyloid fibril exhibiting a cross-sheet helix structure (Blake and Serpell 1996). Studies on the unit of TTR amyloid fibrils suggested a TTR dimer containing at least one mutant subunit (Serga et al. 2001), intermediate species are also β-sheets (Cardoso et al. 2002), α-sheet may represent a significant transient conformation during amyloidogenesis (Armen et al. 2004), one of the two β-sheets becomes disrupted, and α-sheet structure forms in the other sheet under acidic conditions. Regarding fibril growth, the TTR dimer can still form amyloid fibrils after chemical cross-linking, and the subunit interfaces in amyloid fibrils are similar to the natural dimeric interchain association of native TTR (Serga et al. 2001). STEM mass-per-length measurements showed that the modified monomer is the unit being added to a growing fibril (Cardoso et al. 2002). For misassembly or aggregation reactions before fibrile elongation, nucleated polymerization is often required in non-TTR amyloidogenesis (Jarrett and Lansbury 1993; Masters et al. 1985; Powers and Powers 2008). While, it has been shown that TTR aggregation may be a nucleation-independent process (Hammarström et al. 2003a, b; Hurshman Babbes et al. 2008), and a process in which the highest energy native monomer forms lower-energy polymeric aggregates (Hurshman Babbes et al. 2004). Destabilized TTR variants in the serum are degraded by the endoplasmic reticulum (ER) quality control system, i.e., ER-associated degradation (ERAD), of hepatic cells. The ERAD prevents severe early onset systemic amyloidosis by keeping the concentrations of these variants in serum low (Sekijima et al. 2005). Consequently, this quality control system takes part in determination of the severity of the disease. It was demonstrated that tethering two TTR subunits whose quaternary interface defines the T4 binding site reduces tetramer dissociation (Foss et al. 2005).

Biochemical studies have shown that several amyloidogenic mutations alter protein stability, causing the aggregation and formation of amyloid fibrils (Kelly 1998). TTR amyloid fibrils could be formed from tetramers linked by disulfide bridges through oxidative modification of the thiol residue on the Cys10 of TTR as disulfide bond formation occurs in homozygous and heterozygous patients with the Val30Met mutation (Nakanishi et al. 2010; Terry et al. 1993; Thylén et al. 1993). It has also been shown that a mutation at Cys10Arg also has amyloidogenic potential (Uemichi et al. 1992). It was indicated that structural modification of TTR Glu54Gly and TTR Glu54Lys may sufficiently alter tetramer stability and T_4 binding (Miyata et al. 2010). FAP with Leu55Pro TTR has been focused on as the most aggressive form of the disease. The Leu55Pro TTR monomer undergoes substantial structural changes relative to fluctuations observed in the WT TTR and Val30Met TTR monomers (Yang et al. 2003). The Leu55Pro mutation results in profound conformational changes relative to Val30Met (Lei et al. 2004). Structural studies involving X-ray diffraction on TTR Leu55Pro revealed that monomers are the building block of the amyloid structure (Sebastião et al. 1998). The TTR Val30Met, WT TTR and TTR Leu55Pro

denaturation pathways show that both TTR variants form protofilaments at physiological pH (Lashuel et al. 1998, 1999). This condition was also demonstrated using TTR transgenic mice (Sousa et al. 2002; Teng et al. 2001). Experimentaly, the L55P variant subunit exchange occurs via dimers, but the WT TTR exchange via both monomers and dimmers (Keetch et al. 2005). The report also revealed that the rate of dissociation of WT TTR increases in the presence of TTR Leu55Pro, which leads to acceleration of the formation of hybrid tetramers. The *in vitro* assembly properties of both WT TTR fibrils produced through acidification and TTR Leu55Pro fibrils assembled at physiological pH reflected similar morphological features and dimensions (Cardoso et al. 2002). Foguel et al. (2003) showed the existence of packing defects in the fibril core indicating a source of non-fibrillar protein. Several studies revealed the contribution of WT TTR to amyloid deposition in the hearts of FAP patients (Yazaki et al. 2000). Studies on WT TTR have shown that increased temperature or hydrostatic pressure can cause the formation of amyloidogenic species (Chung et al. 2001; Ferrao-Gonzales et al. 2003).

TTR fibrilogenesis is complicated *in vivo*, because almost all individuals suffering from FAP are heterozygous, co-expressing both WT and variant proteins in the liver (Murakami et al. 1992; Saraiva et al. 1990; Thylén et al. 1993). In heterozygous patients with Val30Met FAP, WT and Val30Met TTR are present in ratios of 2:1 and 1:2 in plasma and amyloid fibrils, respectively (Dwulet and Benson 1984). A compound heterozygous family in Portugal that expressed both Val30Met and Thr119Met TTR with no or very mild symptoms of FAP in the kindred has been reported (Coelho et al. 1993). TTR tetrameric structural stability in patients with the TTR Thr119Met or TTR Val30Met/TTRThr119Met genes was shown by semidenaturing isoelectric focusing (Alves et al. 1997). The inclusion of Thr119Met subunits in disease-associated tetramers kinetically prevents tetramer dissociation substantially, reducing the susceptibility of amyloidogenesis by a process referred to as interallelic trans-supression (Hammarström et al. 2001, 2003a). TTR stability with the TTR Val30Met/TTR Arg104His mutation has also been reported (Almeida 2000). Urea denaturation studies demonstrated that Val122Ile TTR exhibits a destabilized quaternary structure and a stable tertiary structure, Leu55Pro TTR an unstable quaternary and tertiary structure, Val30Met TTR a stable quaternary structure but an unstable tertiary structure, and the Ara25Thr TTR a drastically destabilized quaternary and tertiary structure (Hurshman Babbes et al. 2008). The stability differences among the disease-associated TTR variants highlight the complexity and heterogeneity of TTR amyloid disease. Metal ions including Zn^{2+} may also enhance the amyloidogenesis in FAP (Palmieri et al. 2010).

It was demonstrated that increased glycation of fibrinogen (da Costa et al. 2011) and loss of functional albumin (Kugimiya et al. 2011) triggers acceleration of TTR amyloid fiibrile formation in FAP.

Recently, several simulation studies involving engineered mutations have been carried out to investigate amyloidogenesis. The destabilized variant TTR are as follows; one with destabilization of the AB loop through substitution of Tyr78Phe (Redondo et al. 2000a), mutations Asp18Asn and Leu110Ara (Redondo et al. 2000b), highly amyloidogenic triple D-strand mutant Gly53Ser/Glu54Asp/Leu55Ser, which

resulted in conformational changes in the CD loop, D-strand and the DE loop, designated as the β-silp (Eneqvist et al. 2000), amyloidogenic double mutant Phe87Met/Leu110Met (Hurshman Babbes et al. 2004), WT TTR, and the TTR Tyr116Ser monomer affecting the binding of T4 inducing amyloidogenesis (Banerjee et al. 2010). On the other hand, the stabilizing variant TTR, insufficient for amyloid fibril formation, are as follows; ones with mutations Ser117Cys and Glu92Cys, and the dimer/tetramer interactions (Redondo et al. 2000b), and the Phe87Met/Leu110Met TTR variant (Jiang et al. 2001).

21.3.2 TTR-Induced Neurotoxicity

Although the precise mechanism underlying neurotoxicity of TTR is unknown, a population of unstable, heterogeneous amyloid oligomers and protofibrils is recognized to be mainly responsible for amyloid cytotoxicity. As for the pathogenesis of peripheral nerve disturbances in FAP, various theories, such as local compression by aggregated amyloid, ischemia, neurotoxicity and apoptosis have been presented. Length-dependent impairment of the peripheral nerves was explained by multifocal endoneurial deposits of amyloid (Said 2003). Schwann cell pathology, i.e., segmental degeneration, onion bulb formation and myelin-axonal disproportion, was absent and evidence of regeneration was not obtained (Toyooka et al. 1995). On the other hand, atypical findings have been reported; preferential loss of axons in large myelinated fibers (Misu et al. 1999), significant myelin damage (Sobue et al. 1990), and independent correlation between fiber loss and size (Yamada et al. 1984). Lesions in the dorsal root ganglia neurons (DRG) or Schwann cells have also been proposed to explain axonal and neuronal loss (Sousa and Saraiva 2003). It has been reported that TTR mRNA was detected in the DRG and cauda equina, but not in the sural nerves, lumbar plexus or sympathetic ganglia on RT-PCR, it being concluded that TTR synthesis in the DRG may be important for the development of neuropathy (Murakami et al. 2008).

Toxic species of variant TTR have been identified *in vitro*, as well as *in vivo* in TTR transgenic mice and in *ex vivo* explants from FAP patients (Andersson et al. 2002; Reixach et al. 2004; Sousa et al. 2001a, 2002). Formerly, similar to other amyloids, mature TTR amyloid fibrils were thought to have a cytotoxic effect, having highly organized, insoluble structures that become resistant to proteolysis. Above all, mutant TTR have been shown to be toxic to cells in culture (Hou et al. 2005; Sousa et al. 2001a). In the last decade, one of the subjects of research on amyloid toxicity has been the small oligomeric TTR aggregates, i.e., non-fibrillar and not mature fibrils, formed mainly in the fibrillogenesis pathway (Bucciantini et al. 2002). The Ser112Ile variant TTR was shown to exist as a dimer having a nonnative tertiary structure that leads to spherical aggregates that induce cell death at physiological pH (Matsubara et al. 2005). The toxic nature of the aggregates was evidenced by their ability to induce the expression of oxidative stress and inflammation-related molecules in neuronal cells including many cytokines, e.g. tumor necrosis factor α(TNFα), interleukin-1βb

(IL-1βb), nuclear transcription factor κB (NFκB) and macrophage-colony stimulating factor (MCSF), and a relationship with the cellular receptors for advanced glycation end-products (RAGE), driving them into apoptotic pathways (Sousa et al. 2001b). The engagement of RAGE by a ligand triggers activation of key cell signaling pathways, thereby reprogramming cellular properties (Huttunen et al. 1999; Lander et al. 1997; Yan et al. 1994). It has been reported that activation of RAGE leads to endoplasmic reticulum stress, activation of ERK1/2 and caspase-dependent apoptosis (Monteiro et al. 2006). IL-10 expression increases after fibril deposition in FAP nerves (Sousa et al. 2005). These studies represented the desire to understand the involvement of inflammatory activity and apotosis via cytokines and RAGE in the pathophysiological changes in FAP. On the other hand, ultrastractural and histochemical study by using autopsy tissues and cultured cell lines revealed that neither TTR amyloid fibrils nor prefibrillar aggregates derectly induced apoptosis (Misumi et al. 2012).

Several cell toxic events induced by membrane affinity and calcium influx are obvious. There is evidence that misfolded TTR mediate their toxic effects by binding directly to lipid-rich areas of the plasma membrane, with the resulting disruption of membrane integrity and cell impairment (Hou et al. 2005; Subasinghe et al. 2003). Recently, it was suggested that TTR aggregation into oligomers disrupting intracellular calcium influx by increasing the permeability of the plasma membrane may contribute to the pathophysiology of FAP (Hou et al. 2007). A study also suggested that activation of transient receptor potential M8 channels by Leu55Pro TTR in cultured DRG neurons results in the subsequent opening of voltage-sensitive sodium and calcium channels (Gasperini et al. 2011). On the other hand, evidence has been presented that the mechanism underlying neurotoxicity in amyloidoses is the conformation of the aggregated protein itself, rather than its amyloidogenic amino acid variant that results in altered membrane permeability to calcium (Bucciantini et al. 2002).

Protection against amyloid deposition or cytotoxicity has been demonstrated. In our study on sural nerves, amyloid deposits were found to be surrounded by a long process of fibroblasts that may play a role in preventing amyloid toxicity (Nagasaka et al. 2009). Induction of heat shock proteins (HSP), HSP27 and 70, heat shock factor 1, and αB-crystallin (HspB5), by the extracellular TTR deposits in FAP tissues indicates a protective effect of anti-apoptotic and chaperone properties (Magalhães et al. 2010). Many types of toxic amyloid other than that in FAP are well known and these proteins may help us understand the TTR-induced neurotoxicity or pathogenesis of FAP.

21.4   Tissue-specific Pattern of TTR Deposition

The molecular basis of the susceptibility of certain tissues is not fully understood. Deformation of the amyloid structure, a conformational change or a host tissue mechanism in a variant form of TTR, and interaction between an amyloid and its receptor

may contribute to the pathogenesis. Cell-specific secretion efficiency differences could also be an important parameter for the tissue specificity of FAP (Hurshman et al. 2004; Sekijima et al. 2003).

To determine the site of amyloid deposition, it would be valuable to reveal why TTR is not deposited in the liver, which is the major site of TTR synthesis. An investigation involving transgenic mice of human TTR amyloidosis also revealed the sparing of liver deposition (Teng et al. 2001). One of the lines of evidence is that the binding of RBP, which is secreted by hepatocyte, may stabilize the TTR tetramer sufficiently in the liver (White and kelly 2001). Some TTR variants that are highly unstable as to thermodynamics cause a late-onset central nervous system-selective amyloidosis (CNSA) with a low concentration in the blood (Hammarström et al. 2003b; Sekijima et al. 2003). As to proteostatic differences, cultured choroid plexus cells appear to be more permissive secretors of highly destabilized, misfolding-prone TTR variants than cultured murine hepatocytes, allowing initiation of CNSA (Sekijima et al. 2005). Binding of cysteine and homocysteine with TTR may alter the structure and characteristics of serum TTR, and may facilitate TTR denaturation and deposition more than free TTR (Hanyu et al. 2009).

Other than the protein nature of TTR for amyloidogenesis, factors of deposition sites such as extracellular matrix (ECM) components have been thought to influence the efficiency of amyloidogenesis (Suk et al. 2006). Amyloid deposits, composed mainly of variant and/or WT TTR and universal amyloid-associated molecules including serum amyloid P component (SAP) (Pepys et al. 1994), apolipoprotein E (Gallo et al. 1994), and ECM-related components such as proteoglycans (Lyon et al. 1991) and matrix metalloproteinase (MMP)-9 (Sousa et al. 2004) commonly occur in the endoneurium (Sobue et al. 1990), in which ECM is abundant (Dubový et al. 2002). Two genes related to ECM remodeling, biglycan and neutrophil gelatinase-associated lipocalin (NGAL), are overexpressed, and MMP-9, which exists as a complex with NGAL, is increased in FAP. Biglycan, NGAL, and MMP-9 are transcriptionally up-regulated by NF-κB, which is activated in FAP nerves (Sousa et al. 2004). Although, MMP-9 degrades TTR aggregates and fibrils *in vitro*, TTR fibrils became resistant to MMP-9 proteolysis in the presence of SAP (Sousa et al. 2005). In TTR-related cardiac amyloidosis, it has been demonstrated that a relationship between TTR and ECM proteolytic activation through MMP and tissue inhibitors of metalloproteinase (TIMPs) may play an important role in the pathogenesis (Biolo et al. 2008). Regarding the basement membrane (BM), TTR amyloid fibrils are firstly formed at the BM, followed by further amyloid deposition due to the expression of BM components such as collagen IV, laminin, and fibronectin, as found using autopsy cardiac tissues from patients with FAP and a smooth muscle cell line (Misumi et al. 2009). Glycoasaminoglycans (GAGs) including heparan sulfate (HS), dermatan sulfate, ketaran sulfate, and chondroitin sulfate are highly sulfated linear polysaccharides prevalent in the ECM, and commonly found in association with amyloid deposits including TTR amyloid *in vivo*. A report revealed that TTR can bind to HS proteoglycan perlecan; a large multidomain proteoglycan that binds to and cross-links many ECM and cell-surface components, and consists of three heparan sulphate chains linked to a large core protein (Iozzo et al. 1994), with the

binding probably mediated by a core protein, not a protein-glycosaminoglycan interaction (Smeland et al. 1997). A report has also revealed that HS can promote fibrillogenesis by binding to amyloid precursors (Ancsin 2003). A recent *in vitro* study revealed that sulfated GAGs, especially heparin, accelerate TTR aggregation through quaternary structural conversion based on a mechanism in which the sulfate groups comprising GAGs interact primarily with TTR proteotoxic oligomers through electrostatic interactions, facilitating the formation of less toxic amyloid aggregates. This model raises the possibility that GAGs may play a protective role in human amyloid diseases (Bourgault et al. 2011). Molecular chaperones, including the extracellular protein clusterin, are proteins that assist in the maintenance of proteostasis with stabilizing non-native protein conformations, thereby preventing aggregation. In patients with familial TTR, senile systemic, and Ig light chain amyloidosis, the association of clusterin with extracellular myocardial amyloid deposits and decreased serum clusterin concentrations revealed the amyloidogenic properties of clusterin (Greene et al. 2011).

21.5 TTR-Related FAP

Several types of amyloidoses related to mutant forms of TTR are recognized, including FAP, FAC, and CNSA. These amyloidoses can appear as early as the second decade of life. A common age-related amyloidosis associated with WT TTR is called senile systemic amyloidosis (SSA, see below for further details).

21.5.1 Familial Amyloidotic Polyneuropathy

Classically, FAP has been classified into four types according to ethnic origin, differing amyloid proteins and patterns of neuropathy; FAP type I (Portuguese, Japanese, and Swedish type) and FAP type II (Indiana/Swiss and Maryland/German type) are associated with TTR. Several TTR mutations associated with FAP type II with the Leu58HisTTR, in the Maryland/German kindred (Nichols et al. 1989b), Ile84SerTTR, in the Indiana/Swiss kindred (Nichols et al. 1989a; Wallace et al. 1988), and Lys70Asn TTR in an FAP pedigree of German ancestry residing in New Jersey (Izumoto et al. 1992), have been reported. Clinically, FAP type II is a systemic disease characterized by carpal tunnel syndrome (CTS), followed by a generalized polyneuropathy developing with vitreous opacity, and death due to myocardial impairment. However, it is inappropriate to classify FAP into type I or II because of the diversity of its symptoms with various TTR mutations, and it has been designated as a TTR-related amyloidosis recently. FAP types III (Iowa type) and IV (Finnish type) involve apolipoplotein A1 and gelsolin, respectively. Although several phenotypes showing correlation with the mutant or WT TTR variation are known at present, an outline of the clinical manifestations of TTR-related FAP has already been presented

the perspicuous report of Andrade in 1952. The hallmarks of the disease are it is inherited, with onset in the second or third decade, an unremitting course to death due to cachexia and infection an average of 7–10 years later, and a characteristic pattern of symptoms; paresis, early impairment of thermal and pain sensibilities, and anhidrosis in the lower limbs. Concomitant symptoms are nausea, vomiting, abdominal pain, and sexual and sphincter disorders. A unique feature is the dissociated sensory loss. Andrade found that the various sensation modalities were lost in the following order: temperature, pain, touch, and, finally, joint position. On postmortem examination, amyloid deposits are found in large amounts in the kidneys and peripheral nerves, and to a lesser extent in other organs (Dyck and Lambert 1969). TTR-related FAP patients have been confirmed in more than 30 countries. Among several TTR mutations, only a few including Val30Met are present in extended kindreds and multiple loci, which are prominent in northern Portugal, northern Sweden, and Japan, although many have been found in single individuals or families (Andrade 1952; Benson and Cohen 1977; Holmgren et al. 1988; Tawara et al. 1983). Penetrance differences have been explained by that there may be variations in either the nuclear or mitochondrial genomes (Soares et al. 2005). The penetrance of the Val30Met TTR mutation shows considerable variation between families and localities; above 80 % in Portugal and Japan, and much lower in Sweden. The mean Val30Met TTR carrier frequency in counties in northern Sweden is 1.5 % (Holmgren et al. 1994). As for FAP with non-Val30Met TTR, the Ile84Ser mutation in an Indiana/Swiss kindred was reported to exhibit essentially 100 % penetrance by age 50. The Thr60Ala mutation is prominent in the United States, but originated in northern Ireland and is now distributed in many countries with families of Irish descent (Benson et al. 1987). Leu58His is also frequently found in the United States in families originating in Germany (Goebel et al. 1997; Nichols et al. 1989b). Genotype-phenotype correlations remain largely unknown in spite of several intensive investigations (Connors et al. 2003; Nakazato et al. 1989; Saraiva 2001). The complexity is increased normal allelic variants in individuals of various ethnic backgrounds (Connors et al. 2003).

21.5.2   Clinical Symptoms

FAP usually presents as a peripheral neuropathy including autonomic failure. The neuropathy usually starts with small-fiber dysfunction in the lower extremities with a lack of thermal sensation being an early feature, like the neuropathy of diabetes mellitus. Walking difficulty, weakness, and cardiac or gastrointestinal manifestations are less common at onset. Dysesthesia in the distal parts of the four extremites may be prominent with or without varying degrees of pain. The level of sensory symptoms slowly progresses from the disatal to the proximal parts of the four extremities bilaterally. Motor function tends to be relatively preserved until the sensory manifestations have advanced considerably. Muscular atrophy is remarkable compared with motor weakness (Benson and Cohen 1977). Autonomic neuropathy tends to occur relatively early in the course, and results in orthostatic hypotension, gastrointestinal

motility problems (constipation alternating with diarrhea, attacks of nausea and vomiting, and delayed gastric emptying), anhidrosis, urinary retention or incontinence, and sexual impotence. Mortality is dependent on complications such as pneumonia, leg ulcers, malnutrition due to gastrointestinal tract dysfunction, and cardiac involvement including restricted cardiomyopathy, cardiac arrhythmias (atrial fibrillation, atrial ventricular block, and sinus exit blockage). Carpal tunnel syndrome (CTS) is often an early feature. The eyes are one of the sites of amyloid deposition. Vitreous opacity is much more common, being seen with approximately 20 % of TTR mutations. This symptom is a part of the oculoleptomeningeal syndrome of amyloid deposition and amyloidotic cardiomyopathy without leptomeningeal involvement (described below). Deposition of amyloid TTR in the vitreous has been found in at least one individual with intact WT TTR genes without systemic involvement (Barouch et al. 2004).

It is unclear whether the molecular mechanism is due to the position of the TTR mutation, although no correlation between the severity and quantity of variant TTR has been proposed (Nakazato et al. 1992; Saraiva 2001). Some FAP cases with a progressive course have been reported. Jacobson et al. (1992) reported a type of FAP with TTR Leu55Pro, early-onset, and rapidly progressive amyloidosis with severe cardiac involvement. In that study, four of seven autopsy proven cases showed heavy amyloid deposition limited to the blood vessels and nerves in the heart, the thyroid, blood vessels, peripheral nerves, and the gastrointestinal tract. Biochemically, several lines of evidence of severe amyloidogenicity of TTR Leu55Pro have been reported (Keetch et al. 2005; McCutchen et al. 1993; Sebastião et al. 1998). Variant TTRGlu54Lys was reported to be an aggressive form of FAP (Togashi et al. 1999). Alternatively, TTR Arg104His was found to induce structural alterations leading to an increase the stability of the tetramer in compound heterozygotes for TTR Val30Met in a study of mild-type FAP with TTR Val30Met/Arg104His (Almeida et al. 2000). These characteristics are very similar to those found in compound heterozygotic carriers of TTR Val30Met/Thr119Met. TTR Asp99Asn in a Danish kindred has been reported to be a non-pathogenic benign mutation with WT properties (Groenning et al. 2011). These findings emphasize that a specific mutation is important for the severity of FAP.

The heart and kidneys are the main sites of amyloid deposition other than peripheral nerves in FAP. It has been reported that serious cardiac amyloidosis is commonly seen in patients with FAP of the non-Val30Met TTR type (Ikeda et al. 2002). Cardiomyopathy is a prominent feature in patients with the Asp45, Ile50, Lys59, Ala60, Leu68, Ser84, and Gln89 variants among FAP ones. An FAP patient with TTR Val30Met who primarily developed amyloid cardiomyopathy, with less amyloid polyneuropathy, has also been reported (Nakamura et al. 1999). A report pointed out that autonomic denervation may contribute to the maintenance and progression of cardiomyopathy, because of the lower norepinephrine contents in myocardial bioptic specimens from FAP Val30Met patients (Fiori et al. 1994). The detection of subclinical cardiac involvement may facilitate the diagnosis of late-onset FAP Val30Met in patients without a family history of the disease (Koike et al. 2011). A large amount of amyloid in the kidneys has been observed in almost all cases of

the progressive form of FAP (Jacobson et al. 1992), and the common form of FAP Val30Met. The combination of severe cardiac involvement and mild deposition in the kidneys was reported in an autopsy case of FAP Ser50Ile, Glu54 Lys (Nagasaka et al. 2009; Sakashita et al. 2001). The severity might not be necessarily attributed to both the kidneys and heart. Endocrinologically, the pituitary, thyroid and adrenal gland can be affected in FAP. Impairment of the hypothalamic-pituitary function in FAP patients; abnormal growth hormone and prolactin regulation in the thyrotropin-releasing hormone test, has been reported (Olofsson et al. 1991). Olofsson et al. also showed no abnormality of the thyroid function, and subclinical adrenal insufficiency in the short ACTH-stimulation test and on measurement of dehydroepiandrosterone (Olofsson et al. 1989). A significant amyloid content in the thyroid gland has been detected (Nakazato et al. 1989). The endocrine system may help explain the lack of correlation between amyloid deposition and the function of the amyloidogenic organs. In the gastrointestinal tract, causable evidence of severe disturbance in motility and absorption, i.e., the amyloid deposition in the nerves leading to loss of neural control (Yoshimatsu et al. 1998), and the impairment of the neuroendocrine system including the secretion of serotonin and cholecystokinin/gastrin, and the immunoreactive cell content in endocrine cells (El-Salhy et al. 1994), has been reported. In the pancreas, amyloid deposits are more prominent in the inner nerves than in the Langerhans islets (Ando et al. 1991). Excessive secretion of insulin in 75-g OGTT, which may reflect the abnormality of nerve regulation in the pancreatic endocrine system, with amyloid deposition has been observed (Nagasaka et al. 2009). The spleen and hepatic parenchyma do not have amyloid deposits. Muscular involvement is an infrequent feature (Prashantha et al. 2010). In the spinal cord, minimal to moderate myelinated fiber loss has been observed in the posterior columns in early- and late-onset FAP TTR Val30Met (Koike et al. 2004).

21.5.3 FAP TTR Val30Met

FAP with Val30Met TTR is the most common FAP-related mutation present in 5,000–10,000 symptomatic patients, and exhibits an extensive distribution in more than 15 countries in the world. In Portugal, over 500 kindreds had been identified, constituting the largest focus of FAP Val30Met worldwide (Saraiva 2001). The second largest focus is northern Sweden, where more than 350 families have been diagnosed (Holmgren et al. 1994). In Japan, the first case with FAP TTR Val30Met in a focus of patients in a southwest district was reported in 1968 (Araki et al. 1968), and more than 25 variant TTR have been reported (Ando et al. 2005; Araki and Ando 2010; Ikeda et al. 2002). The disease has became widely known, FAP TTR Val30Met is now prevalent in areas other than those seen within conventional endemic foci (Koike et al. 2011). Differences in clinical manifestations between races were found for FAP TTR Val30Met, that is, Swedish patients appeared to develop vitreous opacity later and more frequently compared with Japanese patients (Kawaji et al. 2010a). As for the onset of polyneuropathy, there is a similar age difference in the report. Many

differences are also present as to the disease course. FAP with Val30MetTTR is associated with late-onset clinical disease (mean age, 55–60 years), and exhibits less than 50 % penetrance in northern Sweden (Hellman et al. 2008; Holmgren et al. 1994), in contrast, with early-onset (mean age, 30–35 years) and much higher penetrance in northern Portugal (Coelho et al. 1994). In Japanese families with late onset at ages over 50 years, the neuropathy shows a male preponderance, low penetrance, little relationship to endemic foci, sensorimotor symptoms beginning distally in the lower extremities with disturbance of both superficial and deep sensation, and relatively mild autonomic symptoms. In contrast, families with early onset show concentration in endemic foci, predominant loss of superficial sensation, severe autonomic dysfunction, and atrioventricular nodal blockage requiring pacemaker implantation (Koike et al. 2002). Underlying pathologic features of peripheral nerves and heart in regard to the clinical differences has also been reported (Koike et al. 2004). These differences, other than mutant TTR, are important for the pathogenesis as to clinical genetics.

21.5.4 Familial Amyloid Cardiomyopathy

FAC, which is characterized by prominent cardiomyopathy and the absence of polyneuropathy, is associated with the apparently preferential amyloid deposition of some TTR mutants in the heart causing congestive heart failure. The diagnosis of FAC can be made by means of endomyocardial biopsy (Jacobson et al. 2011). Three TTR mutations, the Ala45Thr, Leu111Met and Val122Ile ones, are associated predominantly with FAC. Val122Ile FAC, which is found in approximately 3–4 % of African Americans and causes late-onset restrictive cardiomyopathy, originated on the west coast of Africa, and its clinical penetrance is thought to be high (Afolabi et al. 2000; Jacobson et al. 1997; Yamashita et al. 2005). Amyloidotic cardiomyopathy without leptomeningeal involvement is also associated with vitreous opacity (Falls et al. 1955).

21.5.5 Central Nervous System-selective Amyloidosis

CNSA, leptomeningeal amyloidosis or meningocerebrovascular amyloidosis is a form of hereditary TTR amyloidosis characterized by primary involvement of the central nervous system. The clinical features include seizures, stroke-like episodes, dementia, psychomotor deterioration, hydrocephalus, spinal cord infarction, and variable amyloid deposition in the vitreous humor. Pathologically, amyloid is detected in the walls of leptomeningeal vessels and surrounding connective tissue structures, and in the pia arachnoid and subpial region, which leads to cerebral infarction and, in later stages, cerebral hemorrhage (Blevins et al. 2003; Goren et al. 1980). Several mutations of TTR genes (e.g., Leu12Pro, Asp18Gly, Ala25Thr, Val30Gly,

Ala36Pro, Gly53Glu, Gly53Ala, Phe64Ser, Tyr69His, or Tyr114Cys) have been reported to be associated with this phenotype (Blevins et al. 2003; Connors et al. 2003; Hagiwara et al. 2009; Uemichi et al. 1999). Mild systemic amyloidosis may also be a complication. Familial oculoleptomeningeal amyloidosis (FOLMA) is leptomeningeal amyloidosis in association with vitreous amyloid deposits (Petersen et al. 1997).

21.6 Pathological Features of FAP

The systemic distribution of amyloid deposition has been revealed by histochemical, immunohistochemical and ultrastructural studies of materials obtained by autopsy or biopsy (Ikeda et al. 1987; Inoue et al. 1998; Nagasaka et al. 2009; Takahashi et al. 1991).

21.6.1 Sural Nerve Biopsy

The nerve pathology does not differ among FAP patients with several mutant TTR essentially. Several reports have revealed that axonal fiber degeneration begins in unmyelinated and small myelinated fibers, the large myelinated fibers only being affected in an advanced stage. Specifically, unmyelinated fibers are severely affected. Amyloid deposition was observed in the capillary walls and connective tissue of the endoneurium, epineurium and perineurium (Fig. 21.1a, b) (Coimbra and Andrade 1971; Dick and Lambert 1969). Schwann cells are in contact with amyloid deposits and some of them have been observed to present degenerative changes, including disappearance of the basement membrane and proliferation of distorted processes (Dick and Lambert 1969). In later stages of disease progression, severely affected nerves exibit endoneurium contents replaced by amyloid, abundant collagen bundles, and Schwann cells without axons and fibroblasts, only a few nerve fibers retaining viability. In ganglia, TTR amyloid deposits are present in the stroma in close contact with satellite cells, and a progressive loss of neurons is observed (Hanyu et al. 1989; Hofer and Anderson 1975; Ikeda et al. 1987; Takahashi et al. 1991). In an ultrastructural study, TTR amyloid deposits were found to be very dense and disorganized, with a hard-edged profile and surrounded by a long process of fibroblasts (Fig. 21.2a, b). The amyloid seemed not to invade nerve fibers or Schwann cells for the most part, but some nerve fibers or Schwann cells seemed to have been destroyed (Fig. 21.2c–f). Amyloid fibrils from the sural nerve of patient for FAP Glu54Lys comprise rigid, straight and nonbranching tubules with a diameter of 7 nm, and with a periodicity of about 200 nm (Fig. 21.2g). The fibrils from the vitreous humours of patients homozygous for TTRVal30Met has been shown to have a uniform width of about 130 Å, and are composed of four parallel protofilaments of 50–60 Å diameter in a square-section array (Serpell et al. 1995). The variation of the size of amyloid fibrils may be due
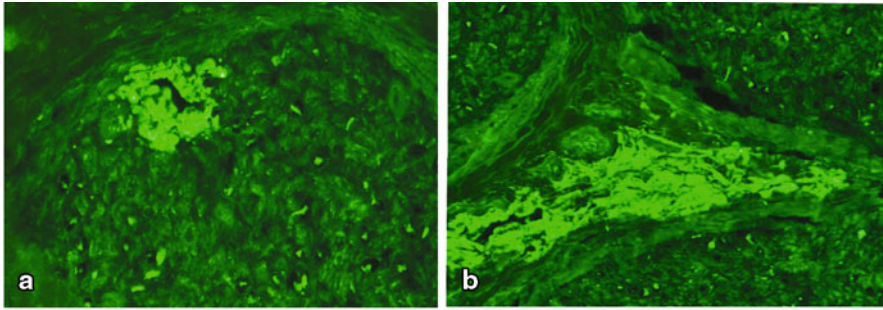
Fig. 21.1 Sural nerve obtained by biopsy in FAP with Glu54LysTTR. In immunofluorescence staining for TTR. **a** Amyloid deposition is seen in the endoneurium adjacent to the perineurium. **b** Amyloid is detected in the perineurium

to the composition of WT and variant TTR being characterized by the mutation, or the sites of deposition. The formation of amyloid fibrils being composed of a surface layers and a core obtained from FAP Val30Met has been proposed morphologically (Inoue et al. 1998). On immuno-electronmicroscopy, gold labeling-positive TTR were observed in both pre-fibrillar and fibrillar structures along or intermingled with collagen fibers (Fig. 21.2g–i).

21.6.2 Autopsy

On autopsy, the distribution of amyloid deposited organs differs among FAP patients with several mutant TTR to various degrees. As an example, a comparison of the regions of amyloid deposition between FAP Val30Met and Glu54Lys is summarized in Table 21.1 (Nagasaka et al. 2009). The major difference between the two types of FAP is renal involvement. Common findings are as follows. In the peripheral nervous system, TTR fibrils are diffusely distributed in nerve trunks, plexuses, and ganglia. The vagus nerves and celiac ganglia show extensive endoneurial and intra-ganglionic deposition of amyloid (Ikeda et al. 1987). The cardiac muscle is atrophic with complemental hypertrophy. Amyloid deposition is detected in the endoneurium of cardiac nerves, and in the capillary walls and perimyofibrils in the heart. In the cardiac conduction system, amyloid deposition is prominent in the sino-arterial node and in the limbs of the intraventricular bundle. The kidneys are also a major site of amyloid deposition. Other organs containing amyloid include the sympathetic nerve trunk, vagal nerves, celiac plexus, pelvic plexus, DRG, bladder, gastrointestinal tract, tongue, pancreas (more prominent in the nerves than Langerhans islets), lungs, pituitary (anterior lobe), gall bladder, adrenals and muscles. Deposition limited to blood vessels in the arachnoid, subarachnoid, and choroid plexuses, but lacking in the parenchyma including blood vessels, is generally seen in the brain. (Ando et al. 1991; Nagasaka et al. 2009).
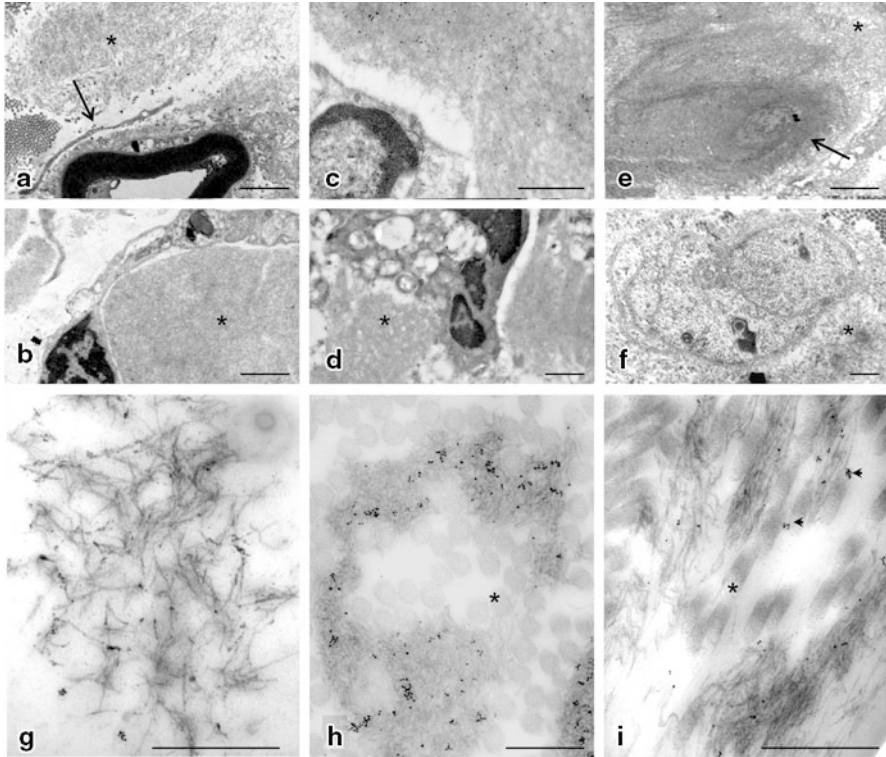
Fig. 21.2 Electronmicroscopic study using sural nerve in FAP with Glu54LysTTR. (**a**, **b**, **d**, **e**, **f**: Routine electronmicroscopy **c**, **g**, **h**, **i**: immunoelectronmicroscopy by immuno-gold labeling for TTR). **a** A myelinated fiber and amyloid aggregation (*asterisk*) are separated by the process of fibroblast (*arrow*). Bar = 1 μm. **b** Amyloid aggregation (*asterisk*) is surrounded by the fibroblast. Bar = 2 μm. **c** Fibrile aggregation is positive of TTR. Schwann cell is intact near amyloid aggregation. Bar = 0.5 μm. **d** Schwann cell seems to be destructed near amyloid aggreagation (*asterisk*). Bar = 2 μm. **e** Ghost structure (*arrow*) which is covered by amyloid (*asterisk*) seems to be myelinated fiber. Bar = 1 μm. **f** Slightly shrinked unmyelinated nerve is seen surrounded by amyloid fibrils (*asterisk*). Bar = 1 μm. **g** The fibrils were straight, nonbranching tubules with a diameter of 7 nm, and with a periodicity of about 200 nm. Bar = 0.5 μm. **h** Amyloid fibrils intermingled with collagen fibers (*asterisk*) are seen. Bar = 0.2 μm. **i** Pre-fibrillar amyloid (*arrow head*) deposited in collagen fiber (*asterisk*) is seen. Bar = 0.5 μm. (From Nagasaka et al. (2009), with permission from Elsevier)

21.7   Diagnosis of FAP

The clinical course, family history, and nerve conduction study, sural nerve biopsy, cardiac examination, including ultrasound cardiography and scintigraphy, and genetic test results are helpful for diagnosis of FAP. Nerve conduction studies can be a useful conventional tool for detecting and evaluating peripheral neuropathy (Said et al. 1984). Reduced nerve conduction velocity, and lowered amplitudes of

Table 21.1 Comparison of the region of amyloid deposition between FAPMet30 and FAPLys54. The degree of amyloid deposition is classified into the following 4 grades: − negative; + slight; ++ moderate; +++ severe. (From Nagasaka et al. (2009), with permission from Elsevier)

Tissue	Glu54Lys (our case)	Val 30 Met
Heart	+++	++
Lung	+	−
Liver	−	−
Kidneys	−	++
Spleen	−	+
Pancreas	++	++
Digestive tract	++	++
Bladder	++	+++
Adrenals	++	+
Testis	+++	+
Thyroid gland	+++	+++
Prostate	+++	Unknown
Pituitary	++	+
Skeletal muscle	++	+
Cerebrum	−	−
Cerebellum	−	−
Arachnoid membrane	+	+
Choroid plexus	−	+++
Spinal cord	−	−
Peripheral nerves	+	++

compound muscle action potential and sensory nerve action potential, reflecting demyelination and axonal impairment, can be observed in affected nerves. Among the polyneuropathies, localized abnormalities such as CTS can be differentiated with this technique. For evaluation of the heart, echocardiography is the most useful noninvasive test for cardiac amyloidosis, i.e., for visualization of the ventricular wall thickness, ventricular septal thickness, and hyperrefractile myocardial echoes; granular sparkling appearance. Electrocardiography (ECG) shows characteristic findings of cardiac amyloidosis including low voltage in the standard limb leads and a QS pattern in the right precordial leads with or without conduction blocks. Gadolinium-enhanced MRI of the heart can be used to identify cardiac amyloidosis (Di Bella et al. 2010; Perugini et al. 2006). RI scintigraphy involving 123-I-iodine-meta-iodobenzylguanidine (MIBG) is adequate for detecting cardiac sympathetic nerve impairment. RI scintigraphy involving (99 m)Tc-(V)-dimercaptosuccinic acid, (99 m)Tc-DPD (3,3-diphosphono-1,2-propanodicarboxylic acid) (Perugini et al. 2005), and (99 m)Tc-pyrophosphate (Ikeda 2004) has been proposed for detecting cardiac TTR deposits. (99 m)Tc-DPD imaging is useful for distinguishing TTR from AL amyloidosis, in which the uptake is usually less. Enhanced MRI of the brain and spinal cord is a convenient method for evaluating CNS amyloidosis (Mitsuhashi et al. 2004). Ophthalmologic evaluation is also useful. To confirm TTR amyloidosis, the demonstration of amyloid deposition in biopsied tissues is essential.

Deposition of amyloid in tissues can be demonstrated by Congo red staining of biopsy materials. With Congo red staining, amyloid deposits show characteristic yellow-green birefringence under polarized light. It is ideal to show that these

amyloid deposits are specifically immunolabeled by anti-TTR antibodies. For many years, a rectal biopsy was the favored procedure when systemic amyloidosis was suspected. Tissues suitable for biopsy include subcutaneous fatty tissue of the abdominal wall (the capillaries are often involved in amyloidgenic TTR), skin, gastric or rectal mucosa, sural nerve, and peritendinous fat from specimens obtained on carpal tunnel surgery. Biopsy of an organ with impaired function, such as the heart or gastrointestinal tract, has the advantage of a definitive diagnosis of some phenotype. Data have revealed that the sensitivity of endoscopic biopsy of the gastrointestinal mucosa is around 85 %; biopsy of a sural nerve is less sensitive because the amyloid deposition is often patchy (Hund et al. 2001; Koike et al. 2004; Vital et al. 2004). Salivary gland biopsy is also useful tool (Do Amaral et al. 2009; Planté-Bordeneuve et al. 2007).

Determination of whether or not a TTR variant is present is important because the treatment options is different. Information about a TTR variant can also be of use for other family members, ensuring early diagnosis and treatment. Clinical genetical testing, including targeted mutation analysis, sequence analysis of exons, and deletion/duplication analysis, is a siginificant method for TTR-related amyloidosis. SELDI-TOF MS (a variation of matrix-assisted laser desorption/ionization; MALDI) is a reliable tool for the diagnosis and investigation of the pathogenesis of FAP with quantitative evaluation of TTR variants, in both tissues and body fluids (Ueda et al. 2009).

In sporadic (non-familial) cases of neuropathy, careful diagnosis should be made (Ando 2010). Of 90 non-familial FAP patients with 17 different mutations of the TTR gene including Val30Met, Ser77Tyr, Ile107Val and Ser77Phe, 18 were mistakenly diagnosed as having chronic inflammatory demyelinating polyneuropathy (CIDP), which was the most common diagnostic error (Planté-Bordeneuve et al. 2007). The clinical aspects in 15 non-familial FAP patients with TTR Val30Met were reported, including somatic neuropathy as an initial symptom in all patients, minor prevalence of small-fiber predominant loss in peripheral nerves, and findings suggestive of subclinical amyloidotic cardiomyopathy (Koike et al. 2011). The report also stated that some patients were initially suspected to have CIDP. An atypical case with FAP TTRTyr78Phe was also reported who presented with rapidly progressive axonal motor neuropathy with subclinical signs of sensory involvement (Riboldi et al. 2011). These data indicate that genetical examination for TTR FAP is a significant tool for the diagnosis of patients with a progressive polyneuropathy of undetermined etiology, especially, with autonomic dysfunction or subclinical cardiac involvement.

21.8   Therapeutic Approaches for FAP

Even now, no specific treatment for TTR amyloidosis has been proven to prevent or cure the disease. There are various symptoms in FAP, so several conservative therapies for orthostatic hypotension, gastro-intestinal symptoms, anemia, heart failure, neuropathic pain, hypothyroidism, hypoglycemia, decubitus ulcers, and vitreous opacity are needed. Araki and Ando (2010) categorized the therapies on

the basis of the amyloid formation mechanism as follows: replacement of the variant TTR gene with a normal TTR gene (liver transplantation (LT), gene therapy), down-regulation of TTR gene mRNA (injection of a large amont of normal TTR (Ando et al. 1997; Ando 2005)), reduction of variant TTR levels (plasma exchange, affinity column chromatography, TTR column chromatography, laser photo coagulation (Kawaji et al. 2010b)), stabilization of the tetrameric TTR structure (NSAIDs, Cr^{3+} (Sato et al. 2006)), Fx-1006A, prevention of amyloid formation (the fluorescent Congo red derivative (trans, trans)-1-bromo-2,5-bis-(3-hydroxycarbonyl-4-hydroxy)styrylbenzene (BSB), antibody therapy, radical scavengers), and dissolution of amyloid fibrils (IDOX (Sebastião et al. 2000), tetracycline). Methods for elimination of amyloidogenic TTR from the blood circulation, including plasma exchange, affinity column chromatography, and TTR column chromatography, were concluded not to be effective for TTR amyloidosis, because decreased serum TTR levels returned to the same levels as before the treatment because of the rapid turnover of TTR.

21.8.1   Liver Transplantation

LT is the only effective treatment for ameliorating FAP, an FAP-associated mutant TTR/WT-TTR liver being replaced by a WT-TTR/WT-TTR secreting one, eliminating the presence of amyloidogenic mutant TTR in the blood. The first LT performed for FAP was performed in 1990 by Holmgren et al. (Holmgren et al. 1991). Since then, LT has widely spread, and had been performed in approximately 1,800 FAP patients in 18 countries by the end of 2009, according to the Familial Amyloidotic Polyneuropathy World Transplant Registry in Sweden (FAPWTR: http://www.fapwtr.org/). Better results have been obtained for Val30Met FAP and much less effective ones for non-V30M FAP, that is, 82 % 5-year survival for patients with Val30Met compared to 61 % for patients with other TTR mutations (Benson et al. 2006; Stangou and Hawkins 2004). LT in FAP Val30Met decreases the TTR plasma level to less than 5 % of the pre-surgical level followed by prevention of further deposition of amyloid in most tissues (Ando et al. 1995; Herlenius et al. 2004; Holmgren et al. 1993; Stangou and Hawkins 2004; Suhr et al. 2002). On peripheral neuropathy, halting of the progression for 10 years after LT was observed for FAPVal30Met (Shimojima et al. 2008). Pathological examination showed that the rate of axonal loss of myelinated fibers was markedly lower in transplanted patients than in non-transplanted ones on 2 years follow-up, while autonomic dysfunction exhibited no significant change (Adams et al. 2000). It was suggested that survival after LT may be associated with progression of neuropathy due to deposition of WT TTR fibrils (Liepnieks et al. 2010). On the other hand, a number of limitations and problems has been recognized for LT, including a shortage of donors, a requirement for surgery for both the recipient and living donor, high cost, incompatibility of patients, and lifelong administration of immunosuppressants (Sekijima et al. 2008). The most important evidence is that the progression of the disease in many patients after LT has been obvious, and is

hypothesized to be the result of continued amyloid fibril formation and deposition of WT TTR after amyloid deposits have been initiated by variant TTR (Olofssoon et al. 2002; Pomfret et al. 1998; Yazaki et al. 2000). It has also been shown that approximately 5–10 % of recipients develop TTR amyloid deposits in several tissues within 5–8 years after LT (Bittencourt et al. 2002; Stangou et al. 2005; Takei et al. 2007). More compelling evidence is that greater amounts of WT TTR than variant TTR were found in amyloid deposits from patients heterozygous for a TTR mutation who died more than 3 years after LT, whereas amyloid deposits from organs of patients who did not undergo LT usually contained significantly more variant than WT TTR (Dwulet and Benson 1986; Wallace et al. 1986; Yazaki et al. 2000). Moreover, cardiac amyloidosis often progresses in Val30Met FAP patients after LT due to the lasting deposition of WT TTR in the heart (Herlenius et al. 2004; Hörnsten et al. 2004; Olofsson et al. 2002; Stangou and Hawkins 2004). It was also shown that in cardiac tissue after LT, fibrils with fragmented amyloidotic TTR contains a higer WT propotion than fibrils without (Ihse et al. 2011). Although the presence of recipient-derived cells in transplanted livers has been reported, the effect of the cells does not contribute to the post-transplant progression of FAP symptoms (Ohya et al. 2010). It has also been demonstrated that progression of amyloid deposition in the eyes and CNS, in which TTR is produced by itself and there is a barrier, e.g., the BBB, could not be halted by LT (Ando et al. 2004). Because of the shortage of donors, more than 330 domino LT (the liver of an individual with familial TTR amyloidosis can be grafted into an individual with liver cancer or end-stage liver disease) have been performed (Stangou et al. 2005). A recent report revealed that a case developed systemic amyloidosis 8 years after domino LT received from a Val30Met heterozygote with familial TTR amyloidosis. Subclinical cutaneous TTR deposits have been confirmed in five other recipients of domino LT (Sousa et al. 2004). The poor outcomes in LT individuals can be summarized as follows, poor nutritional condition (mean body mass index, < 600), severe polyneuropathy (Norris score, <55/81), permanent urinary incontinence, marked postural hypotension, and a fixed pulse rate (Ikeda et al. 2003). Based on this experience, adequate requirements for LT have been proposed.

21.8.2 Approaches Other than Liver Transplantation

Recent studies suggested new therapeutic approaches other than LT such as native TTR tetramer kinetic stabilization through small molecule binding (e.g. diflunisal and Fx-1006A), immune therapy, and gene therapy with small interfering RNAs, antisense oligonucleotides, and single-stranded oligonucleotides (Sekijima et al. 2008). Inhibition or slowing of amyloid fibril formation through stabilization of the native form of the protein TTR over the dissociative transition state is a viable approach for the treatment of FAP (Johnson et al. 2005; Simões et al. 2010). It has been shown that, in cerebral spinal fluid, the TTR binding T4 is in the stable tetrameric conformation, which does not lead to detectable TTR amyloid formation in the brain. Previous work

has shown that binding of T4 and derivatives dramatically stabilizes the native conformation of the TTR tetramer and consequently inhibits amyloid formation *in vitro* (Miroy et al. 1996). Several compounds known to bind TTR in its T4 pocket also bind albumin, non-steroidal anti-inflammatory drugs (NSAIDs), or agents like bromophenol blue, salicylates, and furosemide, among others (Baures et al. 1998, 1999; Larsen 1972; Steinrauf et al. 1991; Stockigt et al. 1985). NSAIDs, the structure of which resemble the T4 one, including flufenamic acid, diflunisal, diclofenac, flurbiprofen, and resveratrol, and structurally similar compounds has been shown to have potential as to stabilization of the tetrameric TTR and strong inhibition of the formation of TTR amyloid fibrils *in vitro* (Almeida et al. 2004; Hammarström et al. 2003a; Miller et al. 2004; Sekijima et al. 2008; Tojo et al. 2006). A study on the effect of diflunisal on familial amyloidosis is ongoing (Clinical Trials.gov, a service of the U.S. National Institutes of Health: http://clinicaltrials.gov/ct2/show/study/NCT00409175 last updated on Dec 16, 2010). FX-1006A (tafamidis meglumine; an amino sugar derived from sorbitol) has completed a phase II/III clinical trial and preliminary results suggest it slows progression of the disease (Clinical Trials.gov, a service of the U.S. National Institutes of Health: http://clinicaltrials.gov/ct2/show/study/NCT00409175 last updated on May 25, 2011).

Small molecules of a diverse variety of scaffolds (stilbens, flavones, benzoxazoles, tetrahydroquinolines, dihydropyridines, benzodiazepines, and phenoxazines), which are able to bind the T4 binding sites of TTR with high affinity, are also expected to kinetically stabilize the native quaternary structure, and thereby preclude TTR disassembly, misfolding, and aggregation (Adamski-Werner et al. 2004; Johnson et al. 2008a, b, 2009). These small molecules typically comprise two appropriately substituted aromatic rings connected by hydrophobic linkers of variable chemical composition, and usually have polar substituents on their aryl rings, which interact with carboxyl or hydroxyl groups located in the T4 binding site (Johnson et al. 2008a). *In vivo* studies involving cultured tissues have shown that such compounds reduce amyloidogenic TTR-induced cytotoxicity in amyloid deposited cells (Reixach et al. 2004, 2006).

Other mechanisms and therapeutic approaches for stabilizing TTR have been proposed.

A report demonstrated that clusterin prevents the amyloid fibril formation by TTR variants under acidic conditions through stabilization of the TTR tetrameric structure by strongly interacting with WT TTR, and TTR variants Val30Met and Leu55Pro (Lee et al. 2009).

Another study has demonstrated that (-)-epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea, binds to TTR, resulting in inhibition of aggregation *in vitro* and *ex vivo* (Ferreira et al. 2009). Targeting the misfolding step, CyDs (cyclodextrins), cyclic oligosaccharides composed of glucose units, various branched β-CyDs, and GUG-β-CyD [6-*O*-α-(4-*O*-α-D-glucuronyl)-D-glucosyl-β-CyD] showed potent inhibition of TTR misfolding by stabilizing its conformation, resulting in suppression of TTR amyloid formation (Jono et al. 2011). It was found on mass spectrometry that retinol stabilizes the quaternary structure of TTR, through

its interactions with RBP, thereby reducing the rate of TTR disassembly (Hyung et al. 2010).

Recently, anti-oxidant and apoptosis therapies have been proposed. Carvedilol, a β-antagonist with a strong anti-oxidant effect on lipids, proteins and superoxide production, reduces TTR deposition in a FAP mouse model (Macedo et al. 2010). Tauroursodeoxycholic acid (TUDCA), a unique natural compound that acts as a potent anti-apoptotic and anti-oxidant agent, reduced TTR toxic aggregates with decreasing apoptotic and oxidative biomarkers in a transgenic mouse model of FAP (Macedo et al. 2008). Moreover, combined doxycyclin and TUDCA treatment significantly lowered fibrillar TTR amyloid deposition in transgenic TTR mice models (Cardoso et al. 2010).

Suppression of TTR expression in hepatocyte cell cultures with ribozymes has been reported (Nakamura and Ando 2004; Pröpsting et al. 2000; Tanaka et al. 2001). Instead of LT, gene therapy has been attempted to prevent the production of variant TTR in the liver. Several studies on mice in which the TTR gene had been inactivated indicated no ill effects due to the lack of TTR (Episkopou et al. 1993), therefore, it could be an effective therapeutic approach due to the limitation of TTR as the amyloid fibril precursor, followed by inhibition of production of both variant and WT TTR. An examination demonstrated endogenous gene repair of genomic deoxyribonucleic acid induced by single-stranded oligonucleotides in transgenic murine liver with the murine TTR Val30Met gene (Nakamura et al. 2004). TTR antisense oligonucleotides suppress the hepatic TTR mRNA levels and serum TTR levels in a transgenic mouse model carrying the human TTR Ile84Ser mutation (Benson et al. 2006). Intraventricular administration of TTR-specific antisense oligonucleotides into the cerebral ventricular system in mice transgenic as to human TTR Ile84Ser significantly reduces the choroid human TTR mRNA levels, and TTR in choroid plexus epithelial cells (Benson et al. 2010).

A few studies have been performed on specific therapies for TTR amyloid cardiomyopathy. Several new chemical scaffolds, identified using a fluorescence polarization-based high-throughput screenig assay, effectively inhibit the proteotoxicity of Val122Ile-TTR toward human cardiomyocytes by preventing TTR tetramer dissociation, partial unfolding, and aggregation of both WT and Val122Ile-TTR (Alhamadsheh et al. 2011).

Structure-based design of kinetic stabilizers that ameliorate the TTR amyloidoses has been performed (Connelly et al. 2010). *Ortho*-trifluormethylphenyl anthranilic acid and N-(*meta*-trifluoromethylphenyl) phenoxazine 4,6-dicarboxylic acid have been discovered to be very potent and specific TTR fibril formation inhibitors (Klabunde et al. 2000). The isosteric 3,5-dibromo-4-aminophenyl substructure binds to the inner T4 binding pocket of TTR to protect cells from the cytotoxic effect of amyloidgenic TTR (Choi et al. 2010). Inhibition of variant TTR mRNA expression by small interferingRNAs (siRNA) is also under investigation. 4′-Iodo-4′-deoxydoxorubicin (IDOX) has been reported to bind to several types of amyloid and to lead to the catabolism of amyloid in deposits. In a multicenter clinical trial, IDOX was administered to persons with AL amyloidosis, however, no obvious benefit was detected.

21.9   TTR-derived Senile Systemic Amyloidosis

SSA is a common age-related amyloidosis that involves the accumulation of WT TTR (Westermark et al. 1990) predominantly in the heart as well as in other organs, e.g., the lungs, kidneys and gastrointestinal tract (Westermark et al. 1979; Cornwell et al. 1983). 10–15 % of individuals older than 65 years and one-quarter of people aged over 85 years are affected (Tanskanen et al. 2008; Ueda et al. 2011) and findings are frequently observed at autopsy. Pathologically, the TTR amyloid deposits exhibit a patchy plaque-like shape and develop mainly inside the ventricular wall in cases with SSA, whereas in FAP cases they develop mainly in the pericardium and the surrounding muscle fascicles (Ueda et al. 2011). A report revealed a close association between the presence of amyloid and the basement membrane around myocardial cells (Sawabe et al. 2003). However, the clinicopathological features of SSA remain to be completely elucidated. TTR in SSA is fragmented more than 90 % in contrast with full length TTR in most hereditary amyloidosis cases with variant TTR. From the tissues with amyloid deposits in SSA patients, a series of C-terminal TTR fragments with N-terminals ranging from 46 to 55 amino acids in length have been identified (Ando and Ueda 2008). A report suggested that myocardial infarction, and variations in the genes for alpha2M and tau may be associated with SSA (Tanskanen et al. 2008). The pre-mortem diagnosis of SSA is rare in spite of the disease frequency, so it is important to develop several diagnostic methods. In addition to routine cardiac examination, radiological evaluation, RI scintigraphy involving (99 m)Tc-DPD for detecting cardiac amyloid deposits (Perugini et al. 2005), and magnetic resonance imaging to detect delayed gadolinium enhancement (Perugini et al. 2006) can lead an accurate diagnosis. Subcutaneous fat biopsies allow the detection of amyloid deposits in only 20 % of SSA patients, but the detection rate on combined biopsy evaluation of gastrointestinal tract and subcutaneous tissue samples from SSA patients is 44 % (Ueda et al. 2011). The treatment of SSA focuses on conservative care including diuretics, etc. Also, implantation of a permanent pacemaker (Mathew et al. 1997) and also orthotopic heart transplantation (Fuchs et al. 2005) have been considered. Even so, as for FAP, novel treatments should be developed.

   The WT TTR protein has been shown to undergo conformational changes and resulting fibril formation similar to amyloidogenic variants under denaturing conditions (Cardoso et al. 2002). Studies on WT TTR after heat denaturation have shown that several key changes occur during the process that are not completely reversed on renaturation, leading to the hypothesis that these permanent conformational events may promote subsequent fibrile formation (Chung et al. 2001). Kinetic stabilization by small molecules has been shown to stabilize the WT TTR tetramer, and to prevent or slow the formation of amyloid fibrils *in vitro* (Johnson et al. 2005).

21.10 Conclusion

In recent years, a lot of molecular biological data concerning not only TTR but the other proteins involved in protein misfolding disease have accumulated, and thus unceasing efforts of research on TTR-related amyloisosis has been continued. FAP is now recognized not to be a rare disease, and to have many variations based on genetical and biochemical variations of TTR, although the genotype-phenotype correlation is still unclear. Moreover, progress has been made in diagnostic procedures including genetical approaches for the early accurate diagnosis. As for treatment, in addition to liver transplantation, several TTR stabilizers has been applied for as the results of fundamental molecular biological studies. The radical treatment is expected to be established in the future.

References

Adams D, Samuel D, Goulon-Goeau C, Nakazato M, Costa PM, Feray C, Planté V, Ducot B, Ichai P, Lacroix C, Metral S, Bismuth H, Said G (2000) The course and prognostic factors of familial amyloid polyneuropathy after liver transplantation. Brain 123:1495–1504.

Adamski-Werner SL, Palaninathan SK, Sacchettini JC, Kelly JW (2004) Diflunisal analogues stabilize the native state of transthyretin. Potent inhibition of amyloidogenesis. J Med Chem 47:355–374

Afolabi I, Hamidi Asl K, Nakamura M, Jacobs P, Hendrie H, Benson MD (2000) Transthyretin isoleucine-122 mutation in African and American blacks. Amyloid 7:121–125

Alhamadsheh MM, Connelly S, Cho A, Reixach N, Evan T, Powers ET, Pan DW, Wilson IA, Kelly JF, Graef IA (2011) Potent kinetic stabilizers that prevent transthyretin-mediated cardiomyocyte proteotoxicity. Sci Transl Med 3:97ra81

Almeida MR, Alves IL, Terazaki H, Ando Y, Saraiva MJ (2000) Comparative studies of two transthyretin variants with protective effects on familial amyloidotic polyneuropathy: TTR R104H and TTR T119M. Biochem Biophys Res Commun 270:1024–1028

Almeida MR, Macedo B, Cardoso I, Alves I, Valencia G, Arsequell G, Planas A, Saraiva MJ (2004) Selective binding to transthyretin and tetramer stabilization in serum from patients with familial amyloidotic polyneuropathy by an iodinated diflunisal derivative. Biochem J 381:351–356

Alves IL, Altland K, Almeida MR, Winter P, Saraiva MJ (1997) Screening and biochemical characterization of transthyretin variants in the Portuguese population. Hum Mutat 9:226–233

Ancsin JB (2003) Amyloidogenesis: historical and modern observations point to heparan sulfate proteoglycans as a major culprit. Amyloid 10:67–79

Andersson K, Olofsson A, Nielsen EH, Svehag SE, Lundgren E (2002) Only amyloidogenic intermediates of transthyretin induce apoptosis. Biochem Biophys Res Commun 294:309–314

Ando Y (2005) Liver transplantation and new therapeutic approaches for familial amyloidotic polyneuropathy (FAP). Med Mol Morphol 38:142–154

Ando Y (2010) FAP is a candidate disease in patients with undetermined polyneuropathy. Intern Med 49:1841–1842

Ando Y, Ueda M (2008) Novel methods for detecting amyloidogenic proteins in transthyretin related amyloidosis. Front Biosci 13:5548–5558

Ando Y, Yi S, Nakagawa T, Ikegawa S, Hirota M, Miyazaki A, Araki S (1991) Disturbed metabolism of glucose and related hormones in familial amyloidotic polyneuropathy: hypersensitivities of the autonomic nervous system and therapeutic prevention. J Auton Nerv Syst 35:63–70

Ando Y, Tanaka Y, Nakazato M, Ericzon BG, Yamashita T, Tashima K, Sakashita N, Suga M, Uchino M, Ando M (1995) Change in variant transthyretin levels in patients with familial amyloidotic polyneuropathy type I following liver transplantation. Biochem Biophys Res Commun 211:354–358

Ando Y, Yamashita T, Nakamura M, Tanaka Y, Hashimoto M, Tashima K, Suhr O, Uemura Y, Obayashi K, Terazaki H, Suga M, Uchino M, Ando M (1997) Down regulation of a harmful variant protein by replacement of its normal protein. Biochim Biophys Acta 1362:39–46

Ando Y, Terazaki H, Nakamura M, Ando E, Haraoka K, Yamashita T, Ueda M, Okabe H, Sasaki Y, Tanihara H, Uchino M, Inomata Y (2004) A different amyloid formation mechanism: de novo oculoleptomeningeal amyloid deposits after liver transplantation. Transplantation 77:345–349

Ando Y, Nakamura M, Araki S (2005) Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol 62:1057–1062

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

Araki S, Ando Y (2010) Transthyretin-related familial amyloidotic polyneuropathy. Progress in Kumamoto, Japan (1967–2010). Proc Jpn Acad Ser B 86:694–706

Araki S, Mawatari S, Ohta M, Nakajima A, Kuroiwa Y (1968) Polyneuritic amyloidosis in a Japanese family. Arch Neurol 18:593–602

Armen RS, DeMarco ML, Alonso DO, Daggett V (2004) Pauling and Corey's alpha-pleated sheet structure may define the prefibrillar amyloidogenic intermediate in amyloid disease. Proc Natl Acad Sci USA 101:11622–11627

Banerjee A, Bairagya HR, Mukhopadhyay BP, Nandi TK, Bera AK (2010) Structural insight to mutated Y116S transthyretin by molecular dynamics simulation. Indian J Biochem Biophys 47:197–202

Barouch FC, Benson MD, Mukai S (2004) Isolated vitreoretinal amyloidosis in the absence of transthyretin mutations. Arch Ophthalmol 122:123–125

Baures PW, Peterson SA, Kelly JW (1998) Discovering transthyretin amyloid fibril inhibitors by limited screening. Bioorg Med Chem 6:1389–1401

Baures PW, Oza VB, Peterson SA, Kelly JW (1999) Synthesis and evaluation of inhibitors of transthyretin amyloid formation based on the non-steroidal anti-inflammatory drug, flufenamic acid. Bioorg Med Chem 7:1339–1347

Benson MD, Cohen AS (1977) Generalized amyloid in a family of Swedish origin. A study of 426 family members in seven generations of a new kinship with neuropathy, nephropathy, and central nervous system involvement. Ann Intern Med 86:419–424

Benson MD, Wallace MR, Tejada E, Baumann H, Page B (1987) Hereditary amyloidosis: description of a new american kindred with late onset cardiomyopathy. Arthritis Rheum 30:195–200

Benson MD, Kluve-Beckerman B, Liepniesks JJ, Murrell JR, Hanes D, Uemichi T (1996) Metabolism of amyloid proteins. Ciba Found Symp 199:104–113

Benson MD, Kluve-Beckerman B, Zeldenrust SR, Siesky AM, Bodenmiller DM, Showalter AD, Sloop KW (2006) Targeted suppression of an amyloidogenic transthyretin with antisense oligonucleotides. Muscle Nerve 33:609–618

Benson MD, Kincaid JC (2007) The molecular biology and clinical features of amyloid neuropathy. Muscle Nerve 36:411–423

Benson MD, Smith RA, Hung G, Kluve-Beckerman B, Showalter AD, Sloop KW, Monia BP (2010) Suppression of choroid plexus transthyretin levels by antisense oligonucleotide treatment. Amyloid 17:43–49

Berg I, Thor S, Hammarström P (2009) Modeling familial amyloidotic polyneuropathy (transthyretin V30M) in *Drosophila melanogaster*. Neurodegener Dis 6:127–138

Biolo A, Ramamurthy S, Connors LH, O'Hara CJ, Meier-Ewert HK, Soo Hoo PT, Sawyer DB, Seldin DS, Sam F (2008) Matrix metalloproteinases and their tissue inhibitors in cardiac amyloidosis: relationship to structural, functional myocardial changes and to light chain amyloid deposition. Circ Heart Fail 1:249–257

Bittencourt PL, Couto CA, Leitão MC, Siqueira SA, Farias AQ, Massarolo PCB, Mies S (2002) No evidence of de novo amyloidosis in recipients of domino liver transplantation: 12 to 40 (mean 24) month follow-up. Amyloid 9:194–196

Blake CCF, Geisow MJ, Swan ID, Rérat C, Rérat B (1974) Structure of human plasma prealbumin at 2–5 Å resolution: a preliminary report on the polypeptide chain conformation, quaternary structure and thyroxine binding. J Mol Biol 88:1–12

Blake CC, Oatley SJ (1977) Protein-DNA and protein-hormone interactions in prealbumin: a model of the thyroid hormone nuclear receptor? Nature 268:115–120

Blake C, Serpell L (1996) Synchrotron X-ray studies suggest that the core of the transthyretin amyloid fibril is a continuous beta-sheet helix. Structure 4:989–998

Blake CCF, Geisow MJ, Oatley SJ, Rérat B, Rérat C (1978) Structure of prealbumin: secondary, tertiary and quaternary interactions determined by Fourier refinematat 1.8 Å. J Mol Bio 121:339–356

Blevins G, Macaulay R, Harder S, Fladeland D, Yamashita T, Yazaki M, Hamidi Asl K, Benson MD, Donat JR (2003) Oculoleptomeningeal amyloidosis in a large kindred with a new transthyretin variant Tyr69His. Neurology 60:1625–1630

Bourgault S, Solomon JP, Reixach N, Kelly JW (2011) Sulfated glycosaminoglycans accelerate transthyretin amyloidogenesis by quaternary structural conversion. Biochemistry 50:1001–1015

Bucciantini M, Giannoni E, Chiti F, Baroni F, Formigli L, Zurdo J, Taddei N, Ramponi G, Dobson CM, Stefani M (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. Nature 416:507–511

Buxbaum JN (2009) Animal models of human amyloidoses: are transgenic mice worth the time and trouble? FEBS Lett 583:2663–2673

Buxbaum JN, Ye Z, Reixach N, Friske L, Levy C, Das P, Golde T, Masliah E, Roberts AR, Bartfai T (2008) Transthyretin protects Alzheimer's mice from the behavioral and biochemical effects of Abeta toxicity. Proc Natl Acad Sci USA 105:2681–2686

Cardoso I, Goldsbury CS, Müller SA, Olivieri V, Wirtz S, Damas AM, Aebi U, Saraiva MJ (2002) Transthyretin fibrillogenesis entails the assembly of monomers, a molecular model for in vitro assembled transthyretin amyloid-like fibrils. J Mol Biol 317:683–685

Cardoso I, Martins D, Tania Ribeiro T, Merlini G, Saraiva MJ (2010) Synergy of combined doxycycline/TUDCA treatment in lowering transthyretin deposition and associated biomarkers: studies in FAP mouse models. J Transl Med 8:74–84

Cavallaro T, Martone RL, Dwork AJ, Schon EA, Herbert J (1990) The retinal pigment epithelium is the unique site of transthyretin synthesis in the rat eye. Invest Ophthalmol Vis Sci 31:497–501

Choi S, Reixach N, Connelly S, Johnson SM, Wilson IA, Kelly JW (2010) A substructure combination strategy to create potent and selective transthyretin kinetic stabilizers that prevent amyloidogenesis and cytotoxicity. J Am Chem Soc 132:1359–1370

Chung CM, Connors LH, Benson MD, Walsh MT (2001) Biophysical analysis of normal transthyretin: implications for fibril formation in senile systemic amyloidosis. Amyloid 8:75–83

Coelho T, Carvalho M, Saraiva MJ, Alvas I, Almedia MR, Costa PP (1993) Familial amyloidotic polyneuropathy and other transthyretin related disorders. Proceedings of the Second International Symposium, Skelleftea, Sweden, 1992. J Rheumatol 20:179

Coelho T, Sousa A, Lourenço E, Ramalheira J (1994) A study of 159 Portuguese patients with familial amyloidotic polyneuropathy (FAP) whose parents were both unaffected. Med Genet 31:293–299

Coimbra A, Andrade C (1971) Familial amyloid polyneuropathy: an electron microscope study of the peripheral nerve in five cases. II. Nerve fibre changes. Brain 94:207–212

Colon W, Kelly JW (1992) Partial denaturation of transthyretin is sufficient for amyloid fibril formation in vitro. Biochemistry 31:8654–8660

Connelly S, Choi S, Johnson SM, Kelly JW, Wilson IA (2010) Structure-based design of kinetic stabilizers that ameliorate the transthyretin amyloidoses. Curr Opin Struct Biol 20:54–62

Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE (2003) Tabulation of human transthyretin (TTR) variants. Amyloid 10:160–184

Cornwell GG, Murdoch WL, Kyle RA, Westermark P, Pitkänen P (1983) Frequency and distribution of senile cardiovascular amyloid: a clinicopathologic correlation. Am J Med 75:618–623

Costa PP, Figueira AS, Bravo FR (1978) Amyloid fibril protein related to prealbumin in familial amyloidotic polyneuropathy. Proc Natl Acad Sci USA 75:4499–4503

da Costa G, Gomes RA, Guerreiro A, Mateus É, Monteiro E, Barroso E, Coelho AV, Freire AP, Cordeiro C (2011) Beyond genetic factors in familial amyloidotic polyneuropathy: protein glycation and the loss of fibrinogen's chaperone activity. PLoS One 6:e24850

Dardiotis E, Koutsou P, Zamba-Papanicolaou E, Vonta I, Hadjivassiliou M, Hadjigeorgiou G, Cariolou M, Christodoulou K, Kyriakides T (2009) Complement C1Q polymorphisms modulate onset in familial amyloidotic polyneuropathy TTR Val30Met. J Neurol Sci 284:158–162

Di Bella G, Minutoli F, Mazzeo A, Vita G, Oreto G, Carerj S, Anfuso C, Russo M, Gaeta M (2010) MRI of cardiac involvement in transthyretin familial amyloid polyneuropathy. Am J Roentgenol 195:394–399

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

Du J, Murphy RM (2010) Characterization of the interaction of β-amyloid with transthyretin monomers and tetramers. Biochemistry 49:8276–8289

Dubový P, Klusáková I, Svíženská I (2002) A quantitative immunohistochemical study of the endoneurium in the rat dorsal and ventral spinal roots. Histochem Cell Biol 117:473–480

Dwulet FE, Benson MD (1984) Primary structure of an amyloid prealbumin and its plasma precursor in a heredofamilial polyneuropathy of Swedish origin. Proc Natl Acad Sci USA 81:694–698

Dwulet FE, Benson MD (1986) Characterization of prealbumin variant associated with familial amyloidotic polyneuropathy type II (Indiana/Swiss). J Clin Invest 78:880–886

Dyck PJ, Lambert EH (1969) Compound action potential, quantitative histologic and teased-fiber, and electron microscopic studies of sural nerve biopsies. Arch Neurol 20:490–507

El-Salhy M, Suhr O, Stenling R, Wilander E, Grimelius L (1994) Impact of familial amyloid associated polyneuropathy on duodenal endcrine cells. Gut 35:1413–1418

Eneqvist T, Andersson K, Olofsson A, Lundgren E, Sauer-Eriksson AE (2000) The beta-slip: a novel concept in transthyretin amyloidosis. Mol Cell 6:1207–1218

Episkopou V, Maeda S, Nishiguchi S, Shimada K, Gaitanaris GA, Gottesman ME, Robertson EJ (1993) Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. Proc Natl Acad Sci USA 90:2375–2379

Falls HF, Jackson J, Carey JH, Rukavina JG, Block WD (1955) Ocular manifestations of hereditary primary systemic amyloidosis. Arch Ophthalmol 54:660–664

Ferrao-Gonzales AD, Palmieri L, Valory M, Silva JL, Lashuel H, Kelly JW, Foguel D (2003) Hydration and packing are crucial to amyloidogenesis as revealed by pressure studies on transthyretin variants that either protect or worsen amyloid disease. J Mol Biol 328:963–974

Ferreira N, Cardoso I, Domingues MR, Vitorino R, Bastos M, Bai G, Maria Saraiva J, Almeida MR (2009) Binding of epigallocatechin-3-gallate to transthyretin modulates its amyloidogenicity. FEBS Lett 583:3569–3576

Fiori MG, Salvi F, Plasmati R, Tessari F, Bianchi R, Tassinari CA (1994) Amyloid deposits inside myocardial fibers in transthyretin-Met30 familial amyloidotic polyneuropathy. A histological and biochemical study. Cardiology 85:145–153

Fleming CE, Saraiva MJ, Sousa MM (2007) Transthyretin enhances nerve regeneration. J Neurochem 103:831–839

Foguel D, Suarez MC, Ferrão-Gonzales AD, Porto TC, Palmieri L, Einsiedler CM, Andrade LR, Lashuel HA, Lansbury PT, Kelly JW, Silva JL (2003) Dissociation of amyloid fibrils of alpha-synuclein and transthyretin by pressure reveals their reversible nature and the formation of water-excluded cavities. Proc Natl Acad Sci USA 100:9831–9836

Foss TR, Kelker MS, Wiseman RL, Wilson IA, Kelly JW (2005) Kinetic stabilization of the native state by protein engineering: implications for inhibition of transthyretin amyloidogenesis. J Mol Biol 347:841–854

Fuchs U, Zittermann A, Suhr O, Holmgren G, Tenderich G, Minami K, Koerfer R (2005) Heart transplantation in a 68-year-old patient with senile systemic amyloidosis. Am J Transplant 5:1159–1162

Gallo G, Wisniewski T, Choi-Miura N-H, Ghiso J, Frangione B (1994) Potential role of apolipoprotein-E in fibrillogenesis. Am J Pathol 145:526–530

Gambetti P, Russo C (1998) Human brain amyloidosis. Nephrol Dial Transplant 13:33–40

Gasperini RJ, Hou X, Parkington H, Coleman H, Klaver DW, Vincent AJ, Foa LC, Small DH,(2011) TRPM8 and Nav1.8 sodium channels are required for transthyretin-induced calcium influx in growth cones of small-diameter TrkA-positive sensory neurons. Mol Neurodegener 6:19

Goebel HH, Seddigh S, Hopf HC, Uemichi T, Benson MD, McKusick VA (1997) A European family with histidine 58 transthyretin mutation in familial amyloid polyneuropathy. Neuromuscul Disord 7:229–230

Goren H, Steinberg MC, Farboody GH (1980) Familial oculoleptomeningeal amyloidosis. Brain 103:473–495

Greene MJ, Sam F, Soo Hoo PT, Patel RS, Seldin DC, Connors LH (2011) Evidence for a functional role of the molecular chaperone clusterin in amyloidotic cardiomyopathy. Am J Pathol 178:61–68

Groenning M, Campos RI, Fagerberg C, Rasmussen AA, Eriksen UH, Powers ET, Hammarström P (2011) Thermodynamic stability and denaturation kinetics of a benign natural transthyretin mutant identified in a Danish kindred. Amyloid 18:35–46

Gustavsson A, Engström U, Westermark P (1991) Normal transthyretin and synthetic transthyretin fragments form amyloid-like fibrils in vitro. Biochem Biophys Res Commun 175:1159–1164

Hagiwara K, Ochi H, Suzuki S, Shimizu Y, Tokuda T, Murai H, Shigeto H, Ohyagi Y, Iwata M, Iwaki T, Kira J (2009) Highly selective leptomeningeal amyloidosis with transthyretin variant Ala25Thr. Neurology 72:1358–1360

Hamilton JA, Benson MD (2001) Transthyretin: a review from a structural perspective. Cell Mol Life Sci 58:1491–1521

Hamilton JA, Steinrauf LK, Braden BC, Liepnieks J, Benson MD, Holmgren G, Sandgren O, Steen L (1993) The X-ray crystal structure refinements of normal human transthyretin and the amyloidogenic Val-30®Met variant to 1.7-Å resolution. J Biol Chem 268:2416–2424

Hammarström P, Schneider F, Kelly JW (2001) Trans-suppression of misfolding in an amyloid disease. Science 293:2459–2462

Hammarström P, Wiseman RL, Powers ET, Kelly JW (2003a) Prevention of transthyretin amyloid disease by changing protein misfolding energetics. Science 299:713–716

Hammarström P, Sekijima Y, White JT, Wiseman RL, Lim A, Costello CE, Altland K, Garzuly F, Budka H, Kelly JW (2003b) D18G transthyretin is monomeric, aggregation prone, and not detectable in plasma and cerebrospinal fluid: a prescription for central nervous system amyloidosis? Biochemistry 42:6656–6663

Hanyu N, Ikeda S, Nakadai A, Yanagisawa, N, Powell HC (1989) Peripheral nerve pathological findings in familial amyloid polyneuropathy: a correlative study of proximal sciatic nerve and sural nerve lesions. Ann Neurol 25:340–350

Hanyu N, Shimizu T, Yamauchi K, Okumura N, Hidaka H (2009) Characterization of cysteine and homocysteine bound to human serum transthyretin. Clin Chim Acta 403:70–75

Harats N, Robert M, Worth RM, Benson MD (1989) Hereditary amyloidosis: evidence against early amyloid deposition. Arthritis Rheum 32:1474–1476

Hellman U, Alarcon F, Lundgren HE, Suhr OB, Bonaiti-Pellié C, Planté-Bordeneuve V (2008) Heterogeneity of penetrance in familial amyloid polyneuropathy, ATTR Val30Met, in the Swedish population. Amyloid 15:181–186

Herbert J, Wilcox JN, Pham KT, Fremeau RT Jr, Zeviani M, Dwork A, Soprano DR, Makover A, Goodman DS, Zimmerman EA, Roberts JL, Schon EA (1986) Transthyretin: a choroid plexus-specific transport protein in human brain. Neurology 36:900–911

Herlenius G, Wilczek HE, Larsson M, Ericzon B-G (2004) Ten years of international experience with liver transplantation for familial amyloidotic polyneuropathy: results from the familial amyloidotic polyneuropathy world transplant registry. Transplantation 77:64–71

Hofer PA, Anderson R (1975) Postmortem findings in primary familial amyloidosis with polyneuropathy. Acta Pathol Microbiol Scand A 83:309–322

Holmgren G, Holmberg E, Lindström A, Lindström E, Nordenson I, Sandgren O, Steen L, Svensson B, Lundgren E, von Gabain A (1988) Diagnosis of familial amyloidotic polyneuropathy in Sweden by RFLP analysis. Clin Genet 33:176–180

Holmgren G, Steen L, Ekstedt J, Groth C-G, Ericzon B-G, Eriksson S, Andersen O, Karlberg I, Nordén G, Nakazato M, Hawkins P, Richardson S, Pepys M (1991) Biochemical effect of liver transplantation in two Swedish patients with familial amyloidotic polyneuropathy (FAP-met30). Clin Genet 40:242–246

Holmgren G, Bergström S, Drugge U, Lundgren E, Nording-Sikström C, Sandgren O, Steen L (1992) Homozygosity for the transthyretin-Met30-gene in seven individuals with familial amyloidosis with polyneuropathy detected by restriction enzyme analysis of amplified genomic DNA sequences. Clin Genet 41:39–41

Holmgren G, Steen L, Suhr O, Ericzon B-G, Groth C-G, Andersen O, Wallin BG, Seymour A, Richardson S, Hawkins PN, Pepys MB (1993) Clinical improvement and amyloid regression after liver transplantation in hereditary transthyretin amyloidosis. Lancet 342:1113–1116

Holmgren G, Costa PMP, Andersson C, Asplund K, Steen L, Beckman L, Nylander P-O, Teixeira A, Saraiva MJM, Costa PP (1994) Geographical distribution of TTR met30 carriers in northern Sweden: discrepancy between carrier frequency and prevalence rate. J Med Genet 31:351–354

Hörnberg A, Eneqvist T, Olofsson A, Lundgren E, Sauer-Eriksson AE (2000) A comparative analysis of 23 structures of the amyloidogenic protein transthyretin. J Mol Biol 302:649–669

Hörnsten R, Wiklund U, Olofsson B-O, Jensen SM, Suhr OB (2004) Liver transplantation does not prevent the development of life-threatening arrhythmia in familial amyloidotic polyneuropathy, Portuguese-type (ATTR Val30Met) patients. Transplantation 78:112–116

Hou X, Richardson SJ, Aguilar MI, Small DH (2005) Binding of amyloidogenic transthyretin to the plasma membrane alters membrane fluidity and induces neurotoxicity. Biochemistry 44:11618–11627

Hou X, Parkington HC, Coleman HA, Mechler A, Martin LL, Aguilar M-I, Small DH (2007) Transthyretin oligomers induce calcium influx via voltage-gated calcium channels. J Neurochem 100:446–457

Hund E, Linke RP, Willig F, Grau A (2001) Transthyretin-associated neuropathic amyloidosis. Pathogenesis and treatment. Neurology 56:431–435

Hurshman Babbes AR, White JT, Powers ET, Kelly JW (2004) Transthyretin aggregation under partially denaturing conditions is a downhill polymerization. Biochemistry 43:7365–7381

Hurshman Babbes AR, Powers ET, Kelly JW (2008) Quantification of the thermodynamically linked quaternary and tertiary structural stabilities of transthyretin and its disease-associated variants: the relationship between stability and amyloidosis. Biochemistry 47:6969–6984

Huttunen HJ, Fages C, Rauvala H (1999) Receptor for advanced glycation end products (RAGE)-mediated neurite outgrowth and activation of NF-kappaB require the cytoplasmic domain of the receptor but different downstream signaling pathways. J Biol Chem 274:19919–19924

Hyung S-J, Deroo S, Robinson CV (2010) Retinol and retinol-binding protein stabilize transthyretin via formation of retinol transport complex. ACS Chem Biol 5:1137–1146

Ihse E, Suhr OB, Hellman U, Westermark P (2011) Variation in amount of wild-type transthyretin in different fibril and tissue types in ATTR amyloidosis. J Mol Med (Berl.) 89:171–180

Ikeda S (2004) Cardiac amyloidosis: heterogenous pathogenic backgrounds. Intern Med 43:1107–1114

Ikeda S, Hanyu N, Hongo M, Yoshioka J, Oguchi H, Yanagisawa N, Kobayashi T, Tsukagoshi H, Ito N, Yokota T (1987) Hereditary generalized amyloidosis with polyneuropathy, clinicopathological study of 65 Japanese patients. Brain 110:315–337

Ikeda S, Nakazato M, Ando Y, Sobue G (2002) Familial transthyretin-type amyloid polyneuropathy in Japan: clinical and genetic heterogeneity. Neurology 58:1001–1007

Ikeda S, Takei Y, Hashikura Y (2003) Liver transplantation as treatment for neurological disorders. Expert Rev Neurother 3:547–555

Ingenbleek K, Young V (1994) Transthyretin (prealbumin) in health and disease: nutritional implications. Annu Rev Nutr 14:495–533

Inoue S, Kuroiwa M, Saraiva MJ, Guimarães A, Kisilevsky R (1998) Ultrastructure of familial amyloid polyneuropathy amyloid fibrils: examination with high-resolution electron microscopy. J Struct Biol 124:1–12

Inoue S, Ohta M, Li Z, Zhao G, Takaoka Y, Sakashita N, Miyakawa K, Takada K, Tei H, Suzuki M, Masuoka M, Sakaki Y, Takahashi K, Yamamura K (2008) Specific pathogen free conditions prevent transthyretin amyloidosis in mouse models. Transgenic Res 17:817–826

Inouye H, Domingues FS, Damas AM, Saraiva MJ, Lundgren E, Sandgren O, Kirschner DA (1998) Analysis of x-ray diffraction patterns from amyloid of biopsied vitreous humor and kidney of transthyretin (TTR) Met30 familial amyloidotic polyneuropathy (FAP) patients: axially arrayed TTR monomers constitute the protofilament. Amyloid 5:163–174

Iozzo RV, Cohen IR, Grassel S, Murdoch AD (1994) The biology of perlecan: the multifaceted heparan sulphate proteoglycan of basement membranes and pericellular matrices. Biochem J 302:625–639

Izumoto S, Younger D, Hays AP, Martone RL, Smith RT, Herbert J (1992) Familial amyloidotic polyneuropathy presenting with carpal tunnel syndrome and a new transthyretin mutation, asparagine 70. Neurology 42:2094–2102

Jacobsson B, Collins VP, Grimelius L, Pettersson T, Sandstedt B, Carlström A (1989) Transthyretin immunoreactivity in human and porcine liver, choroid plexus, and pancreatic islets. J Histochem Cytochem 37:31–37

Jacobson DR, McFarlin DE, Kane I, Buxbaum JN (1992) Transthyretin Pro55, a variant associated with early-onset, aggressive, diffuse amyloidosis with cardiac and neurologic involvement. Hum Genet 89:353–356

Jacobson DR, Pastore RD, Yaghoubian R, Kane I, Gallo G, Buck FS, Buxbaum JN,(1997) Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. N Engl J Med 336:466–473

Jacobson D, Tagoe C, Schwartzbard A, Shah A, Koziol J, Buxbaum J (2011) Relation of clinical, echocardiographic and electrocardiographic features of cardiac amyloidosis to the presence of the transthyretin V122I allele in older African-American men. Am J Cardiol 108:440–444

Jarrett JT, Lansbury PT Jr (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73:1055–1058

Jiang X, Smith CS, Petrassi HM, Hammarström P, White JT, Sacchettini JC, Kelly JW (2001) An engineered transthyretin monomer that is nonamyloidogenic, unless it is partially denatured. Biochemistry 40:11442–11452

Jinno Y, Matsumoto T, Kamel T, Kondoh T, Maeda S, Araki S, Shimada K, Niikawa N (1986) Localization of the human prealbumin gene to 18p11.1-q12.3 by gene dose effect study of southern blot hybridization. Jpn J Hum Genet 31:243–248

Johnson SM, Wiseman RL, Sekijima Y, Green NS, Adamski-Werner SL, Kelly JW (2005) Native state kinetic stabilization as a strategy to ameliorate protein misfolding diseases: a focus on the transthyretin amyloidoses. Acc Chem Res 38:911–921

Johnson SM, Connelly S, Wilson IA, Kelly JW (2008a) Biochemical and structural evaluation of highly selective 2-arylbenzoxazole-based transthyretin amyloidogenesis inhibitors. J Med Chem 51:260–270

Johnson SM, Connelly S, Wilson IA, Kelly JW (2008b) Toward optimization of the linker sub-structure common to transthyretin amyloidogenesis inhibitors using biochemical and structural studies. J Med Chem 51:6348–6358

Johnson SM, Connelly S, Wilson IA, Kelly JW (2009) Toward optimization of the second aryl substructure common to transthyretin amyloidogenesis inhibitors using biochemical and structural studies. J Med Chem 52:1115–1125

Jono H, Anno T, Motoyama K, Misumi Y, Tasaki M, Toshinori Oshima T, Mori Y, Mizuguchi M, Ueda M, Shono M, Obayashi K, Arima H, Ando Y (2011) Cyclodextrin, a novel therapeutic tool for suppressing amyloidogenic transthyretin misfolding in transthyretin-related amyloidosis. Biochem J 437:35–42

Kanda Y, Goodman DS, Canfield RE, Morgans FJ (1974) The amino acid sequence of human plasma prealbumin. Biol Chem 249:6796–6806

Kassem NA, Deane R, Segal MB, Preston JE (2006) Role of transthyretin in thyroxine transfer from cerebrospinal fluid to brain and choroid plexus. Am J Physiol Regul Integr Comp Physiol 291:R1310-R1315

Kawaji T, Ando Y, Ando E, Sandgren O, Suhr OB, Tanihara H (2010a) Transthyretin-related vitreous amyloidosis in different endemic areas. Amyloid 17:105–108

Kawaji T, Ando Y, Hara R, Tanihara H (2010b) Novel therapy for transthyretin—related ocular amyloidosis. A pilot study of retinal laser photocoagulation. Ophthalmology 117:552–555

Keetch CA, Bromely EH, McCammon MG, Wang N, Christodoulou J, Robinson CV (2005) L55P transthyretin accelerates subunit exchange and leads to rapid formation of hybrid tetramers. J Biol Chem 280:41667–41674

Kelly JW (1998) The alternative conformations of amyloidogenic proteins and their multi-step assembly pathways. Curr Opin Struct Biol 8:101–106

Klabunde T, Petrassi HM, Oza VB, Raman P, Kelly JW, Sacchettin JC (2000) Rational design of potent human transthyretin amyloid disease inhibitors. Nat Struct Biol 7:312–321

Kohno K, Palha JA, Miyakawa K, Saraiva MJM, Ito S, Mabuchi T, Blaner WS, Iijima H, Tsukahara S, Episkopou V, Gottesman ME, Shimada K, Takahashi K, Yamamura K, Maeda S (1997) Analysis of amyloid deposition in a transgenic mouse model of homozygous familial amyloidotic polyneuropathy. Am J Pathol 150:1497–1508

Koike H, Misu K, Ikeda S, Ando Y, Nakazato M, Ando E, Yamamoto M, Hattori N, Sobue G, For the Study Group for Hereditary Neuropathy in Japan (2002) Type I (Transthyretin Met30) familial amyloid polyneuropathy in Japan. Early- vs late-onset form. Arch Neurol 59:1771–1776

Koike H, Misu K, Sugiura M, Iijima M, Mori K, Yamamoto M, Hattori N, Mukai E, Ando Y, Ikeda S, Sobue G (2004) Pathology of early- vs late-onset TTR Met30 familial amyloid polyneuropathy. Neurology 63:129–138

Koike H, Hashimoto R, Tomita M, Kawagashira Y, Iijima M, Tanaka F, Sobue G (2011) Diagnosis of sporadic transthyretin Val30Met familial amyloid polyneuropathy: a practical analysis. Amyloid 18:53–62

Kugimiya T, Jono H, Saito S, Maruyama T, Kadowaki D, Misumi Y, Hoshii Y, Tasaki M, Su Y, Ueda M, Obayashi K, Shono M, Otagiri M, Ando Y (2011) Loss of functional albumin triggers acceleration of transthyretin amyloid fibril formation in familial amyloidotic polyneuropathy. Lab Invest 91:1219–1228

Lai Z, Colon W, Kelly JW (1996) The acid-mediated denaturation pathway of transthyretin yields a conformational intermediate that can self-assemble into amyloid. Biochemistry 35:6470–6482

Lai Z, McCulloch J, Lashuel HA, Kelly JW (1997) Guanidine hydrochloride-induced denaturation and refolding of transthyretin exhibits a marked hysteresis: equilibria with high kinetic barriers. Biochemistry 36:10230–10239

Lander HM, Tauras JM, Ogiste JS, Hori O, Moss RA, Schmidt AM (1997) Activation of the receptor for advanced glycation end products triggers a p21(ras)-dependent mitogen-activated protein kinase pathway regulated by oxidant stress. J Biol Chem 272:17810–17814

Larsen PR (1972) Salicylate-induced increases in free triiodothyronine in human serum. Evidence of inhibition of triiodothyronine binding to thyroxine-binding prealbumin. J Clin Invest 51:1125–1134

Lashuel HA, Lai Z, Kelly JW (1998) Characterization of the transthyretin acid denaturation pathways by analytical ultracentrifugation: implications for wild-type, V30M, and L55P amyloid fibril formation. Biochemistry 37:17851–17864

Lashuel HA, Wurth C, Woo L, Kelly JW (1999) The most pathogenic transthyretin variant, L55P, forms amyloid fibrils under acidic conditions and protofilaments under physiological conditions. Biochemistry 38:13560–13573

Lee KW, Lee DH, Son H, Kim YS, Park JY, Roh GS, Kim HJ, Kang SS, Cho GJ, Choi WS (2009) Clusterin regulates transthyretin amyloidosis. Biochem Biophys Res Commun 388:256–260

Lei M, Yanga M, Huo S (2004) Intrinsic versus mutation dependent instability/flexibility: a comparative analysis of the structure and dynamics of wild-type transthyretin and its pathogenic variants. J Struct Biol 148:153–168

Li X, Masliah E, Reixach N, Buxbaum JN (2011) Neuronal production of transthyretin in human and murine Alzheimer's disease: is it protective? J Neurosci 31:12483–12490

Liepnieks JJ, Zhang LQ, Benson MD (2010) Progression of transthyretin amyloid neuropathy after liver transplantation. Neurology 75:324–327

Liu L, Murphy RM (2006) Kinetics of inhibition of beta-amyloid aggregation by transthyretin. Biochemistry 45:15702–15709

Lyon AW, Narindrasorasak S, Young ID, Anastassiades T, Couchman JR, McCarthy KJ, Kisilevsky R (1991) Co-deposition of basement membrane components during the induction of murine splenic AA amyloid. Lab Invest 64:785–90

Macedo B, Batista AR, Ferreira N, Almeida MR, Saraiva MJ (2008) Anti-apoptotic reatment reduces transthyretin deposition in a transgenic mouse model of familial amyloidotic polyneuropathy. Biochim Biophys Acta 1782:517–22

Macedo B, Magalhães J, Batista AR, Maria João Saraiva MJ (2010) Carvedilol treatment reduces transthyretin deposition in a familial amyloidotic polyneuropathy mouse model. Pharmacol Res 62:514–522

Magalhães J, Santos SD, Saraiva MJ (2010) αB-crystallin (HspB5) in familial amyloidotic polyneuropathy. Int J Exp Pathol 91:515–521

Makover A, Moriwaki H, Ramakrishnan R, Saraiva MJM, Blaner WS, Goodman DS (1988) Plasma transthyretin. Tissue sites of degradation and turnover in the rat. J Biol Chem 263:8598–8603

Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, Beyreuther K (1985) Amyloid plaque core protein in Alzheimer disease and Down syndrome. Proc Natl Acad Sci USA 82:4245–4249

Mathew V, Olson LJ, Gertz MA, Hayes DL (1997) Symptomatic conduction system disease in cardiac amyloidosis. Am J Cardiol 80:1491–1492

Matsubara K, Mizuguchi M, Igarashi K, Shinohara Y, Takeuchi M, Matsuura A, Saitoh T, Mori Y, Shinoda H, Kawano K (2005) Dimeric transthyretin variant assembles into spherical neurotoxins. Biochemistry 44:3280–3288

McCutchen SL, Colon W, Kelly LW (1993) Transthyretin mutation Leu55-Pro significantly alters tetramer stability and increases amyloidgenicity. Biochemistry 32:12119–12127

Miller SR, Sekijima Y, Kelly JW (2004) Native state stabilization by NSAIDs inhibits transthyretin amyloidogenesis from the most common familial disease variants. Lab Invest 84:545–552

Miroy GJ, Lai Z, Lashuel HA, Peterson SA, Strang C, Kelly JW (1996) Inhibiting transthyretin amyloid fibril formation via protein stabilization. Proc Natl Acad Sci USA 93:15051–15056

Misu K, Hattori N, Nagamatsu M, Ikeda S, Ando Y, Nakazato M, Takei Y, Hanyu N, Usui Y, Tanaka F, Harada T, Inukai A, Hashizume Y, Sobue G (1999) Late-onset familial amyloid polyneuropathy type I (transthyretin Met30-associated familial amyloid polyneuropathy) unrelated to endemic focus in Japan. Clinicopathological and genetic features. Brain 122:1951–1962

Misumi Y, Ando Y, Ueda M, Obayashi K, Jono H, Su Y, Yamashita T, Uchino M (2009) Chain reaction of amyloid fibril formation with induction of basement membrane in familial amyloidotic polyneuropathy. J Pathol 219:481–490

Misumi Y, Ueda M, Obayashi K, Jono H, Su Y, Yamashita T, Ohshima T, Ando Y, Uchino M (2012) Relationship between amyloid deposition and intracellular structural changes in familial amyloidotic polyneuropathy. Hum Pathol 43:96–104

Mita S, Maeda S, Shimada K, Araki S (1984) Cloning and sequence analysis of cDNA for human prealbumin. Biochem Biophys Res Commun 124:558–564

Mitsuhashi S, Yazaki M, Tokuda T, Yamamoto K, Ikeda S (2004) MRI analysis on a patient with the V30M mutation is characteristic of leptomeningeal amyloid. Amyloid 11:265–267

Miyata M, Sato T, Mizuguchi M, Nakamura T, Ikemizu S, Nabeshima Y, Susuki S, Suwa Y, Morioka H, Ando Y, Suico MA, Shuto T, Koga T, Yamagata Y, Kai H (2010) Role of the glutamic acid 54 residue in transthyretin stability and thyroxine binding. Biochemistry 49:114–123

Monteiro FA, Sousa MM, Cardoso I, Barbas do Amaral J, Guimarães A, Saraiva MJ (2006) Activation of ERK1/2 MAP kinases in familial amyloidotic polyneuropathy. J Neurochem 97:151–161

Murakami T, Yi S, Maeda S, Tashiro F, Yamamura K, Takahashi K, Shimada K, Araki S (1992) Effect of serum amyloid P component level on transthyretin-derived amyloid deposition in a transgenic mouse model of familial amyloidotic polyneuropathy. Am J Pathol 141:451–456

Murakami T, Ohsawa Y, Sunada Y (2008) The transthyretin gene is expressed in human and rodent dorsal root ganglia. Neurosci Lett 436:335–339

Murakami T, Ohsawa Y, Zhenghua L, Yamamura K, Sunada Y (2010) The transthyretin gene is expressed in Schwann cells of peripheral nerves. Brain Res 1348:222–225

Nagasaka T, Togashi S, Watanabe H, Iida H, Nagasaka K, Nakamura Y, Miwa M, Kobayashi F, Shindo K, Shiozawa Z (2009) Clinical and histopathological features of progressive-type familial amyloidotic polyneuropathy with TTR Lys54. J Neurol Sci 276:88–94

Nagata Y, Tashiro F, Yi S, Murakami T, Maeda S, Takahashi K, Shimada K, Okamura H, Yamamura K (1995) A 6-kb upstream region of the human transthyretin gene can direct developmental, tissue-specific, and quantitatively normal expression in transgenic mouse. J Biochem 117:169–175

Nakamura M, Ando Y (2004) Applications of gene therapy for familial amyloidotic polyneuropathy. Expert Opin Biol Th 4:1621–1627

Nakamura Y, Yutani C, Nakazato M, Date Y, Baba T, Goto Y (1999) A case of hereditary amyloidosis transthyretin variant Met30 with amyloid cardiomyopathy, less polyneuropathy, and the presence of giant cells. Pathol Int 49:898–902

Nakamura M, Ando Y, Nagahara S, Sano A, Ochiya T, Maeda S, Kawaji T, Ogawa M, Hirata A, Terazaki H, Haraoka K, Tanihara H, Ueda M, Uchino M, Yamamura K (2004) Targeted conversion of the transthyretin gene in vitro and in vivo. Gene Ther 11:838–846

Nakanishi T, Yoshioka M, Moriuchi K, Yamamoto D, Tsuji M, Takubo T (2010) S-sulfonation of transthyretin is an important trigger step in the formation of transthyretin-related amyloid fibril. Biochim Biophys Acta 1804:1449–1456

Nakazato M, Tanaka M, Matsukura S, Kangawa K, Matsuo H (1989) Quantitative analysis of amyloid fibril protein in systemic organs of patients with familial amyloidotic polyneuropathy. J Neurol Sci 89:235–242

Nakazato M, Shiomi K, Miyazato M, Matsukura S (1992) Type I familial amyloidotic polyneuropathy in Japan. Intern Med 31:1335–1338

Nichols WC, Padilla L-M, Benson MD (1989a) Prenatal detection of a gene for hereditary amyloidosis. Am J Med Genet 34:520–524

Nichols WC, Liepnieks JJ, McKusick VA, Benson MD (1989b) Direct sequencing of the gene for Maryland/German familial amyloidotic polyneuropathy type II and genotyping by allele-specific enzymatic amplification. Genomics 5:535–540

Noguchi H, Ohta M, Wakasugi S, Noguchi K, Nakamura H, Nakamura O, Miyakawa K, Takeya M, Suzuki M, Nagata N, Urano T, Oon T, Yamamura K (2002) Effect of the intestinal flora on amyloid deposition in a transgenic mouse model of familial amyloidotic polyneuropathy. Exp Anim 51:309–316

Ohya Y, Jono, H, Nakamura M, Hayashida S, Ueda M, Obayashi K, Misumi S, Asonuma K, Ando Y, Inomata Y (2010) Effect of recipient-derived cells on the progression of familial amyloidotic polyneuropathy after liver transplantation: a retrospective study. Ann Clin Biochem 47:529–534

Olofsson B-O, Grankvist K, Boman K, Forsberg K, Lafvas I, Lithner F (1989) Assessment of thyroid anad adrenal function in patients with familial amyloidotic polyneuropathy. J Intern Med 225:337–341

Olofsson B-O, Grankvist K, Olsson T, Boman K, Forsberg K, Lafvas I, Lithner F (1991) Assessment of hypothalamic-pituitary function in patients with familial amyloidotic polyneuropathy. J Intern Med 229:55–59

Olofsson B-O, Backman C, Karp K, Suhr OB (2002) Progression of cardiomyopathy after liver transplantation in patients with familial amyloidotic polyneuropathy, Portuguese type. Transplantation 73:745–751

Palmieri L de C, Lima LM, Freire JB, Bleicher L, Polikarpov I, Almeida FC, Foguel D (2010) Novel Zn2+ -binding sites in human transthyretin: implications for amyloidogenesis and retinol-binding protein recognition. J Biol Chem 285:31731–31741

Pepys MB, Rademachero TW, Amatayakul-Chantler S, Williams P, Noble GE, Hutchinson WL, Hawkins PN, Nelson SR, Gallimore JR, Herbert J, Hutton T, Dwek RA (1994) Human serum amyloid P component is an invariant constituent of amyloid deposits and has a uniquely homogeneous glycostructure. Proc Nat Acad Sci USA 91:5602–5606

Perugini E, Guidalotti PL, Salvi F, Cooke RMT, Pettinato C, Riva L, Leone O, Farsad M, Ciliberti P, Bacchi-Reggiani L, Fallani F, Branzi A, Rapezzi C (2005) Noninvasive etiologic diagnosis of cardiac amyloidosis using 99 m Tc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. J Am Coll Cardiol 46:1076–1084

Perugini E, Rappezi C, Piva T, Leone O, Bacchi-Reggiani L, Riva L, Salvi F, Lovato L, Branzi A, Fattori R (2006) Non-invasive evaluation of the myocardial substrate of cardiac amyloidosis by gadolinium cardiac magnetic resonance. Heart 92:343–349

Petersen RB, Goren H, Cohen M, Richardson SL, Tresser N, Lynn A, Gali M, Estes M, Gambetti P (1997) Transthyretin amyloidosis: a new mutation associated with dementia. Ann Neurol 41:307–313

Pfeffer BA, Becerra SP, Borst DE, Wong P (2004) Expression of transthyretin and retinol binding protein mRNAs and secretion of transthyretin by cultured monkey retinal pigment epithelium. Mol Vis 10:23–30

Planté-Bordeneuve V, Ferreira A, Lalu T, Zaros C, Lacroix C, Adams D, Said G (2007) Diagnostic pitfalls in sporadic transthyretin familial amyloid polyneuropathy (TTR-FAP). Neurology 69:693–698

Pomfret E, Lewis WD, Jenkins R, Bergethon P, Dubrey Simon W, Reisinger J, Falk R, Skinner M,(1998) Effect of orthotopic liver transplantation on the progression of familial amyloidotic polyneuropathy. Transplantation 65:918–925

Powers ET, Powers DL (2008) Mechanisms of protein fibril formation: nucleated polymerization with competing off-pathway aggregation. Biophys J 94:379–391

Pras M, Prelli F, Franklin EC, Frangione B (1983) Primary structure of an amyloid prealbumin variant in familial polyneuropathy of Jewish origin. Proc Natl Acad Sci USA 80:539–542

Prashantha DK, Taly AB, Sinha S, Yasha TC, Gayathri N, Kovur J, Vijayan J (2010) Familial amyloidotic polyneuropathy with muscle, vitreous, leptomeningeal, and cardiac involvement: Phenotypic, pathological, and MRI description. Ann Indian Acad Neurol 13:142–144

Prelli F, Pras M, Frangione B (1985) The primary structure of human tissue amyloid P component from a patient with primary idiopathic amyloidosis. J Biol Chem 280:12895–12898

Pröpsting MJ, Kubicka S, Genschel J, Manns MP, Lochs H, Schmidt HH-J (2000) Inhibition of transthyretin-met30 expression using inosine15.1-hammerhead ribozymes in cell culture. Biochem Biophys Res Comm 279:970–973

Quintas A, Saraiva MJ, Brito RM (1997) The amyloidogenic potential of transthyretin variants correlates with their tendency to aggregate in solution. FEBS Lett 418:297–300

Quintas A, Saraiva MJ, Brito RM (1999) The tetrameric protein transthyretin dissociates to a non-native monomer in solution. A novel model for amyloidogenesis. J Biol Chem 74:32943–32949

Quintas A, Vaz DC, Cardoso I, Saraiva MJM, Brito RMM (2001) Tetramer dissociation and monomer partial unfolding precedes protofibril formation in amyloidogenic transthyretin variants. J Biol Chem 276:27207–27213

Raz A, Goodman DS (1969) The interaction of thyroxine with human plasma prealbumin and with the prealbumin-retinal-binding protein complex. J Biol Chem 244:3230–3237

Redondo C, Damas AM, Olofsson A, Lundgren E, Saraiva MJ (2000a) Search for intermediate structures in transthyretin fibrillogenesis: soluble tetrameric Tyr78Phe TTR expresses a specific epitope present only in amyloid fibrils. J Mol Biol 304:461–470

Redondo C, Damas AM, Saraiva MJ (2000b) Designing transthyretin mutants affecting tetrameric structure: implications in amyloidogenicity. Biochem J 348(Pt1):167–172

Reixach N, Deechongkit S, Jiang X, Kelly JW, Buxbaum JN (2004) Tissue damage in the amyloidoses: transthyretin monomers and non-native oligomers are the major cytotoxic species in tissue culture. Proc Natl Acad Sci USA 101:2817–2822

Reixach N, Adamski-Werner SL, Kelly JW, Koziol J, Buxbaum JN (2006) Cell based screening of inhibitors of transthyretin aggregation. Biochem Biophys Res Commun 348:889–897

Reixach N, Foss TR, Santelli E, Pascual J, Kelly JW, Buxbaum JN (2008) Human-murine transthyretin heterotetramers are kinetically stable and non-amyloidogenic. A lesson in the generation of transgenic models of diseases involving oligomeric proteins. J Biol Chem 283:2098–2107

Riboldi G, Del Bo R, Ranieri M, Magri F, Sciacco M, Moggio M, Bresolin N, Corti S, Comi GP (2011) Tyr78Phe transthyretin mutation with predominant motor neuropathy as the initial presentation. Case Rep Neurol 3:62–68

Richardson SJ, Lemkine GF, Alfama G, Hassani Z, Demeneix BA (2007) Cell division and apoptosis in the adult neural stem cell niche are differentially affected in transthyretin null mice. Neurosci Lett 421:234–238

Said G (2003) Familial amyloid polyneuropathy: mechanisms leading to nerve degeneration. Amyloid 10(Suppl. 1):7–12

Said G, Ropert A, Faux N (1984) Length-dependent degeneration of fibers in Portuguese amyloid poly neuropathy: a clinicopathologic study. Neurology 34:1025–1032

Sakashita N, Ando Y, Jinnouchi K, Yoshimatsu M, Terazaki H, Obayashi K, Takeya M (2001) Familial amyloidotic polyneuropathy (ATTR Val30Met) with widespread cerebral amyloid angiopathy and lethal cerebral hemorrhage. Pathol Int 51:476–480

Saraiva MJM (2001) Transthyretin mutations in hyperthyroxinemia and amyloid diseases. Hum Mutat 17:493–503

Saraiva MJ, Costa PP, Goodman DS (1983) Studies on plasma transthyretin (prealbumin) in familial amyloidotic polyneuropathy, Portuguese type. J Lab Clin Med 102:590–603

Saraiva MJ, Birken S, Costa PP, Goodman DS (1984) Amyloid fibril protein in familial amyloidotic polyneuropathy, Portuguese type. Definition of molecular abnormality in transthyretin (prealbumin). J Clin Invest 74:104–119

Saraiva MJ, Sherman W, Marboe C, Figueira A, Costa P, de Freitas AF, Gawinowicz MA (1990) Cardiac amyloidosis: report of a patient heterozygous for the transthyretin isoleucine 122 variant. Scand J Immunol 32:341–346

Sasaki H, Yoshioka N, Takagi Y, Sakaki Y (1985) Structure of the chromosomal gene for human serum prealbumin. Gene 37:191–197

Sato T, Ando Y, Susuki S, Mikami F, Ikemizu S, Nakamura M, Suhr O, Anraku M, Kai T, Suico MA, Shuto T, Mizuguchi M, Yamagata Y, Kai H (2006) Chromium (III) ion and thyroxine cooperate to stabilize the transthyretin tetramer and suppress in vitro amyloid fibril formation. FEBS Lett 580:491–496

Sawabe M, Hamamatsu A, Ito T, Arai T, Ishikawa K, Chida K, Izumiyama N, Honma N, Takubo K, Nakazato M (2003) Early pathogenesis of cardiac amyloid deposition in senile systemic

amyloidosis: close relationship between amyloid deposits and the basement membranes of myocardial cells. Virchows Arch 442:252–257

Schneider F, Hammarström P, Kelly JW (2001) Transthyretin slowly exchanges subunits under physiological conditions: a convenient chromatographic method to study subunit exchange in oligomeric proteins. Protein Sci 10:1606–1613

Schormann N, Murrell JR, Benson MD (1998) Tertiary structures of amyloidogenic and non-amyloidogenic transthyretin variants: new model for amyloid fibril formation. Amyloid 5:175–187

Schreiber G, Aldred AR, Jaworowski A, Nilsson C, Achen MG, Segal MB (1990) Thyroxine transport from blood to brain via transthyretin synthesis in choroid plexus. Am J Physiol 258:R338–R345

Schwarzman AL, Tsiper M, Wente H, Wang A, Vitek MP, Vasiliev V, Goldgaber D (2004) Amyloidogenic and anti-amyloidogenic properties of recombinant transthyretin variants. Amyloid 11:1–9

Sebastião MP, Saraiva NJ, Damas AM (1998) The crystal structure of amyloidogenic Leu55Pro transthyretin variant reveals a possible pathway for transthyretin polymerization into amyloid fibrils. J Biol Chem 273:24715–24722

Sebastião MP, Merlini G, Saraiva MJ, Damas AM (2000) The molecular interaction of 4'-iodo-4'-deoxydoxorubicin with Leu-55Pro transthyretin 'amyloid-like' oligomer leading to disaggregation. Biochem J 351:273–279

Sekijima Y, Hammarström P, Matsumura M, Shimizu Y, Iwata M, Tokuda T, Ikeda S, Kelly JW (2003) Energetic characteristics of the new transthyretin variant A25T may explain its atypical central nervous system pathology. Lab Invest 83:409–417

Sekijima Y, Wiseman RL, Balch WE, Kelly JW (2005) The biological and chemical basis for tissue-selective amyloid disease. Cell 121:73–85

Sekijima Y, Kelly JW, Ikeda S (2008) Pathogenesis of and therapeutic strategies to ameliorate the transthyretin amyloidoses. Curr Pharm Des 14:3219–3230

Editors

Sekijima Y, Yoshida K, Tokuda T, Ikeda S (2001) (Last updated 2012). Familial transthyretin amyloidosis. In: Pagon RA, Bird TD, Dolan CR et al (eds) GeneReviews (Internet). University of Washington, Seattle, 1993

Serga AA, Altenbach C, Gingery M, Hubbell WL, Yeates TO (2001) Identification of a subunit interface in transthyretin amyloid fibrils: evidence for self-assembly from oligomeric building blocks. Biochemistry 40:9089–9096

Serot JM, Christmann D, Dubost T, Couturier M (1997) Cerebrospinal fluid transthyretin: aging and late onset Alzheimer's disease. J Neurol Neurosurg Psychiatry 63:506–508

Serpell LC, Sunde M, Fraser PE, Luther PK, Morris EP, Sangren O, Lundgren E, Blake CCF (1995) Examination of the structure of the transthyretin amyloid fibril by image reconstruction from electron micrographs. J Mol Biol 254:113–118

Shimojima Y, Morita H, Kobayashi S, Talei Y, Ikeda S (2008) Ten-year follow-up of peripheral nerve function in patients with familial amyloid polyneuropathy after liver transplantation. J Neurol 255:1220–1225

Shnyrov VL, Villar E, Zhadan GG, Sanchez-Ruiz JM, Quintas A, Saraiva MJ, Brito RM (2000) Comparative calorimetric study of non-amyloidogenic and amyloidogenic variants of the homotetrameric protein transthyretin. Biophys Chem 88:61–67

Simões CJV, Mukherjee T, Brito RMM, Jackson RM (2010) Toward the discovery of functional transthyretin amyloid inhibitors: application of virtual screening methods. J Chem Inf Model 50:1806–1820

Smeland S, Kolset SO, Lyon M, Kaare R, Norum KR, Blomhoff R (1997) Binding of perlecan to transthyretin in vitro. Biochem J 326:829–836

Soares ML, Coelho T, Sousa A, Batalov S, Conceição I, Sales-Luís ML, Ritchie MD, Williams SM, Nievergelt CM, Schork NJ, Saraiva MJ, Buxbaum JN (2005) Susceptibility and modifier genes in Portuguese transthyretin V30M amyloid polyneuropathy: complexity in a single-gene disease. Hum Mol Genet 14:543–553

Sobue G, Nakao N, Murakami K, Yasuda T, Sahashi K, Mitsuma T, Sasaki H, Sakaki Y, Takahashi A (1990) Type I familial amyloid polyneuropathy. A pathological study of the peripheral nervous system. Brain 113:903–919

Sousa MM, Saraiva MJ (2003) Neurodegeneration in familial amyloid polyneuropathy: from pathology to molecular signaling. Prog Neurobiol 71:385–400

Sousa MM, Saraiva MJ (2008) Transthyretin is not expressed by dorsal root ganglia cells. Exp Neurol 214:362–365

Sousa MM, Cardoso I, Fernandes R, Guimaraes A, Saraiva MJ (2001a) Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy: evidence for toxicity of non-fibrillar aggregates. Am J Pathol 159:1993–2000

Sousa MM, Du Yan S, Fernandes R, Guimarães A, Stern D, Saraiva MJ (2001b) Familial amyloid polyneuropathy: receptor for advanced glycation endproducts-dependent triggering of neuronal inflammatory and apoptotic pathways. J Neurosci 21:7576–7586

Sousa MM, Fernandes R, Palha JA, Taboada A, Vieira P, Saraiva MJ (2002) Evidence for early cytotoxic aggregates in transgenic mice for human transthyretin Leu55Pro. Am J Pathol 161:1935–1948

Sousa MM, Ferrao J, Fernandes R, Guimaraes A, Geraldes JB, Perdigoto R, Tome L, Mota O, Negrao L, Furtado AL, Saraiva MJ (2004) Deposition and passage of transthyretin through the blood-nerve barrier in recipients of familial amyloid polyneuropathy livers. Lab Invest 84:865–873

Sousa MM, Barbas do Amaral J, Guimarães A, Saraiva MJ (2005) Up-regulation of the extracellular matrix remodeling genes, biglycan, neutrophil gelatinase-associated lipocalin and matrix metalloproteinase-9 in familial amyloid polyneuropathy. FASEB J 19:124–126

Skare J, Yazici H, Erken E, Debe H, Cohen A, Milunsky A, Skinner M (1990) Homozygosity for the met30 transthyretin gene in a Turkish kindred with familial amyloidotic polyneuropathy. Hum Genet 86:89–90

Snow CM, Senior A, Gerace L (1987) Monoclonal antibodies identify a group of nuclear pore complex glycoproteins. J Cell Biol 104:1143–1156

Stangou AJ, Hawkins PN (2004) Liver transplantation in transthyretin-related familial amyloid polyneuropathy. Curr Opin Neurol 17:615–620

Stangou AJ, Heaton ND, Hawkins PN (2005) Transmission of systemic transthyretin amyloidosis by means of domino liver transplantation. N Engl J Med 352:2356

Steinrauf LK, Cao Y, Hamilton J, Murrell J, Liepnieks JJ, Benson MD (1991) Preparation and crystallization of human transthyretin (prealbumin) variants. Biochem Biophys Res Commun 179:804–809

Stockigt JR, Lim CF, Barlow JW, Wynne KN, Mohr VS, Topliss DJ, Hamblin PS, Sabto J (1985) Interaction of furosemide with serum thyroxine binding sites: in vivo and in vitro studies and comparison with other inhibitors. J Clin Endocrinol Metab 60:1025–1031

Subasinghe S, Unabia S, Barrow CJ, Mok SS, Aguilar MI, Small DH (2003) Cholesterol is necessary both for the toxic effect of Abeta peptides on vascular smooth muscle cells and for Abeta binding to vascular smooth muscle cell membranes. J Neurochem 84:471–479

Suhr OB, Ericzon BG, Friman S (2002) Long-term follow-up of survival of liver transplant recipients with familial amyloid polyneuropathy (Portuguese type). Liver Transplant 8:787–794

Suk JY, Zhang F, Balch WE, Linhardt RJ, Kelly JW (2006) Heparin accelerates gelsolin amyloidogenesis. Biochemistry 45:2234–2242

Takahashi K, Yi S, Kimura Y, Araki S (1991) Familial amyloidotic polyneuropathy type 1 in Ku-
     mamoto, Japan: a clinicopathologic, histochemical, immunohistochemical, and ultrastructural
     study. Hum Pathol 21:519–527
Takaoka Y, Tashiro F, Yi S, Maeda S, Shimada K, Takahashi K, Sakaki Y, Yamamura K (1997) Com-
     parison of amyloid deposition in two lines of transgenic mouse that model familial amyloidotic
     polyneuropathy, type I. Transgenic Res 6:261–269
Takei Y, Gono T, Yazaki M, Ikeda S, Ikegami T, Hashikura Y, Miyagawa S, Hoshii Y (2007)
     Transthyretin-derived amyloid deposition on the gastric mucosa in domino recipients of familial
     amyloid polyneuropathy liver. Liver Transplant 13:215–218
Tanaka K, Yamada T, Ohyagi Y, Asahara H, Horiuchi I, Kira J (2001) Suppression of transthyretin
     expression by ribozymes: a possible therapy for familial amyloidotic polyneuropathy. J Neurol
     Sci 183:79–84
Tanskanen M, Peuralinna T, Polvikoski T, Notkola I-L, Sulkava R, Hardy J, Singleton A, Kiuru-
     Enari S, Paetau A, Tienari PJ, Myllykangas L (2008) Senile systemic amyloidosis affects 25 %
     of the very aged and associates with genetic variation in *alpha2-macroglobulin* and *tau*: a
     population-based autopsy study. Ann Med 40:232–239
Tawara S, Nakazato M, Kangawa K, Matsuo H, Araki S (1983) Identification of amyloid prealbumin
     variant in familial amyloidotic polyneuropathy (Japanese type). Biochem Biophys Res Commun
     116:880–888
Teng MH, Yin JY, Vidal R, Ghiso J, Kumar A, Rabenou R, Shah A, Jacobson DR, Tagoe C,
     Gallo G, Buxbaum J (2001) Amyloid and nonfibrillar deposits in mice transgenic for wild-type
     human transthyretin: a possible model for senile systemic amyloidosis. Lab Invest 81:385–396
Terry CJ, Damas AM, Oliveira P, Saraiva MJM, Alves IL, Costa PP, Matias PM, Sakaki Y, Blake
     CCF (1993) Structure of Met30 variant of transthyretin and its amyloidogenic implications.
     EMBO J 12:735–741
Thylén C, Wahlqvist J, Haettner E, Sandgren O, Holmgren G, Lundgren E (1993) Modifications
     of transthyretin in amyloid fibrils: analysis of amyloid from homozygous and heterozygous
     individuals with the Met30 mutation. EMBO J 12:743–748
Togashi S, Watanabe H, Nagasaka T, Shindo K, Shiozawa Z, Maeda S, Tawata M, Onaya T (1999)
     An aggressive familial amyloidotic polyneuropathy caused by a new variant transthyretin Lys54.
     Neurology 53:637–639
Tojo K, Sekijima Y, Kelly JW, Ikeda S (2006) Diflunisal stabilizes familial amyloid polyneuropathy-
     associated transthyretin variant tetramers in serum against dissociation required for amyloido-
     genesis. Neurosci Res 56:441–449
Toyooka K, Fujimura H, Ueno S, Yoshikawa H, Kaido M, Nishimura T, Yorifuji S, Yanagihara T
     (1995) Familial amyloid polyneuropathy associated with transthyretin Gly42 mutation: a quan-
     titative light and electron microscopic study of the peripheral nervous system. Acta Neuropathol
     90:516–525
Tsuzuki T, Mita S, Maeda S, Araki S, Shimada K (1985) Structure of the human prealbumin gene.
     J Biol Chem 260:12224–12227
Ueda M, Misumi Y, Mizuguchi M, Nakamura M, Yamashita T, Sekijima Y, Ota K, Shinriki S,
     Jono H, Ikeda S, Suhr OB, Ando Y (2009) SELDI-TOF mass spectrometry evaluation of variant
     transthyretins for diagnosis and pathogenesis of familial amyloidotic polyneuropathy. Clin Chem
     55:1223–1227
Ueda M, Horibata Y, Shono M, Misumi Y, Oshima T, Su Y, Tasaki M, Shinriki S, Kawahara S,
     Jono H, Obayashi K, Ogawa H, Ando Y (2011) Clinicopathological features of senile systemic
     amyloidosis: an ante- and post-mortem study. Mod Pathol 24:1533–1544 (electronical form)
Uemichi T, Murrell JR, Zeldenrust S, Benson MD (1992) A new mutant transthyretin (Arg 10)
     associated with familial amyloid polyneuropathy. J Med Genet 29:888–891
Uemichi T, Liepnieks JJ, Benson MD (1997) A trinucleotide deletion in the transthyretin gene
     (AV122) in a kindred with familial amyloidotic polyneuropathy. Neurology 48:1667–1670
Uemichi T, Uitti RJ, Koeppen AH, Donat JR, Benson MD (1999) Oculoleptomeningeal amyloidosis
     associated with a new transthyretin variant Ser64. Arch Neurol 56:1152–1155

Vital C, Vital A, Bouillot-Eimer S, Brechenmacher C, Ferrer X, Lagueny A (2004) Amyloid neuropathy: a retrospective study of 35 peripheral nerve biopsies. J Peripher Nerv Syst 9:232–241

Wakasugi S, Inomoto T, Yi S, Naito M, Uehira M, Iwanaga T, Maeda S, Araki K, Miyazaki J, Takahashi K, Shimada K, Yamamura K (1987) A transgenic mouse model of familial amyloidotic polyneuropathy. Proc Jpn Acad 63(B):344–347

Wallace MR, Naylor SL, Kluve-Beckerman B, Long GL, Mc-Donald L, Shows TB, Benson MD (1985) Localization of the human prealbumin gene to chromosome 18. Biochem Biophys Res Commun 129:753–758

Wallace MR, Dwulet FE, Conneally PM, Benson MD (1986) Biochemical and molecular genetic characteristics of a new variant prealbumin associated with hereditary amyloidosis. J Clin Invest 78:6–12

Wallace MR, Conneally PM, Benson MD (1988) A DNA test for Indiana/Swiss hereditary amyloidosis (FAP II). Am J Hum Genet 43:182–187

Westermark P, Johansson B, Natvig JB (1979) Senile cardiac amyloidosis: evidence of two different amyloid substances in the ageing heart. Scand. J Immunol 10:303–308

Westermark P, Sletten K, Johansson B, Cornwell GG 3rd (1990) Fibril in senile systemic amyloidosis is derived from normal transthyretin. Proc Natl Acad Sci USA 87:2843–2845

White JT, Kelly JW (2001) Support for the multigenic hypothesis of amyloidosis: the binding stoichiometry of retinol-binding protein, vitamin A, and thyroid hormone influences transthyretin amyloidogenicity in vitro. Proc Natl Acad Sci USA 98:13019–13024

Whitehead AS, Skinner M, Bruns GA, Costello W, Edge MD, Cohen AS, Sipe JD (1984) Cloning of human prealbumin complementary DNA. Localization of the gene to chromosome 18 and detection of a variant prealbumin allele in a family with familial amyloid polyneuropathy. Mol Biol Med 2:411–423

Wojtczak A, Cody V, Luft JR, Pangborn W (1996) Structures of human transthyretin complexed with thyroxine at 2.0 Å resolution and 3',5'-dinitro-N-acetyl-L-thyronine at 2.2 Å resolution. Acta Crystallogr D 52:758–765

Yamada M, Hatakeyama S, Tsukagoshi H (1984) Peripheral and autonomic nerve lesions in systemic amyloidosis. Three pathological types of amyloid polyneuropathy. Acta Pathol Jpn 34:1251–1266

Yamamura K, Wakasugi S, Maeda S, Inomoto T, Iwanaga T, Uehira M, Araki K, Miyazaki J, Shimada K (1987) Tissue-specific and developmental expression of human transthyretin gene in transgenic mice. Dev Genet 8:195–205

Yamashita T, Hamidi Asl K, Yazaki M, Benson MD (2005) A prospective evaluation of the transthyretin Ile122 allele frequency in an African-American population. Amyloid 12:127–130

Yan C, Costa RH, Darnell JE Jr, Chen J, Van Dyke TA (1990) Distinct positive and negative elements control the limited hepatocyte and choroid plexus expression of transthyretin in transgenic mice. EMBO J 9:869–878

Yan SD, Schmidt AM, Anderson GM, Zhang J, Brett J, Zou YS, Pinsky D, Stern D (1994) Enhanced cellular oxidant stress by the interaction of advanced glycation end products with their receptors/binding proteins. J Biol Chem 269:9889–9897

Yang M, Lei M, Huo S (2003) Why is Leu55→Pro55 transthyretin variant the most amyloidogenic: insights from molecular dynamics simulations of transthyretin monomers. Protein Sci 12:1222–1231

Yazaki M, Tokuda T, Nakamura A, Higashikata T, Koyama J, Higuchi K, Harihara Y, Baba S, Kametani F, Ikeda S (2000) Cardiac amyloid in patients with familial amyloid polyneuropathy consists of abundant wild-type transthyretin. Biochem Biophys Res Commun 274:702–706

Yi S, Takahashi K, Naito M, Tashiro F, Wakasugi S, Maeda S, Shimada K, Yamamura K, Araki S (1991) Systemic amyloidosis in transgenic mice carrying the human mutant transthyretin (Met30) gene. Pathologic similarity to human familial amyloidotic polyneuropathy, Type I. Am J Patbol 138:403–412

Yoshimatsu S, Amdo Y, Terazaki H, Sakashita N, Tada S, Yamashita T, Suga M, Uchino M, Ando M (1998) Endoscopic and pathological manifestation of the gastrointestinal tract in familial amyloidotic polyneuropathy type I (Met30). J Intern Med 243:65–72

Yoshinaga T, Nakazato M, Ikeda S, Ohnishi A (1992) Homozygosity for the transthyretin-Met30 gene in three Japanese siblings with type I familial amyloidotic polyneuropathy. Neurology 42:2045–2047

Zhao G, Li Z, Araki K, Haruna K, Yamaguchi K, Araki M, Takeya M, Ando Y, Yamamura K (2008) Inconsistency between hepatic expression and serum concentration of transthyretin in mice humanized at the transthyretin locus. Genes Cells 13:1257–1268