RARE DISEASES

Hereditary transthyretin amyloidosis: a model of medical progress for a fatal disease

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Abstract | Hereditary amyloidogenic transthyretin (ATTRv) amyloidosis with polyneuropathy (also known as familial amyloid polyneuropathy) is a condition with adult onset caused by mutation of transthyretin (TTR) and characterized by extracellular deposition of amyloid and destruction of the somatic and autonomic PNS, leading to loss of autonomy and death. This disease represents a model of the scientific and medical progress of the past 30 years. ATTRv amyloidosis is a worldwide disease with broad genetic and phenotypic heterogeneity that presents a diagnostic challenge for neurologists. The pathophysiology of the neuropathy is increasingly understood and includes instability and proteolysis of mutant TTR leading to deposition of amyloid with variable lengths of fibrils, microangiopathy and involvement of Schwann cells. Wild-type TTR is amyloidogenic in older individuals. The main symptoms are neuropathic, but the disease is systemic; neurologists should be aware of cardiac, eye and kidney involvement that justify a multidisciplinary approach to management. Infiltrative cardiomyopathy is usually latent but present in half of patients. Disease-modifying therapeutics that have been developed include liver transplantation and TTR stabilizers, both of which can slow progression of the disease and increase survival in the early stages. Most recently, gene-silencing drugs have been used to control disease in the more advanced stages and produce some degree of improvement.

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Hereditary amyloidogenic transthyretin (ATTRv) amyloidosis with polyneuropathy, also known as familial amyloid polyneuropathy (FAP), is a severe systemic disease with predominant PNS and autonomic nervous system involvement caused by mutation of the transthyretin protein, which is encoded by the *TTR* gene[1]. The condition is characterized by extracellular deposition of amyloid and by progressive and extensive destruction of the somatic and autonomic PNS (FIG. 1) that leads to loss of autonomy and death.

Mutant forms of other proteins, such as gelsolin[2], apolipoprotein A1 (REF.[3]), β2-microglobulin[4] and prion protein[5], can also cause amyloid neuropathy with distinct characteristics (TABLE 1). Although patients often present with neuropathy and the neurologist is the primary specialist involved in the initial management of patients, these conditions are systemic, and organ involvement is variable. Indeed, the Nomenclature Committee of the International Society of Amyloidosis recommends use of a general designation for all forms of extracellular amyloidosis (for example, amyloidogenic TTR (ATTRv) amyloidosis and amyloidogenic gelsolin amyloidosis), emphasizing the systemic involvement of these conditions. Here, we focus on ATTRv amyloidosis because this condition represents a model of the scientific and medical progress of the past 30 years.

ATTRv amyloidosis was first described (as FAP) in northern Portugal[6] and subsequently in Japan[7] and Sweden[8]. Discovery of causal mutations and wider application of advances in genetic tests have enabled identification of other clusters of the disease. Extensive genetic heterogeneity of the condition and sporadic cases are now recognized[9]. Increasing knowledge of the pathogenic mechanisms of ATTRv amyloidosis has led to the development of several disease-modifying therapies, including liver transplantation[10], kinetic TTR stabilizers[11] and gene-silencing drugs.

In this Review, we discuss the latest insights into various aspects of ATTRv amyloidosis and its management, including the latest epidemiological data, the phenotypic heterogeneity, the genetic heterogeneity and how this influences the age of onset, the pathophysiology of the disease and the success of phase III studies of gene-silencing therapies.

Epidemiology

Until 1990, ATTRv amyloidosis was considered to be a rare disease that was endemic to northern Portugal[12], northern Sweden[8] and two regions of Japan[13] and that had a prevalence in these regions of 1–10 in 10,000

Key points

- Hereditary amyloidogenic transthyretin (ATTRv) amyloidosis is an autosomal dominant, adult-onset systemic disease that usually presents as a progressive peripheral neuropathy and is caused by point mutations in the gene that encodes transthyretin (TTR).
- ATTRv amyloidosis was initially considered to be endemic to certain regions but is now known to occur worldwide; there are many variants of the *TTR* gene, which creates large genetic and phenotypic heterogeneity.
- Dissociation of mutant TTR homotetramers, disruption of the blood–nerve barrier and misfolding and aggregation of TTR that causes endoneurial toxicity are major events in the pathogenesis of ATTRv amyloidosis.
- Clinical presentation is diverse, including length-dependent small-fibre polyneuropathy, all-fibre polyneuropathy, pseudo-chronic inflammatory demyelinating polyneuropathy, upper-limb-onset neuropathy and motor neuropathy; half of patients also have cardiac amyloidosis.
- Diagnosis is based on *TTR* gene sequencing to detect causal mutations and biopsy to detect amyloid deposits or scintigraphy to assess cardiac uptake of bone tracers when biopsy samples are negative.
- Disease-modifying therapy includes liver transplantation, TTR stabilizers and *TTR* gene-silencing therapies; trials of RNA interference therapy have produced improvements in neuropathy and quality of life, suggesting reversal of the disease.

(REFS[12,13]). Since the 1990s, new endemic areas have been reported in Cyprus[14] and Majorca[15]. However, an increasing number of late-onset, often sporadic, cases have also been diagnosed through biopsy findings and *TTR* gene sequencing[9]. ATTRv amyloidosis has now been reported in 29 countries, including many countries in Europe[16], the USA[17], China[18] and India[19].

A nationwide epidemiological study of ATTRv amyloidosis in Portugal, published in 2018, showed that the mean incidence is 0.87 per 100,000 people per year, corresponding to 71 new patients each year[20]. The estimated prevalence of ATTRv amyloidosis in 2016 was 22.93 per 100,000 adults, corresponding to 1,865 individuals with the condition in Portugal (45.8% male, mean age 52.3 ± 15.4 years)[20].

In a study published in 2018, the global population of people with ATTRv amyloidosis was estimated at 10,186, with a range of 5,526–38,468[21]. The estimation was based on the known prevalence in Portugal, Sweden and specific regions of Japan in addition to that in seven core countries (France, Italy, Turkey, Cyprus, Bulgaria, Germany and the Netherlands). With increasing awareness of the condition among clinicians and with wider use of genetic testing, the incidence of ATTRv amyloidosis is likely to increase, particularly in regions where it is not endemic, creating the need to increase epidemiological research.

Pathophysiology

Central to the pathophysiology of ATTR amyloidosis is the TTR protein itself. TTR, which was initially known as prealbumin, is a transport protein for thyroxine and retinol-binding protein associated with vitamin A and is present in the serum and cerebrospinal fluid (CSF) of healthy humans[22]. TTR is synthesized in the liver, the choroid plexus of the brain and the retinal and ciliary pigment epithelia of the eye (FIG. 1). It is a tetrameric protein with four identical subunits and a total molecular mass of 55 kDa. Each subunit consists of 127 amino acids arranged in eight antiparallel β-sheets (designated A–H). The tetramer has a central channel that contains the two thyroxine binding sites, only one of which is occupied under physiological conditions[23]. The mature protein is formed after cleavage of a 20-amino-acid signal sequence.

Most cases of ATTRv amyloidosis are caused by a point mutation in the *TTR* gene that leads to substitution of valine by methionine at position 30 of the mature protein[24] (see Genetics below). Historically, the numbering of amino acids was based on the mature protein so that this mutation was referred to as ATTR–Val30Met. Standardized nomenclature means that numbering now begins at the methionine initiation codon and the mutation is formally referred to as p.ATTRVal50Met[25]. Nevertheless, Val30Met continues to be widely used in the literature.

Genetics

Heterogeneity. The *TTR* gene is located on chromosome 18 and comprises four exons[26,27]. Over 130 mutations have been identified in this gene, and the vast majority are pathogenic; few mutations are non-amyloidogenic, although evidence suggests that the Thr119Met mutation is protective, as a compound heterozygotic genotype with both the Val30Met and Thr119Met mutations seems to slow the initial misfolding event (tetramer dissociation)[28]. A registry has been established to determine the importance of the specific mutations and phenotypes in hereditary ATTRv amyloidosis[29]. Most identified mutations are point mutations, and just one deletion has been identified. Patients who are homozygous for pathogenic mutations have been described, as have patients who are compound heterozygous, meaning they carry one pathogenic mutation and one non-pathogenic mutation.

Global distribution. The Val30Met variant of TTR is responsible for ATTRv amyloidosis in the regions where it is endemic (Portugal, Sweden, Japan, Majorca and Cyprus)[14–16,30]. This mutation is also the most commonly identified among patients in smaller disease clusters and scattered families worldwide[17]. However, particular mutations have been associated with small clusters of families with the disease. These associated mutations include Thr60Ala in Northern Ireland and populations with Irish ancestry; Glu89Gln in Bulgaria; Ser50Arg in Mexico; Phe64Leu in Sicily; Ser77Tyr and Ser77Phe in France; and Ala97Ser in Taiwan. Conversely, many other mutations have been described only in single families[16,17].

Penetrance. The penetrance of mutations in the *TTR* gene is highly variable. Penetrance of 100% has most commonly been described in families in which several generations have been affected and that originate from regions where the condition is considered endemic[31]. Outside these regions, incomplete penetrance that increases with age is the general rule[32]. Penetrance studies in which the same methodology has been used and that included patients from Sweden, France, Portugal

Penetrance
The proportion of mutation carriers who will express the associated disease phenotype.

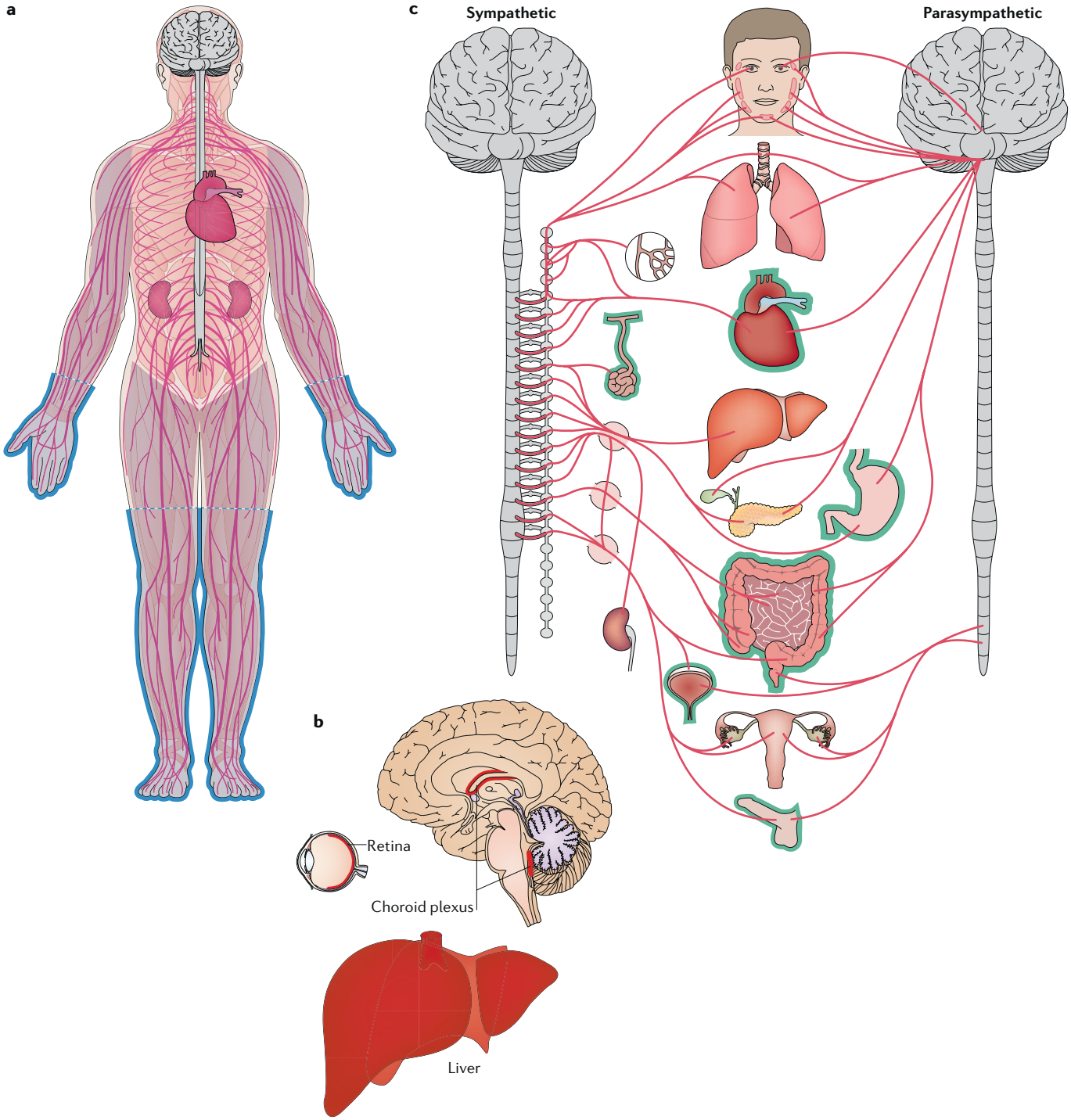


Fig. 1 | **Sites of transthyretin production and sites affected by amyloid deposition and denervation in hereditary transthyretin amyloidosis. a** | Sites of amyloid deposition (dark pink), sensory denervation (blue) and motor denervation (beige). **b** | Sites of transthyretin production (red). **c** | Organs affected by autonomic neuropathy and those affected by autonomic denervation (green outlines).

and Brazil show that penetrance varies with age but increases constantly until the ninth decade of life, when the penetrance approaches 100%, even for genotypes that are associated with a late age of onset[31–33]. Genotype, genetic background, country or region of origin and the gender of the parent from which the mutation was inherited contribute to this variability[31–34].

These studies included only families in which at least one member was affected. A study in which the frequency of two different mutations (mutation of Met30 in northern Sweden and mutation of Ala60 in Northern Ireland) in populations with late-onset disease[35,36] revealed a high mean mutation rate of 1.1–1.5%, much higher than the incidence of the disease. This finding

Table 1 | **Features of different types of familial amyloidosis with polyneuropathy**

Feature	Type of amyloidosis			
	Transthyretin (ATTR)[194]	**Gelsolin (AGel)**[195]	**β_2-Microglobulin (Aβ2M)**[4]	**Apolipoprotein A1 (AApoA1)**[3]
Genetic abnormality	Missense mutation of *TTR* gene[29]	Missense mutation of *GSN* gene[196]	Missense mutation of *B2M* gene[4]	Missense mutation of *APOA1* gene[197]
Number of variants	130 (REFS[27,29])	2 (Asp187Asn and Asp187Tyr)[196,198]	1 (Asp76Asn)[4]	1 (Gly26Arg)
Mode of transmission	Autosomal dominant	Autosomal dominant	Autosomal dominant	Autosomal dominant
Geographical distribution	Worldwide, but endemic to areas of Portugal, Japan, Sweden, Majorca and Cyprus[16,21]	Worldwide, with a high prevalence in Finland	France[4]	USA (Iowa)[199]
Patients identified	10,000 (REF.[21])	400–1,000 (Finland)[2]	1 family[4]	1 family[199]
Age at onset	~30 years for early-onset disease associated with Val30Met mutation, but varies up to 90 years[30,72,73]	~40 years[200]	~50 years	~30 years
Clinical presentation of neuropathy	Length-dependent small-fibre polyneuropathy and/or autonomic neuropathy[6,72]; other phenotypes include all-fibre neuropathy[30], chronic inflammatory demyelinating polyneuropathy[99], motor neuropathy[100] and upper-limb onset polyneuropathy[80]	Cranial neuropathy and/or sensory ataxic polyneuropathy[2]	Autonomic neuropathy, Sicca syndrome and/or sensorimotor polyneuropathy with diarrhoea and/or anal incontinence[4]	Length-dependent polyneuropathy, sensorimotor polyneuropathy and/or autonomic neuropathy[3]
Non-neurological manifestations	Cardiac symptoms (conduction or arrhythmia)[150], cardiomyopathy, ocular involvement[85] and/or renal[90]	Cutaneous involvement, ocular involvement (corneal lattice dystrophy), cardiac symptoms (conduction) and/or renal involvement[200]	None	Renal involvement and/or peptic ulcer[199]
Method of diagnosis	Biopsy and genetic tests[143]	Genetic tests[2]	Biopsy and genetic tests[4]	Biopsy and genetic tests[197]
Survival from onset	~12 years in early-onset disease with Val30Met mutation[72]; ~7 years in late-onset disease with Val30Met mutation and other variants[73,75]	80% at 45 years after onset (not shortened)[200]	10–20 years from onset[4]	Unknown

suggests that mutations can remain completely silent for many generations in many families.

Protein misfolding

Misfolding and aggregation of proteins as a cause of common neurodegenerative diseases, including Alzheimer disease and Parkinson disease[37], is an intense area of research. ATTRv amyloidosis is also a protein misfolding disease: the dissociation of mutant TTR subunits results in misfolding and aggregation of TTR amyloid fibrils in extracellular spaces, leading to systemic organ dysfunction[38,39] (FIG. 2). Amyloid deposition starts before the onset of symptoms, as in the other neurodegenerative diseases. Most *TTR* mutations produce TTR that is less stable than the wild-type protein, leading to development of ATTRv amyloidosis[40]. However, TTR amyloid fibrils can form in individuals who do not have *TTR* mutations, particularly in ageing, which leads to wild-type ATTR (ATTRwt) amyloidosis (also known as senile systemic amyloidosis; see Wild-type transthyretin deposition below)[41].

An alternative suggested pathogenic pathway for ATTRv amyloidosis involves proteolytic cleavage of TTR[42]. Evidence suggests that carboxy-terminal fragments of TTR produced by this cleavage, such as trypsin and plasmin, promote amyloid fibril formation[43]. This process could be an additional pathogenic mechanism.

Amyloid fibrils of variable length

The morphology of amyloid fibrils differs with the age of disease onset and the specific causal mutation[44–47]. In early-onset ATTR–Val30Met amyloidosis, long and thick amyloid fibrils are common (FIG. 3), whereas in late-onset ATTR–Val30Met amyloidosis, the fibrils are usually short and thin[44,46] (FIG. 3d).

An electron microscopic study conducted in Portugal showed that patients with ATTRv amyloidosis exhibited long amyloid fibres similar to those in Japanese patients with early-onset ATTR–Val30Met amyloidosis[48]. Similarly, a study of a specimen from a Brazilian individual with ATTR–Val30Met amyloidosis (said to be of Portuguese origin) revealed long, thick amyloid fibres[47]. By contrast, the characteristics of the amyloid deposits in patients with ATTR amyloidosis associated with other mutations tended to be similar to those of amyloid deposits in patients with late-onset ATTR–Val30Met amyloidosis from non-endemic areas in Japan, irrespective of the age at onset[47,49].

Wild-type transthyretin deposition

Evidence suggests that the mechanism of amyloid deposition in the hearts of patients with ATTR–Val30Met amyloidosis is similar to that of ATTRwt amyloidosis. An autopsy study of Japanese patients with the Val30Met mutation demonstrated that most TTR in

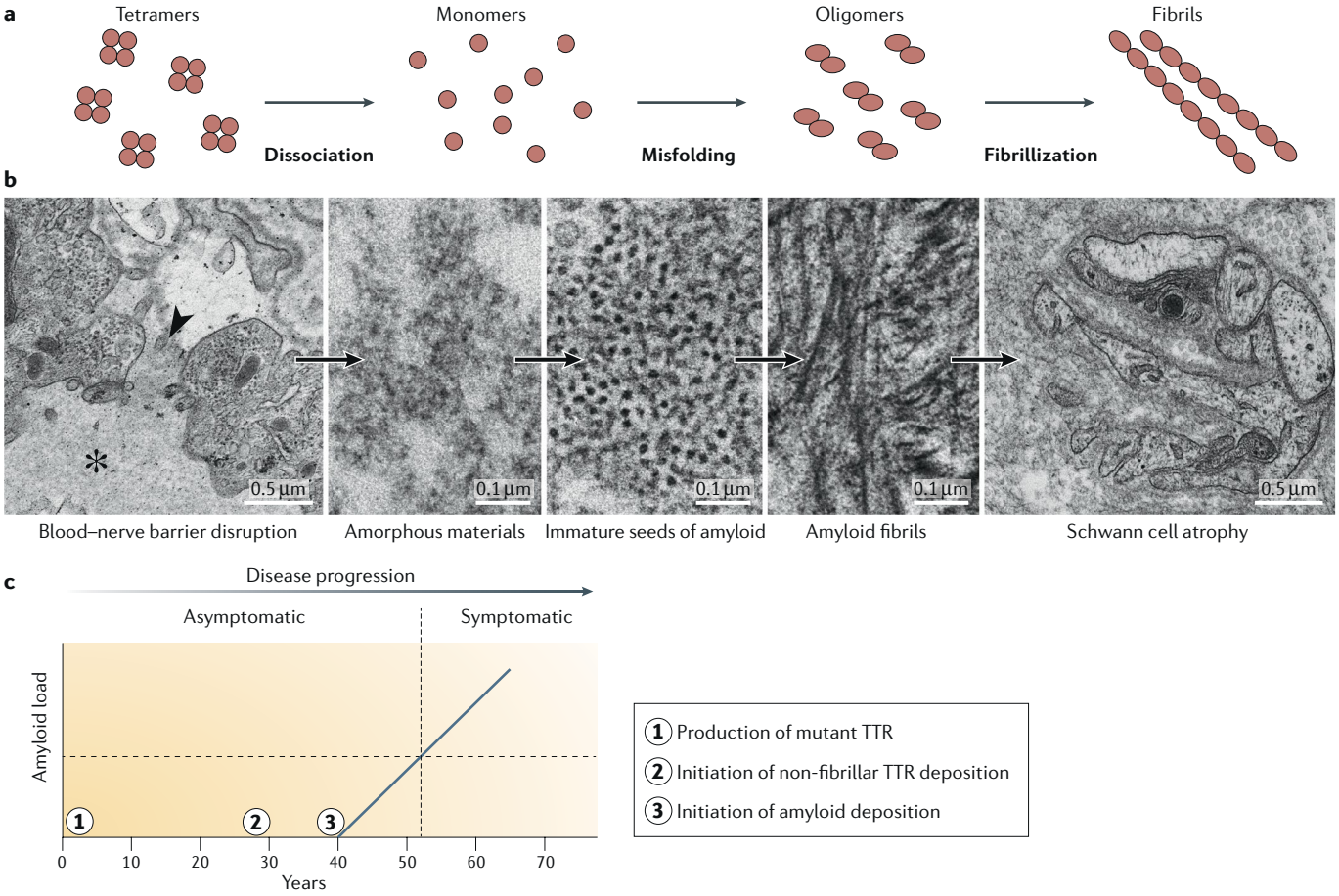


Fig. 2 | **Mechanistic models of amyloid fibril formation and disease progression. a** | Stability of transthyretin (TTR) homotetramers is reduced by TTR mutations, resulting in its dissociation into monomers and deposition in the extracellular spaces of systemic organs. The dissociation results in misfolding of TTR monomers and subsequent aggregation. These oligomers can be present before the completion of mature amyloid fibrils and can have toxic effects on neighbouring tissue. **b** | Disruption of the blood–nerve barrier (arrowhead; vessel lumen indicated by asterisk) can occur in the early phase of neuropathy, resulting in leakage of circulating TTR into the endoneurial space. Consequently, amorphous, electron-dense extracellular material that contains TTR monomers and oligomers becomes abundant in the extracellular spaces of the endoneurium. Aggregation of TTR is subsequently seen as dotty structures among amorphous materials. Finally, elongated fibrillar structures with a thickness similar to the diameter of the dotty structures are formed, leading to adjacent Schwann cell atrophy. **c** | The disease processes, including amyloid deposition, start before onset of the symptoms and signs of amyloidosis.

cardiac amyloid deposits from individuals with early-onset ATTRv amyloidosis was mutant protein, whereas more than half of the TTR in deposits from individuals with late-onset disease was wild-type protein[45]. Another study has shown that in patients with ATTRv amyloidosis who undergo liver transplantation, cardiac amyloidosis can progress after transplantation, particularly in elderly men[50]. This exacerbation of cardiac amyloidosis is attributable to the deposition of wild-type TTR[51]. Analyses of amyloid deposits in the peripheral nerves of patients who have undergone liver transplantation have also revealed wild-type TTR deposition, although its ratio relative to mutant TTR was lower than that in the heart[52,53].

The difference in wild-type TTR involvement between different types of ATTR amyloidosis might be related to differences in proteolytic cleavage[42]. Evidence has shown that a substantial amount of the TTR carboxy-terminal fragments that are present in amyloid deposits in ATTRwt amyloidosis[49,54,55] is also present in amyloid deposits in late-onset ATTR–Val30Met amyloidosis and most cases of ATTR amyloidosis associated with other mutations. By contrast, in early-onset ATTR–Val30Met amyloidosis, amyloid deposits mainly consist of full-length TTR[44,54].

Microangiopathy

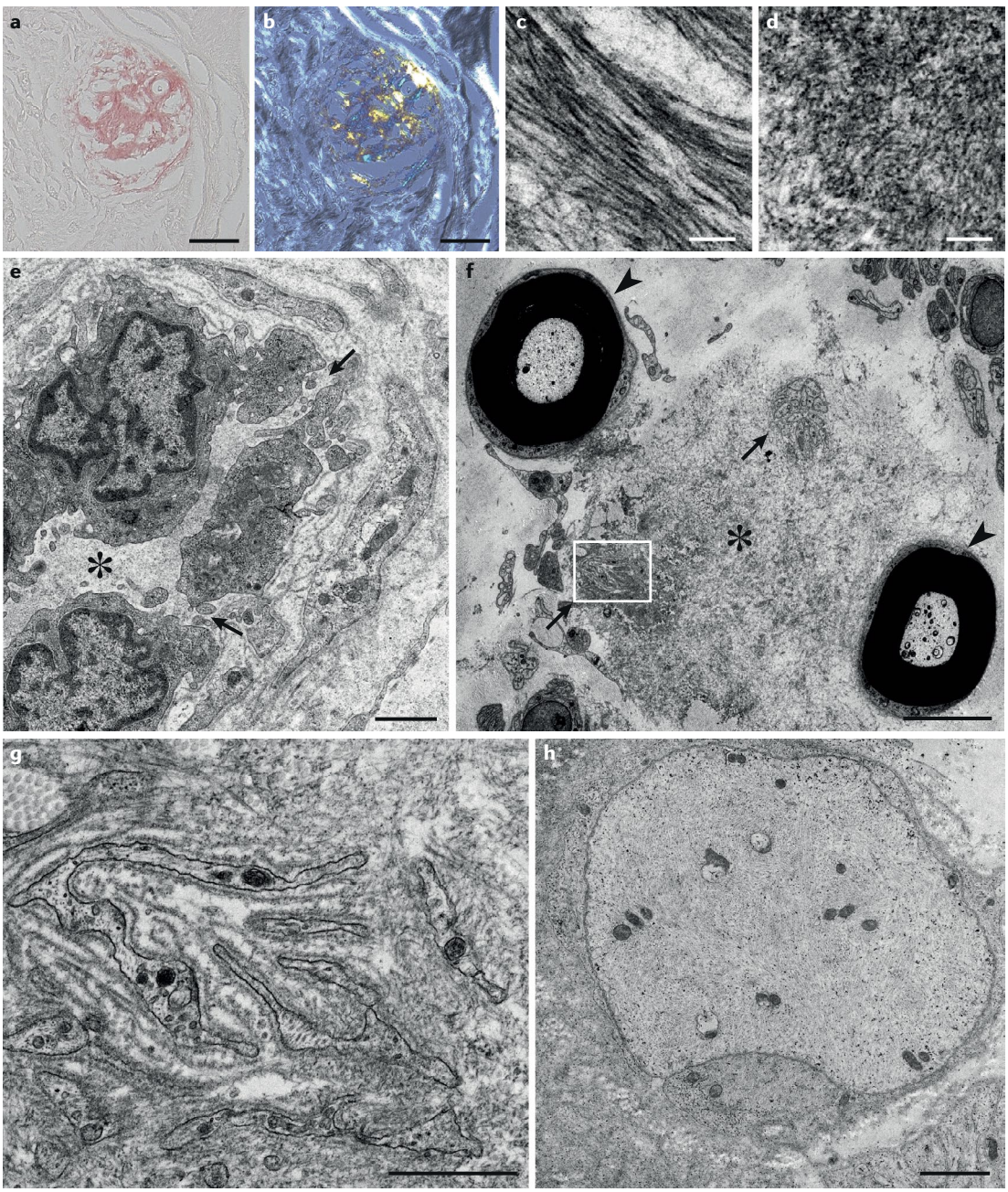
Tight junctions between endothelial cells of the endoneurial microvessels form a blood–nerve barrier between the vascular lumen and interstitium in the PNS[56,57]. A study published in 2016 demonstrated that the blood–nerve barrier is disrupted in ATTR amyloidosis; even amyloid deposits are scarce or absent in the surrounding area[46] (FIG. 3). According to this study, the morphometric indices of the endothelial cells of the endoneurial microvessels, including those that form the blood–nerve barrier, were abnormal in patients with ATTRv amyloidosis compared with those in patients with nutritional

neuropathy associated with similar axonal degeneration[46]. The working assumption is that disruption of the blood–nerve barrier in ATTRv amyloidosis enables entry of circulating TTR into the endoneurial space[46,58]. Indeed, magnetic resonance neurography has revealed swelling of the nerve trunk, even in asymptomatic carriers of mutant *TTR*, suggesting that endoneurial oedema associated with blood–nerve barrier disruption occurs early in the disease[59]. Abnormalities of the retinal and choroidal microvasculature in the eyes of patients with ATTR amyloidosis have also been reported[60], indicating that disruption of blood–retinal barriers also occurs in ATTRv amyloidosis patients.

A widely held opinion is that several factors lead to disruption of the blood–nerve barrier in patients with ATTRv amyloidosis. For example, evidence suggests that inflammatory cytokines[61], mutant TTR[62] and retinol-binding protein[63] induce abnormalities in endothelial cells that lead to disruption of the blood–nerve barrier. Results from studies of microangiopathy associated with diabetes mellitus also suggest that TTR affects endothelial cells by inducing apoptosis[64].

Schwann cell stress

Autopsy studies and analysis of biopsy samples have indicated that axonal damage in ATTRv amyloidosis is length-dependent[58,65,66], suggesting that some factors that affect axons in the nerve trunk are involved in the pathogenesis of the neuropathy. Schwann cells that wrap around small-diameter nerve fibres (non-myelinating Schwann cells) and that are adjacent to amyloid fibril masses become distorted and atrophic, particularly in



early-onset ATTR–Val30Met amyloidosis, in which amyloid fibrils are long and thick[46,47] (FIG. 3f,g). Basement and cytoplasmic membranes of Schwann cells apposed to amyloid fibrils tend to become indistinct, suggesting direct damage of Schwann cells due to invasion of amyloid fibrils[46,47]. The toxic effects of amyloid fibrils on Schwann cell membranes have been attributed to a high affinity of amyloid for Schwann cell membranes owing to their common constituents, such as collagen IV, laminin and fibronectin[67], and to alterations of membrane fluidity[68]. The stress on Schwann cells caused by adjacent amyloid fibrils could explain why small-fibre axonal loss occurs predominantly in early-onset ATTR–Val30Met amyloidosis. By contrast, myelinated fibres, particularly large myelinated fibres, seem to be resistant to such stress because the apposition of these fibres to amyloid fibril aggregates is usually partial (FIG. 3f). Demyelinated axons surrounded by amyloid fibrils are rare (FIG. 3h).

Results of in vitro studies in which Schwannoma cell lines have been used also suggest that TTR is toxic to Schwann cells[69–71], although TTR oligomers rather than mature amyloid fibrils seem to exert this toxicity[69]. Such biochemical stress might contribute to nerve fibre damage in late-onset ATTR–Val30Met amyloidosis, in which fewer amyloid deposits are present and large fibres tend to be lost[58].

Clinical features

Natural history

The natural history of early-onset ATTR–Val30Met amyloidosis in Portugal has been defined as involving three stages[72]. Stage 1 is a progressive sensory polyneuropathy that leads to difficulty walking without assistance. Stage 2 is a sensorimotor polyneuropathy that necessitates assistance for walking. In stage 3, the patient is wheelchair-bound or bedridden until death at ~12 years from disease onset. By contrast, the natural history of late-onset ATTR–Val30Met amyloidosis and ATTRv amyloidosis associated with other mutations[73–75] involves a more rapid course in which gait disability occurs earlier, including decreases in the time until walking assistance is needed and until the patient is wheelchair-bound[74]; this course results in faster progression of the sensorimotor Neuropathy Impairment Scale (NIS) score[76]. Survival is ~7 years from disease onset[73,75,77].

Atrioventricular block is commonly associated with all *TTR* mutations, and cardiac or mixed forms of ATTRv amyloidosis are associated with a worse prognosis than that associated with the neurological phenotype. The life expectancy of a patient with a cardiac phenotype of ATTRv amyloidosis is 2–5 years after symptom onset, and the major cause of death is refractory heart failure due to cardiac infiltration of amyloid deposits into the extracellular matrix[78,79].

Symptoms

The main symptoms of ATTRv amyloidosis are neuropathic, but given that it is a systemic disease, neurologists should be aware of possible cardiac, eye and kidney involvement, which justify a multidisciplinary approach to management. General symptoms of involuntary weight loss or fatigue can be the presenting symptoms of ATTRv amyloidosis, particularly in early-onset ATTR–Val30Met amyloidosis.

The precise symptoms of somatic neuropathy depend on the causal gene mutation and the age of onset. In early-onset ATTR–Val30Met, symptoms include sensory abnormalities (such as paraesthesia and lightning pain) in the lower limbs that start in the feet and extend proximally, impaired pain and/or temperature sensation and plantar ulcers. In late-onset ATTR–Val30Met amyloidosis and other variants, symptoms are sensory or sensorimotor and they start in the feet, distally in all four limbs or in the upper limbs only to mimic carpal tunnel syndrome (CTS)[75,80]. With some other variants, including Ser77Tyr, Ile107Val and Val122Leu, initial symptoms can include gait disorders[73], including gait unsteadiness[81] or weakness in the limbs[73]. A positive history of CTS is common (23–63% of patients) in late-onset ATTR–Val30Met amyloidosis and other variants but is less common in early-onset ATTR–Val30Met amyloidosis[73]. Symptoms of autonomic neuropathy include erectile dysfunction, gastrointestinal symptoms (including constipation, alternating diarrhoea and constipation, daily diarrhoea, early satiety and crisis of vomiting), dysuria, light headedness and fainting upon moving from lying to standing.

Cardiac amyloidosis remains latent in ATTRv amyloidosis with polyneuropathy (ATTRv–PN) for a long time and is underdiagnosed. A study of the natural history of ATTRv–PN revealed the presence of subclinical cardiac amyloidosis at the time of diagnosis and the development of cardiac symptoms later in the disease[75]. When symptoms develop, the pattern is often heart failure with a preserved ejection fraction and/or hypertrophic cardiomyopathy, cardiogenic syncope and peripheral oedema[82]. Two randomized clinical trials have shown that cardiac ATTRv amyloidosis is present as an infiltrative cardiomyopathy with a myocardial thickness >13 mm in 56%[83] and 63%[84].

Eye and CNS disease can occur in isolation, in combination (oculoleptomeningeal amyloidosis) or with the involvement of other organs. Ocular manifestations in ATTR–Val30Met amyloidosis include dry eye

◀ Fig. 3 | **Representative photographs of sural nerve biopsy specimens from patients with hereditary transthyretin amyloidosis.** Cross sections. **a** | Amyloid deposits are positively stained with Congo red. **b** | Congo-red-stained amyloid deposits appear as bright and glittering apple-green birefringence when examined with polarized light. **c** | Amyloid fibrils are long and thick in early-onset hereditary amyloidogenic transthyretin (ATTR–Val30Met) amyloidosis. **d** | Amyloid fibrils are short and thin in late-onset ATTR–Val30Met amyloidosis. **e** | Wide gaps (arrows) are observed between adjacent endothelial cells of the endoneurial microvessels, suggesting disruption of the blood–nerve barrier. Microvessel lumen indicated by an asterisk. **f** | Small Schwann cells (arrows), such as non-myelinating Schwann cells, and bands of Büngner (Schwann cells that previously surrounded myelinated axons) that are apposed to amyloid fibrils become atrophic. By contrast, myelinated fibres, particularly large myelinated fibres (arrowheads), tend to be preserved despite their apposition to amyloid fibrils. Asterisk indicates a mass of amyloid fibrils. White box indicates area magnified in part **g**. **g** | Atrophic non-myelinating Schwann cells become completely surrounded by amyloid fibrils. Some Schwann cell contours become indistinct. **h** | Although rare, unmyelinated large axons (6 µm diameter) that are surrounded by amyloid fibrils can be found. Parts **a** and **b** show Congo-red staining, parts **c**–**h** show uranyl acetate and lead citrate staining. Scale bars represent 20 µm in parts **a** and **b**, 0.1 µm in parts **c** and **d**, 1 µm in parts **e**, **g** and **h** and 5 µm in part **f**.

syndrome (70%), glaucoma (20%) and vitreous amyloidosis (17%)[85]. CNS involvement results from leptomeningeal amyloid angiopathy and can cause episodes of focal neurological deficits, epilepsy, brain haemorrhage and dementia[86].

Genotype–phenotype correlation

Clinical presentation and disease course. The predominance of PNS and cardiac clinical manifestations in ATTRv amyloidosis has led to classification of phenotypes as neurological, cardiac or mixed according to whether presentation is exclusively neuropathy or cardiomyopathy or a combination of both[87]. However, with progression of the disease, the exclusive phenotypes usually become mixed phenotypes. These phenotypes are related to mutations and the age of onset; for example, some mutations (such as Val122Ile and Leu111Met)[88] are associated with a cardiac phenotype, whereas others (such as Ser50Arg[89] and Ala97Ser[74]) are associated with a neurological phenotype. Nevertheless, in two major randomized clinical trials that included patients with polyneuropathy associated with various genotypes, an infiltrative cardiomyopathy (defined as a myocardial thickness >13 mm) was observed in 56%[83] and 63%[84] of the patients enrolled.

Interestingly, age at onset seems to affect the phenotype regardless of the associated mutation. For example, patients with early-onset ATTR–Val30Met amyloidosis have a small-fibre neuropathy at presentation, whereas patients with a late onset often have a large-fibre neuropathy, an isolated cardiac phenotype or a combination of both[58].

The involvement of other organs in association with specific mutations is not clear. Kidney involvement, which manifests as a nephrotic syndrome and/or progressive renal failure, has been described mostly in patients with the Val30Met mutation[90], especially in a subgroup of Portuguese patients.

Age of onset and gender imbalance. The age of onset of ATTRv amyloidosis varies from the late teens to old age. A division between early-onset disease (age <50 years) and late-onset disease (age ≥50 years) was established in the three major regions where the Val30Met mutation is seen (Portugal, Sweden and Japan)[8,30,91,92]. Studies conducted in Japan and Sweden have shown that these two groups correspond to two independent distributions of age of onset and that these two groups have different epidemiological, genetic, pathological and clinical characteristics, although the individuals in both groups have the same genotype[42,58].

A study conducted in Portugal, which included 926 parent and child pairs with predominantly early-onset ATTR–Val30Met amyloidosis, focused on anticipation, a phenomenon in which age at onset is earlier in children of affected adults[93]. The age of onset in the second generation could be predicted on the basis of age at onset in the first generation and the gender of offspring and transmitting parent pairs[93]. Anticipation was more pronounced when the disease was inherited from the mother and when the child was male. Females who inherited a mutation from the father were protected from anticipation. Predicting age of onset on the basis of inheritance of point mutations remains controversial, but studies conducted in Japan[94] and Sweden[95] have produced similar findings.

Diagnosis and assessment

If an individual lives in an area in which ATTRv amyloidosis is endemic, diagnosis of the condition is usually made within 1 year of onset. Families in which ATTRv amyloidosis has been identified are then cared for and monitored regularly in a referral centre. The neuropathy is typically a length-dependent, small-fibre polyneuropathy with predominant loss of thermal and pain sensory fibres and with autonomic dysfunction[6]. In these areas, genetic counselling is well developed for the detection of carriers (BOX 1), and disease onset can be confirmed in these individuals upon onset of symptoms and detection of amyloid deposits in a biopsy sample. Once the diagnosis is confirmed, anti-amyloid therapy can be started.

In regions where ATTRv amyloidosis is not endemic, diagnosis can be delayed by 3–4 years as a result of several factors, including a lack of family history of the condition[81,96,97], the variety of initial presentations that mimic various peripheral neuropathies[80,96,98–100] (TABLE 2), diagnostic wandering of an apparently idiopathic axonal polyneuropathy in elderly individuals[101], misdiagnosis as a chronic inflammatory demyelinating polyneuropathy (CIDP) that leads to time spent testing treatments[102], a delay in asking for diagnostic tests and a biopsy sample that is negative for amyloid deposits[73,81,102]. In these cases, looking early for associated heart involvement (for example, infiltrative cardiomyopathy or heart block) in patients with an undefined peripheral neuropathy can indicate amyloidosis.

Diagnostic and assessment tools

A diagnosis of ATTRv amyloidosis can be confirmed by a biopsy to detect amyloid deposits and by *TTR* gene sequencing to detect amyloidogenic variants[73,75]. These tests also distinguish ATTRv amyloidosis from many peripheral neuropathies[103,104]. Additional approaches and biomarkers can also be used to assess the severity of pathology and symptoms.

Box 1 | Genetic counselling and management of carriers

The children and siblings of heterozygous patients with amyloidogenic transthyretin (ATTR) amyloidosis have a 50% risk of having the pathogenic mutation themselves. The presence of transthyretin gene (*TTR*) mutations is detectable very early in fetal life, which enables prenatal and/or presymptomatic gene testing to be performed. Political and ethical discussions about reproductive options vary from country to country, but decisions must always rely primarily on the carrier and his or her partner[148,192].

Several ATTR amyloidosis reference centres in the main focal regions of the disease have developed programmes for presymptomatic follow-up of mutation carriers. These programmes help to detect the first clinical and paraclinical abnormalities in these individuals and to propose disease-modifying treatments early. Annual tests can provide reassurance for people who have been identified as carriers[148], although people have identified the tests as stressful events. Nevertheless, the presymptomatic tests and the follow-up were recommended by the European Network for TTR–FAP (ATTReuNET), covering ten European countries and nine national reference centres, particularly if multidisciplinary management that includes psychological support was available[193].

Table 2 | **Main misdiagnoses of neuropathy phenotypes among patients with transthyretin amyloidosis**

Phenotype	Genotypes	Misdiagnoses	Refs
Length-dependent small-fibre polyneuropathy and/or autonomic neuropathy	*TTR* Val30Met mutation (early-onset disease)	• Diabetic polyneuropathy • Fibromyalgia • Immunoglobulin light-chain amyloidosis • Chronic digestive disease	72
All-fibre polyneuropathy	*TTR* Val30Met mutation (late-onset disease) and other *TTR* variants	• CIDP • Idiopathic axonal polyneuropathy • Lumbar canal stenosis • Vasculitic peripheral neuropathy • Toxic peripheral neuropathy • Alcoholic neuropathy • Paraproteinaemic peripheral neuropathy	73,81,97,98,102
Upper-limb-onset polyneuropathy	*TTR* Val30Met mutation (43%) and other *TTR* variants (Phe64Leu, Ser77Tyr, Tyr78Phe and Ile107Val)	• Carpal tunnel syndrome • Idiopathic polyneuropathy • CIDP • Paraneoplastic neuropathy • Cervical radiculopathy	80
Motor neuropathy	*TTR* Val30Met, Phe64Leu, Ile68Leu, Tyr78Phe, Val93Met and Ile107Val	• ALS • Motor CIDP • Motor neuropathy • Motor neuron disease	100,112,137

ALS, amyotrophic lateral sclerosis; CIDP, chronic inflammatory demyelinating polyneuropathy.

Biopsy and gene sequencing. Formal diagnosis of ATTRv amyloidosis requires detection of characteristic amyloid deposits in a biopsy sample[105]. The most common tissues that are sampled are the labial minor salivary gland, the nerves, the gastrointestinal tract and aspirated abdominal fat.

Amyloid deposits are detected with Congo-red staining and polarized microscopy, with which they are seen as green–yellow birefringence (FIG. 3a,b). However, a biopsy sample that is negative for amyloid deposits does not eliminate diagnosis of ATTRv amyloidosis: the sensitivity varies according to the tissue sample, the causal mutation and the age of the patient and depends on the skill of the pathologist. Multiple biopsies might be necessary to reach diagnosis for some patients.

In Europe, biopsy of the labial minor salivary gland is used to investigate progressive idiopathic axonal polyneuropathy and look for evidence of Sjogren syndrome[106,107], sarcoidosis[108] and/or amyloidosis[109,110]. Nerve biopsy is considered a second-line strategy for diagnosis[111] but has a sensitivity as high as 80%[96,102,112] that can reach 93% after careful examination of dozens of sections rather than five or six of the biopsy sample[102]. Abdominal fat aspiration for the detection of amyloid deposits has a variable sensitivity of 15–83%[97,112,113].

Immunohistochemistry can be used to determine the biochemical nature of amyloid deposits but can contribute to false-negative or false-positive labelling. Laser microdissection and mass spectrometry-based proteomic analysis have been used to identify specific types of amyloidosis[114].

For *TTR* gene sequencing, Sanger sequencing remains the gold standard[115], as this technique enables detection and identification of rare or unknown variants. Some centres are applying next-generation sequencing techniques[116], although these techniques are not as effective as Sanger sequencing.

Assessment of neuropathy. For the assessment of somatic neuropathy, nerve conduction studies that measure the amplitudes of sensory and motor action potentials in the limbs are useful for detecting axonal neuropathy and assessing its severity[73,117]. For objective testing of autonomic neuropathy, measurement of heart rate variability[118], testing for orthostatic hypotension with a fixed pulse rate and testing of sudomotor function via electrochemical skin conductance can all be useful[119]. Useful clinical scales include the NIS sensorimotor compound[120], the Compound Autonomic Dysfunction Test (CADT) questionnaire[121] for assessment of autonomic neuropathy and the polyneuropathy disability (PND) score for assessment of locomotion[122].

Assessment of cardiomyopathy. The goal of cardiac investigations in ATTRv amyloidosis is to detect serious conduction disorders that carry the risk of sudden death and infiltrative cardiomyopathy. Approaches to diagnosis of cardiac involvement include: echocardiogram; 24 h electrocardiogram Holter monitoring; intracardiac electrophysiological studies when necessary (if prolonged conduction and/or bundle branch block is detected with the surface electrocardiogram, even in asymptomatic patients); looking for heart block that requires implantation of a prophylactic pacemaker; multimodal imaging (such as echocardiography with strain imaging[123]); cardiac MRI[124]; and scintigraphy to detect cardiac uptake of bone tracers such as ^{99m}Tc-2,3-dicarboxypropane-1,1-diphosphonate (DPD), ^{99m}Tc-hydroxy methylene diphosphonate (HMDP) or technetium ^{99m}Tc pyrophosphate (PYP)[125,126]. Amyloid deposits induce a pseudohypertrophy with a sparkling appearance on echocardiography owing to an increase in myocardial thickness that is due to amyloid deposits in the extracellular matrix rather than muscular hypertrophy. The ejection fraction is preserved, but the longitudinal deformation rate is abnormal and late gadolinium enhancement is seen on MRI.

Holter monitoring
Continuous electrocardiogram recording using a portable device for the assessment of patients with suspected cardiac arrhythmias.

Strain imaging
Echocardiographic technique used to measure myocardial regional or global deformation during cardiac contraction.

Scintigraphy
Imaging of the heart at the molecular level using radiolabelled ligands.

Detection of DPD or PYP uptake with cardiac scintigraphy is helpful for diagnosing an amyloid origin of hypertrophic cardiomyopathy because this observation is highly specific[126]. This technique is routinely used as a substitute for cardiac biopsy in the diagnosis of TTR cardiac amyloidosis[125,126] because of its high specificity and sensitivity and because it is non-invasive. Furthermore, if systemic amyloidosis is suspected, genetic testing is positive and biopsy samples are negative, a positive cardiac 'bone' scan can be used as an equivalent of a positive biopsy and help therapeutic decisions.

The New York Heart Association (NYHA) classification is of limited value for assessment of patients with cardiac ATTRv amyloidosis because inter-operator variability is high and because the classification is not reliable as soon as patients have physical limitations as a result of neuropathy[127]. Biomarkers of cardiac pathology are more useful. The amino-terminal prohormone of brain natriuretic peptide (NT-proBNP) is a natriuretic peptide with no biological activity but is increased in the plasma in association with high intracardiac pressure, as observed in heart failure with preserved ejection fraction. Plasma levels of NT-proBNP are abnormal even in the early stages of cardiac amyloid infiltration. In addition, high levels of troponin occur later in the disease and are observed in the most severe forms[128]. Both markers have a prognostic value in amyloid cardiomyopathy. In a new staging system for cardiac ATTR amyloidosis, the disease was stratified into three stages on the basis of cut-off points for levels of NT-proBNP and the estimated glomerular filtration rate (eGFR)[78].

Assessment of other systemic involvement. Ophthalmological assessment is warranted for patients with ATTRv amyloidosis for identification of keratoconjunctivitis sicca, secondary glaucoma, vitreous opacities or pupillary abnormalities. Investigations that are useful in this context are the Schirmer test, measurement of intraocular pressure, testing of visual acuity and slit lamp and ocular fundus examinations.

To assess kidney involvement in ATTRv amyloidosis, measurement of proteinuria, microalbuminuria and the eGFR are useful[129]. In another measure, a modification of the BMI (mBMI; $kg/m^2 \times$ albumin (g/l)) corrects for the effect of hypoalbuminaemia and provides nutritional status, which is related to the duration of gastrointestinal disturbances and malabsorption in ATTRv[122].

Assessment in clinical trials

The clinical scales used as primary end points in clinical trials in ATTRv amyloidosis have evolved as clinical trials have progressed. In a trial published in 2012 (REF.[130]), the NIS lower-limb (NIS-LL) score was used; this score was based on examination of the lower limbs only. In a trial published in 2013, the NIS + 7 (REF.[132]) was used, which combines a neurologist's clinical assessment of muscle weakness, sensory loss and decreased muscle stretch reflexes in the limbs with five nerve conduction attributes. In the most recent trials published in 2018, variants of the modified NIS + 7 (mNIS + 7) were used[83,84]. The mNIS + 7 better quantifies sensation over the whole body (rather than at distal sites), autonomic function and nerve conduction changes associated with progression of ATTRv amyloidosis. Two versions of the mNIS + 7 have been developed: one by Ionis and the other by Alnylam[133].

Other clinical scales used in clinical trials include the Composite Autonomic Symptom Score 31 (COMPASS-31) questionnaire for assessment of autonomic symptoms, the Rasch-built Overall Disability Scale (R-ODS) survey[134] for assessment of activities of daily living, the Norfolk Quality of Life-Diabetic Neuropathy (QoL-DN) questionnaire[135] to measure quality of life and the timed 10-minute walk test and handgrip strength test (dynamometer) to assess specific motor function[133].

Cardiac function is assessed in clinical trials with the 6-minute walk test, echocardiograms and cardiac biomarkers (troponin I and NT-proBNP). Kidney function is assessed in clinical trials by measurement of proteinuria and eGFR.

Misdiagnosis

Early-onset ATTR–Val30Met amyloidosis is typically a length-dependent, small-fibre polyneuropathy with autonomic dysfunction (in 90% of patients)[6,72], but late-onset ATTR–Val30Met[30] can present with all modes of sensory loss (in 50% of patients) and rarely involves autonomic dysfunction at onset (in 10% of patients). Other atypical clinical presentations in late-onset ATTR–Val30Met and other variants include upper-limb-onset polyneuropathy (17.6%)[80,96], motor neuropathy (<1%)[100] and gait difficulties (11–23%[73,81]).

As a consequence of the variety in presentation, misdiagnoses are common; 32–74%[96,98,136] of patients with ATTRv amyloidosis have received a misdiagnosis (TABLE 2) and 18% have received multiple misdiagnoses[80,98]. Misdiagnoses are made on the basis of the initial clinical manifestations and nerve conduction studies. The most common misdiagnoses are idiopathic axonal polyneuropathy[96,102], CIDP[98,99,102], lumbar spinal stenosis[96,98] and, infrequently, motor neuron disease[100,112,137]. CIDP is one of the major misdiagnoses[73,96,98] because its clinical features are similar to those of late-onset ATTRv amyloidosis, including sensorimotor impairment with areflexia, frequent albuminocytological dissociation in the CSF[98,99] and, in 12.5–15% of patients, nerve conduction findings that fulfil the European Federation of Neurological Sciences–Peripheral Nerve Society criteria for CIDP[98,99].

Red flags

Early diagnosis of ATTRv amyloidosis is difficult, and studies have been done in an attempt to define red flags to facilitate early diagnosis (reviewed in detail elsewhere[138]). According to the findings of these studies, early-onset ATTR–Val30Met amyloidosis should be suspected in people with progressive symmetrical sensorimotor neuropathy with satisfaction of at least one of the following criteria: a positive family history for ATTRv amyloidosis, early autonomic dysfunction, gastrointestinal complaints, unexplained weight loss, renal abnormalities, vitreous opacities and bilateral CTS. Late-onset ATTR–Val30Met amyloidosis or ATTRv amyloidosis associated with other mutations should be considered in patients with any progressive polyneuropathy that is accompanied by the red flags of previous surgery for

Schirmer test
A test to determine whether the eye produces enough tears or whether a patient has dry eye.

CTS[73] and/or gait disability[73,75,81]. Specific red flags in CIDP-like ATTRv amyloidosis have been reported[99]. Clinical red flags include neuropathic pain, dysautonomia, small-fibre sensory loss above the wrist and weakness in the upper limbs; these signs were more common in demyelinating ATTRv–PN than in idiopathic CIDP and polyneuropathy, organomegaly, endocrinopathy, M protein and skin changes (POEMS) syndrome[99]. Electrophysiology red flags include prolonged distal motor latency of the median nerve, reduced sensory conduction velocity in the median and ulnar nerves and a motor axonal loss most commonly in the median, ulnar and tibial nerves in demyelinating ATTRv–PN[99]. An ulnar nerve motor amplitude <5.4 mV and a sural nerve amplitude <3.95 μV were distinguishing characteristics of demyelinating ATTRv–PN[99].

Diagnosis of sporadic disease

Until now, the diagnosis of ATTRv amyloidosis has been based on amyloid in biopsy samples (FIG. 4), but the approach is now tending towards early genetic testing. An observational study is ongoing in French tertiary referral centres for neuromuscular diseases[139] to assess *TTR* gene testing as a second-line diagnostic tool before a biopsy for patients with idiopathic axonal polyneuropathy or apparent CIDP that is unresponsive to first-line immunotherapy. A *TTR* gene test that is negative for an amyloidogenic variant eliminates a diagnosis of ATTRv amyloidosis[29].

Early diagnosis in carriers

Early markers for the onset of ATTRv amyloidosis are needed to enable earlier initiation of anti-amyloid therapy after the detection of denervation in the skin[140], heart[141], sweat glands[119] or cornea[142]. Currently, one possibility is the use of DPD scintigraphy: in people whose *TTR* genetic tests identify a pathogenic mutation but whose salivary gland, skin or fat aspirate biopsy samples are negative for amyloid, DPD scintigraphy can be used as a surrogate for a cardiac biopsy to detect amyloid deposits. A positive result in this test is usually associated with other signs of cardiac amyloidosis (left ventricular wall thickening and MRI abnormalities) that indicate initiation of anti-amyloid therapy[126].

Therapy

Management of ATTRv amyloidosis requires a multidisciplinary approach[143] (FIG. 5). Care should include symptomatic therapy, anti-amyloid therapy to prevent further production of amyloid deposits and treatment of cardiac, renal and ocular involvement[144,145], including the possibility of heart[146] or kidney transplantation[147] during end-stage disease. Systematic and regular monitoring of

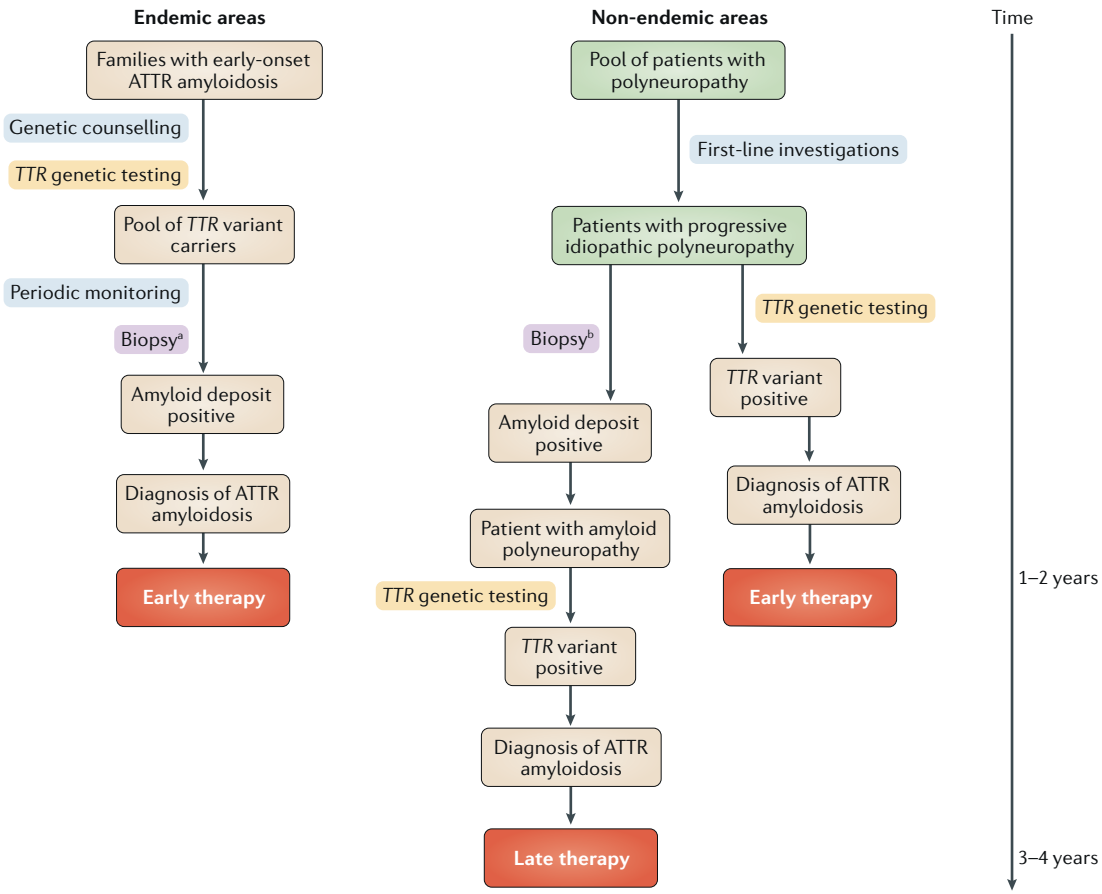


Fig. 4 | **Strategy for diagnosis of hereditary transthyretin amyloidosis with polyneuropathy.** Strategies are provided for areas where hereditary amyloidogenic transthyretin (ATTRv) amyloidosis is endemic (left) and for areas where the condition is not endemic (right). [a]Biopsy of the labial salivary gland is proposed at symptom onset. [b]Biopsy of the labial salivary gland, nerve or aspirated fat tissue. TTR, transthyretin.

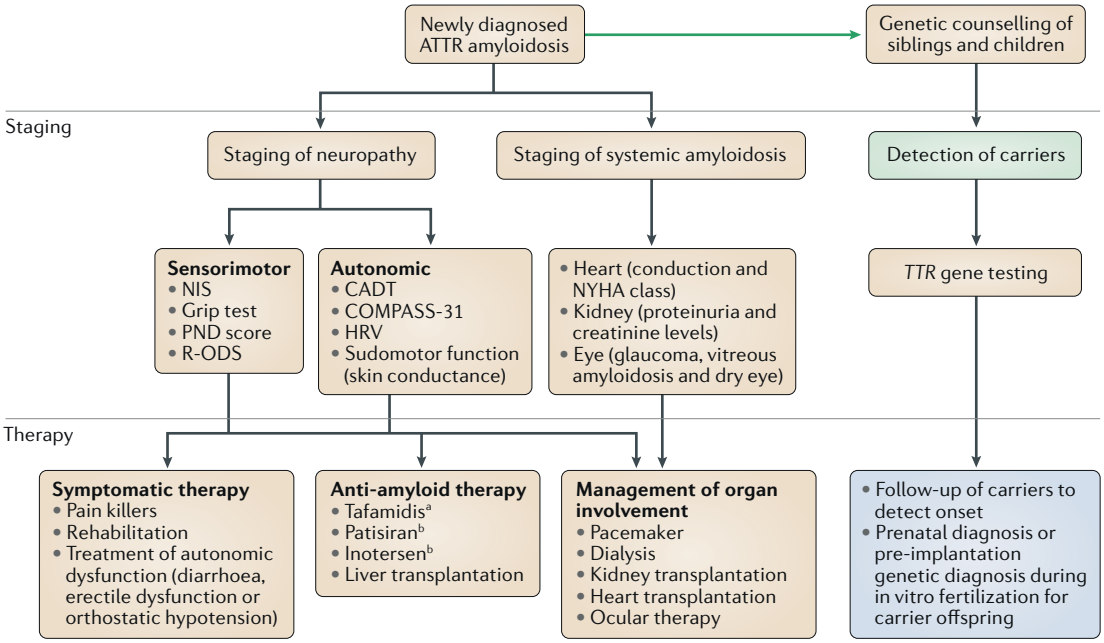


Fig. 5 | **Overall management of hereditary transthyretin amyloidosis with polyneuropathy.** ATTR, amyloidogenic transthyretin; CADT, Compound Autonomic Dysfunction Test; COMPASS-31, Composite Autonomic Symptom Score-31 questionnaire; HRV, heart rate variability; NIS, Neuropathy Impairment Score; NYHA, New York Heart Association; PND, polyneuropathy disability; R-ODS, Rasch-built Overall Disability Scale. [a]This drug has received marketing authorization by the European Medicines Agency (EMA). [b]These drugs have received marketing authorization by the EMA and the FDA.

asymptomatic carriers is necessary to detect early signs of ATTRv amyloidosis, confirm the diagnosis and initiate anti-amyloid therapy[148]. Therapeutic education for patients is also important[149]. Genetic counselling of patients and relatives is also highly recommended[143] (BOX 1).

Most extra-neurological manifestations are silent, therefore systematic screening is required at diagnosis to prevent serious complications, including blindness as a result of glaucoma and sudden death as a result of complete atrioventricular block[150]. Cardiac manifestations can require a prophylactic pacemaker[143].

Various disease-modifying therapies have been developed for ATTRv amyloidosis in the past 28 years (FIG. 6). The first of these treatments was liver transplantation to suppress the main source of mutant TTR[10,151–153]. However, progression of ocular[154] and CNS amyloidosis[155,156], cardiomyopathy[157] and neuropathy[53] after liver transplantation is a major problem. TTR stabilizers have also been developed to stabilize the TTR tetramer[11] and thereby prevent formation of amyloid fibrils. Most recently, TTR gene silencers have been developed to block mRNA synthesis and thereby reduce production of mutant and wild-type TTR; wild-type TTR is also targeted owing to its involvement in progression of disease after liver transplantation in late-onset ATTRv–PN[83,84].

Liver transplantation outcomes

In a 20-year retrospective analysis of 1,940 patients in the FAP World Transplant Registry, the 20-year survival after liver transplantation was 55.3%. Multivariate analysis revealed that a higher mBMI, an early age of onset (<50 years), a short disease duration before liver transplantation and the TTR Val30Met mutation (rather than other TTR mutations) were independent predictors of survival[152]. In a single-centre study that included a cohort of 215 consecutive patients who underwent liver transplantation, a median follow-up of 5.9 years enabled identification of five risk factors for death: a PND score >2, orthostatic hypotension, NYHA classification >I, a QRS complex interval >120 ms and a greater interventricular thickness[153]. These factors were used to build a risk prediction model for accurately estimating the individual risk of death after liver transplantation for patients with ATTRv amyloidosis with an online calculator[153]. Overall, cardiovascular complications are the leading causes of death after liver transplantation for treatment of ATTRv amyloidosis in the world registry of liver transplantation (22%) and in the French Reference Center Registry (38%)[152,158]. In several studies, a poor prognosis has also been associated with cardiac sympathetic denervation, as assessed by iodine-123 metaiodobenzylguanidine (MIBG) cardiac scintigraphy[159,160].

Trials of TTR stabilizers

TTR stabilizers are small molecules that are known to strongly bind to the unoccupied thyroxine (T4)-binding sites within TTR, inducing kinetic stability of the native quaternary structure of the TTR tetramer that limits the rates of tetramer dissociation and amyloidogenesis[161]. Two drugs of this type have undergone trials in ATTRv amyloidosis.

Tafamidis. Studies of the TTR stabilizer tafamidis have suggested that it is effective in disease associated with several TTR variants[162]. On this basis, a multicentre placebo-controlled phase III clinical trial involving

QRS complex
Electrical activity of the ventricles, as recorded by electrocardiogram.

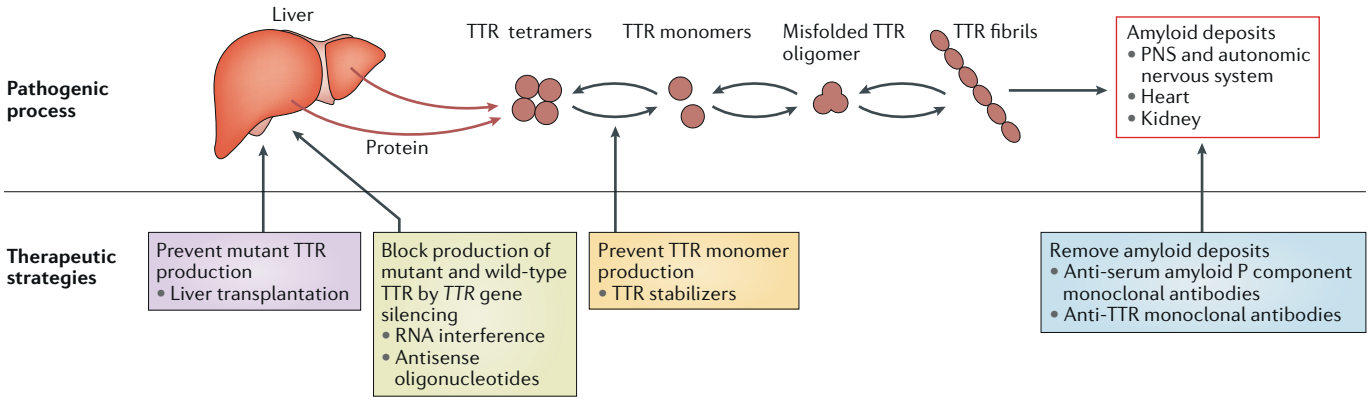


Fig. 6 | **Overview of therapeutic strategies in hereditary transthyretin amyloidosis with polyneuropathy.** Therapeutic approaches developed to date (bottom) target different aspects of the pathogenic process (top). TTR, transthyretin.

128 patients was conducted[130]. All patients expressed the Val30Met TTR variant and had early-onset disease (mean age 39 years); most of the participants were of Portuguese origin. The patients had a recent onset neuropathy with a low NIS-LL neuropathy score of 4 (maximum possible score 88) at baseline[130]. Efficacy analysis revealed a significant effect of tafamidis. No neuropathy progression was seen in 60% of the patients who received tafamidis versus 38% of patients who received placebo. The increase in total scores on the Norfolk Quality of Life Questionnaire–Diabetic Neuropathy (Norfolk QoL–DN) questionnaire from baseline was also greater in the placebo group than in the tafamidis group[130]. Another study has shown that early intervention with tafamidis provides a long-term (5.5-year) delay in neurological progression in ATTRv amyloidosis[163].

The short-term and long-term effects on functional progression and the safety of tafamidis in late-onset ATTR–Val30Met amyloidosis or with disease associated with other TTR variants have been assessed in three open-label studies[164–166]. The mean ages of patients in these studies were 59–63 years. At baseline, 53–77%[164,165] of patients had walking difficulties and 26–38%[164,166] required aid. At 1 year after initiation of treatment, NIS-LL scores and/or NIS-upper limb (NIS-UL) scores increased in most patients who received tafamidis. However, tafamidis did not prevent progression of disability by 43–55% after the first year[164,166]. Gait disorders[164,166] and autonomic dysfunction also progressed[164,166]. Progression of the neuropathy correlated with older age and a poorer clinical status at baseline[165]. Tafamidis was otherwise well tolerated in all studies.

On the basis of the trial results, tafamidis is approved for the treatment of stage 1 ATTRv–PN amyloidosis in Europe and in some countries in South America and Asia. Tafamidis is not yet approved for treatment of cardiac ATTR amyloidosis, but in the recent ATTRACT trial, two doses of tafamidis were compared with placebo in symptomatic patients with cardiac ATTRv amyloidosis (25%) and ATTRwt amyloid cardiomyopathy (75%)[167]. The primary analysis showed that all-cause mortality and rates of cardiovascular-related hospitalizations were lower with treatment with tafamidis than with placebo[167]. Tafamidis has been submitted to the European Medicines Agency (EMA) and is currently under review by the FDA for treatment of cardiac amyloidosis.

Diflunisal. Diflunisal is an NSAID that acts as a TTR stabilizer. A randomized, placebo-controlled, double-blind, multicentre, international study has been conducted to determine whether diflunisal modifies the progression of neurological disease in ATTRv amyloidosis[132]. The enrolment criteria were completely different than those in the phase III study of tafamidis: included patients had late-onset ATTRv amyloidosis associated with different TTR variants and a high NIS at baseline[130]. Progression of scores on the NIS + 7 nerve tests and the NIS score at 2 years after treatment were reduced significantly in patients who received diflunisal compared with that in patients who received placebo, and disease progression was reduced by 60%. However, during this study, 52% of patients discontinued treatment as a result of disease progression and liver transplantation. The conclusions that can be drawn are limited given that half the study population discontinued the treatment, but they confirm that TTR stabilizers cannot stop rapid progression of late-onset ATTRv amyloidosis over 2 years of follow-up[131].

Trials of gene-silencing therapies

RNA interference. RNA interference (RNAi) is a natural cellular process in which small interfering RNAs (siRNAs) mediate the cleavage of specific mRNAs, resulting in a robust and durable reduction in the expression of gene targets[168,169]. Formulations of lipid nanoparticles have emerged as agents for delivery of siRNAs to hepatocytes[169,170]. These parenterally administered lipid nanoparticles are predominantly delivered to the liver, with a small fraction distributed to other organs that have a fenestrated endothelium (for example, the spleen)[170,171]. The therapeutic strategy of mutant and ATTRwt knockdown by RNAi targeting of the liver was supported by the known benefits of lowering levels of mutant TTR in patients with ATTRv amyloidosis by liver transplantation[10,151] and by data that demonstrate improvements in pathology and clinical outcomes upon reducing levels of the amyloidogenic protein[172,173].

Use of RNAi therapy has been tested in successive stages in ATTRv amyloidosis in preclinical models[174] and clinical trials[133,167,175]. In placebo-controlled phase I trials, a single dose of anti-TTR siRNA encapsulated in one of two distinct formulations of lipid nanoparticles (known as ALN-TTR01 and ALN-TTR02) led to rapid, dose-dependent and durable lowering of mutant and non-mutant forms of TTR[175]. For ALN-TTR02, a dose of 0.3 mg/kg body weight reduced TTR levels by a mean of 86.8%. This reduction was shown to be RNAi-mediated with a good safety profile.

In a phase II study that included 29 patients with ATTRv amyloidosis, a dose of 0.3 mg/kg body weight of the RNAi drug patisiran every 3 weeks reduced the mean serum level of TTR by ~80%[167]. An open-label extension study[176] showed that measures of neuropathy impairment remained stable and that patisiran was generally well tolerated over 24 months of treatment[177].

In the phase III placebo-controlled APOLLO study, the efficacy of patisiran at 18 months was tested with a primary outcome of a change in modified NIS + 7 score from baseline[133]. The secondary objectives were to evaluate the effect of patisiran on quality of life and other clinically meaningful markers[133]. A total of 225 patients with different severity of disease were randomly assigned to receive patisiran or placebo and the two groups were balanced for disease severity and genetic variants. Participants were enrolled from 19 countries and carried a total of 39 pathogenic TTR variants to represent the worldwide ATTRv amyloidosis population.

All end points were better with patisiran than with placebo, regardless of the stage of the disease, the associated TTR variant and the age of onset. Improvements from baseline in mNIS + 7 scores occurred in 56% of participants who received patisiran and only 4% of those who received placebo. Improvements relative to baseline were also seen in Norfolk QoL scores (in 51.4% of patients who received patisiran versus in 10.4% in the placebo group), gait speed (in 53% of patients who received patisiran versus 13% of those who received placebo) and in the COMPASS-31 measure of autonomic symptoms[83].

Safety of patisiran in the APOLLO study was excellent[83]. Common adverse events that occurred more frequently with patisiran than with placebo included peripheral oedema (30% versus 22%) and infusion-related reactions (19% versus 9%). These events were mild or moderate. No clinically relevant changes in laboratory values related to patisiran were observed during the trial. On this basis, the EMA and FDA approved patisiran for treatment of polyneuropathy (stage 1 or stage 2) caused by ATTRv amyloidosis[178,179].

In an exploratory study of a prespecified subpopulation in the APOLLO trial with a cardiac phenotype (a baseline left ventricular wall thickness ≥13 mm and no history of hypertension or aortic valve disease), patisiran treatment led to a reduction in ventricular thickness, an improvement in global longitudinal strain parameters in the basal segments of the left ventricle and a decrease in levels of NT-proBNP. A post hoc analysis showed that adverse cardiac outcomes at 18 months after treatment initiation were also lower among patients who received patisiran than among those who received placebo, suggesting that patisiran halts or reverses the progression of the cardiac manifestations of ATTRv amyloidosis[180].

Revusiran is another RNAi drug designed for the treatment of ATTRv amyloidosis, and the ENDEAVOUR randomized, double-blind, placebo-controlled phase III trial was initiated to test its efficacy and safety. Patients enrolled in the trial were those with ATTRv amyloidosis cardiomyopathy; development of revusiran was halted in 2016 owing to greater mortality in the revusiran arm than in the placebo arm.

Antisense oligonucleotides. Inotersen (IONIS-TTRRx) is a second-generation antisense oligonucleotide that targets and reduces the levels of the *TTR* RNA transcript. Systemic biodistribution is broad, but its concentration is typically highest in the liver and kidneys, followed by the bone marrow, adipocytes and lymph nodes[181]. The ability of inotersen to decrease plasma levels of TTR by >80% was demonstrated in transgenic mice that expressed the Ile84Ser human TTR mutation[182]. In a phase I study of healthy volunteers, treatment with inotersen for 4 weeks was well tolerated and plasma levels of TTR were reduced by up to 96%[183].

The safety and efficacy of inotersen in ATTRv amyloidosis has been tested in the phase III randomized, double-blind, placebo-controlled NEURO-TTR study[84]. In this trial, 300 mg inotersen was administered subcutaneously three times in the first week and once weekly for the next 63 weeks. The primary end points were the change in the mNIS + 7 (Ionis) score[184] and in the Norfolk QoL-DN questionnaire compared with baseline. A total of 172 patients were enrolled in the trial. Significant differences in both primary end points were seen between the inotersen group and the placebo group at 15 months. Among patients treated with inotersen, 36.6% had a stable or improved mNIS + 7 score at 15 months versus 19.2% among those who received placebo[84]. However, inotersen did not improve echocardiographic variables when compared with placebo.

In the NEURO-TTR trial, three patients who received inotersen developed severe thrombocytopenia, and one of these three had a fatal cerebral haemorrhage. In addition, three patients who received inotersen developed glomerulonephritis. Four more deaths occurred among the patients who received inotersen, but none of these events were considered to be related to the experimental drug. With close monitoring for thrombocytopenia and renal problems, the drug can be given safely. The EMA and FDA approved inotersen for the treatment of stage 1 or stage 2 ATTRv amyloidosis[185,186].

Emerging treatment approaches

Another strategy for treatment of ATTRv amyloidosis is to facilitate clearance of amyloid deposits with monoclonal antibodies against a component of these deposits. Serum amyloid P component (SAP) is a ubiquitous, non-fibrillar plasma glycoprotein that is present in amyloid deposits and can be targeted with the small-molecule drug miridesap, which depletes circulating SAP, in combination with dezamizumab, a fully humanized anti-SAP monoclonal antibody, to deplete the residual SAP.

Cryptic epitope
Epitopes that are not exposed for presentation to the immune system under normal conditions.

Administration of this antibody to mice with amyloid deposits that contain human SAP triggers a potent, complement-dependent macrophage-derived giant cell reaction that swiftly removes visceral amyloid deposits[187]. An open-label, single-dose escalation, phase I trial demonstrated safety and efficacy of dezamizumab in 15 patients with systemic amyloidosis[188]. However, development of dezamizumab by GlaxoSmithKline has been discontinued[189].

A mouse monoclonal antibody, called T24, that recognizes the cryptic epitope of conformationally changed TTR has also been developed[190]. In model rats that expressed human Val30Met TTR in various tissues and exhibited non-fibrillar deposits of TTR in the gastrointestinal tracts, T24 inhibited TTR accumulation[190]. In addition, in the same rat model, humanized T24 inhibited TTR fibrillation and promoted macrophage phagocytosis of aggregated TTR. This humanized antibody did not bind to ATTRwt that functioned normally in the blood. On the basis of these findings, a phase I, open-label clinical trial will be conducted including people with ATTRv amyloidosis[191].

Conclusions and future prospects

ATTRv amyloidosis is the most severe hereditary polyneuropathy of adult onset. The disease is distributed worldwide and is genetically heterogeneous. The initial clinical presentation of neuropathy is diverse and causes delays in diagnosis. *TTR* gene testing can be used to rule out ATTRv amyloidosis and should be performed early if the condition is suspected. Cardiac ATTRv amyloidosis is underdiagnosed but is present in half of patients and should be looked for as early as possible using cardiac biomarkers and multimodal imaging.

The dissociation of mutant TTR homotetramers into monomers, the disruption of the blood–nerve barrier and the misfolding and subsequent aggregation of TTR are major events in the pathogenesis of neuropathy. The therapeutic arsenal has widened to include liver transplantation, TTR stabilizers and *TTR* gene silencers. Most patients should benefit from active treatment regardless of the stage of their disease. However, the abilities of these treatments to slow or reverse the disease and their safety differ. Further genetic studies of patients with ATTRv amyloidosis are expected to reveal more about the variability of age at onset and male predominance.

The challenges in the study of ATTRv amyloidosis in the coming years will be to assess the effects of amyloid deposit clearance with monoclonal antibodies, the potential of curative gene replacement therapy, the effects of disease-modifying therapies in individuals who are presymptomatic or who have recently become symptomatic and how the different treatment modalities could work together.

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