Transthyretin-associated Familial Amyloid Polyneuropathy—Current and Emerging Therapies

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Abstract

Transthyretin-associated familial amyloid polyneuropathy (TTR-FAP), the most common form of systemic hereditary amyloidosis worldwide, is a late-adult-onset autosomal dominant disease caused by mutations in the *TTR* gene, with peaks in prevalence in endemic areas. The clinical picture is dominated by a progressive length-dependent polyneuropathy with onset in the feet with loss of temperature and pain sensations, accompanied by life-threatening autonomic dysfunction and infiltrative cardiomyopathy, as well as ocular disturbances. Variable expressivity, in terms of age of onset and involvement of extra-neurologic sites, can be due to different mutations, but is also observed among individuals with the same mutation in different countries. Therefore, diagnosis of TTR-FAP is often a challenge and must rely on careful clinical assessment combined with a multidisciplinary approach. Elimination of the synthesis of mutated TTR, through liver transplantation, may arrest the progressive neuropathy but not the cardiac and ocular involvement. Novel drugs have recently been developed based on a better understanding of the molecular mechanisms of the disease. Drugs that prevent the misfolding and deposition of mutated TTR have entered clinical trials, and one of these, tafamidis meglumine, has been approved in Europe and is now clinically available. Other medicines are now in the pipeline aimed at suppressing the expression of the mutated *TTR* gene or at promoting amyloid fibril destructuration, favoring resorption of amyloid deposits. These recent advancements provide grounded hope of an imminent significant improvement in the care of this life-threatening multi-system disease.

Keywords

Amyloidosis, familial amyloid polyneuropathy, transthyretin amyloidosis, hereditary neuropathy, peripheral neuropathy

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Amyloidoses encompass a heterogeneous group of disorders characterized by the accumulation and extracellular deposition of insoluble aggregates of misfolded fibrillar proteins termed amyloid, which can lead to tissue damage and organ dysfunction.[1] They can be exceptionally rare or rather frequent, acquired or hereditary, localized or systemic, quite indolent or life-threatening. Amyloidoses are classified based on the main protein forming the deposits and include, as of today, 28 different forms.[2,3]

Transthyretin (TTR) amyloidoses (ATTR) retain much of the complexity of this family of maladies. Age-related deposition of wild-type TTR causes senile cardiac or systemic amyloidosis (SCA or SSA),[4] affecting up to 25 % of ultra-octogenarian people,[5] whereas mutations in the TTR gene can result in hereditary forms of the disease,[6] characterized either by a predominant neurologic phenotype, also known as familial amyloid polyneuropathy (FAP), by a unique cardiac disease or by a mixture of the two. Several mutations are exceedingly rare and have been described only in single individuals or in single kindreds,[7] whereas others are highly prevalent in certain geographical regions or among certain ethnic groups (such as the Val122Ile mutation, which can lead to isolated heart involvement and is carried by up to 4 % of African-Americans).[8] In addition, TTR-derived amyloid deposits can be focal (in the ligaments and tendons of aged individuals)[9,10] or can be widespread in systemic forms of ATTR.

Here we will review the pathophysiologic and clinical characteristics of TTR-associated familial amyloid polyneuropathy (TTR-FAP), with a focus on current and prospective treatments.

Transthyretin—Intrinsic Instability and Pathogenic Mutations

Transthyretin, formerly termed prealbumin for its electrophoretic migration pattern, is a secreted polypeptide chain consisting of 127 amino acid residues[11] with an approximate mass of 14 kDa and a prominent β-sheet secondary structure.[12] Four monomers associate non-covalently to form a tetramer which can bind thyroxine and holo-retinol-binding protein and circulates in the blood and in cerebrospinal fluid. However, the majority of circulating TTR is not bound. Virtually all TTR in plasma is of hepatic origin, but extrahepatic sites of TTR secretion include the choroid plexus and retinal pigment epithelium. Under physiologic conditions, TTR reaches a plasma concentration of 0.2–0.4 g/l and has a half-life of approximately 1–2 days.[7,13]

Mutations in the TTR gene can be associated with hereditary forms of ATTR that are transmitted as an autosomal-dominant trait (see *Table 1*). Based on *in vitro* studies, disease-associated mutations have been shown to reduce the thermodynamic stability of TTR tetramers, favoring their dissociation into monomers, which can undergo unfolding, self-aggregation and amyloid formation.[6,7,14,15] This concept has been corroborated by observations made on a non-pathogenic variant of TTR, Thr119Met, which confers a stabilizing effect on TTR tetramers in association with both the wild-type and the amyloidogenic Val30Met variant of TTR.[16,17] Remarkably, compound heterozygotes for Thr119Met and Val30Met variants of TTR display a more benign evolution of the TTR-FAP associated with the Val30Met mutation.[16]

A certain degree of instability is an intrinsic feature of TTR, since even wild-type TTR can form amyloid deposits during aging.[4] Such inherent amyloidogenic propensity of TTR is further enhanced in the presence of a disease-related variant of the protein. In affected individuals heterozygous for one of the pathogenic mutations, both normal and variant TTR are found within the deposits[18–21] and this could explain why amyloid deposits can progress in patients for whom liver transplantation has almost eradicated the mutant TTR circulating in the blood.[18–20]

C-terminal fragments of TTR are found in the deposits in addition to full-length TTR molecules in some cases.[22–24] The enzyme responsible for this remains to be identified and it is currently unknown whether proteolysis of TTR precedes or follows amyloid formation.[1] Also, TTR fibrillogenesis and deposition are believed to be influenced by the interaction with glycosaminoglycans and the serum amyloid P component (SAP), which are common constituents of all types of amyloid deposits.[1]

Transthyretin-related Familial Amyloid Polyneuropathy

Epidemiology and Genetics

FAP associated with mutations of TTR is the most common form of hereditary ATTR and of hereditary amyloidoses in general.[25] First reported in 1952 by Andrade in Portugal,[26] it was subsequently described in Japan[27] and Sweden[28] and it is now believed to occur worldwide. At present, 113 different mutations in the *TTR* gene have been associated with amyloid formation (see *Table 1*). Among these, the most common pathogenic mutation by far is Val30Met, which is responsible for the presence of endemic foci of TTR-FAP in northern Portugal,[29] northern Sweden,[28] Japan,[30] and the Balearic Islands,[31] but has also been reported elsewhere. For some mutations there is a strong genotype–phenotype correlation, whereas other variants show higher degrees of phenotypic variation.[7,32,33] For example, the Val30Met mutation has a later clinical onset (at 55–60 years of age on average) and a lower penetrance (69 % at 90 years) in northern Sweden[34] and an earlier clinical manifestation (at 30–35 years of age) and a much higher penetrance (89 % at 60 years and 91 % at 80 years) in Portugal.[35] The gender of the transmitting parent was shown to impact the penetrance of the disease.[34,36] Genetic anticipation has been described in several kindreds in endemic areas.[37–39] The genetic factors underlying these phenomena are currently unknown but are under intense scrutiny.[40–44] Moreover, anecdotal reports of discordant cases of TTR-FAP in monozygotic twins[45–49] suggest the involvement of presently unidentified environmental factors.

Clinical Features

The clinical picture of TTR-FAP is dominated by the association of a sensorimotor and autonomic polyneuropathy with a family history of neuropathy.[7,50,51] The clinical onset is characterized by numbness and spontaneous pains in the feet, impaired thermal sensitivity over the feet and reduced pinprick sensation with preserved light touch sensation and proprioception. The hallmark is the relentless proximal progression of the sensory deficits, which, during the following months and years, extend to the thighs and to the upper limbs. As larger sensory and motor nerve fibers become involved, impairment of light touch and deep sensations, with motor deficits, ensues. These manifestations are often accompanied or preceded by carpal tunnel syndrome. Unassisted walking becomes progressively difficult and autonomic dysfunction emerges with cardiocirculatory, gastrointestinal and genito-urinary dysfunctions including orthostatic hypotension, severe constipation/episodic post-prandial diarrhea or alternation thereof, dysuria, urinary retention, and erectile dysfunction in men.[7,50,51] The latter can often be the first manifestation of the disease.

Differences have been reported in presentation between early-onset cases in endemic areas and late-onset cases in non-endemic areas (reviewed by Plante-Bordeneuve and Said).[51] In the latter cases there is a preponderance of males, milder autonomic dysfunction, and more frequent heart involvement.

Involvement of the ciliary nerve may lead to the so-called 'scalloped pupils', which are almost pathognomonic for FAP[26,52] and involvement of the recurrent laryngeal nerve can cause vocal hoarseness.

Central nervous system (CNS) manifestations—including recurrent cerebral hemorrhage, seizures, psychosis, deafness, and visual impairment—are present in association with some TTR mutations and, when they dominate the clinical picture, they are indicated as TTR-associated leptomeningeal or oculoleptomeningeal amyloidosis or cerebral amyloid angiopathy.[53–56]

Non-neurologic manifestations can arise as a result of TTR amyloid deposition in the heart, eyes, and kidneys. Progressive amyloid deposition within the heart induces electrical disturbances and restrictive cardiomyopathy with distinctive features with respect to other types of amyloid cardiomyopathy and which is often the cause of death in TTR-FAP patients.[57–59] When, in association with specific mutations, the cardiac involvement dominates the clinical picture, the disease is

Table 1: Transthyretin Mutations Associated with Amyloidosis

Mutation	Codon Change	Clinical Features	Geographic Kindreds	Mutation	Codon Change	Clinical Features	Geographic Kindreds
Cys10Arg	TGT – CGT	Heart, eye**, PN	US (PA)	Leu55Pro	CTG – CCG	Heart, AN, eye	US, Taiwan
Leu12Pro	CTG – CCG	LM	UK	Leu55Arg	– CGG	LM	Germany
Asp18Glu	GAT – GAA	PN	South America, US	Leu55Gln	– CAG	Eye, PN	US
Asp18Gly	– GGT	LM	Hungary	Leu55Glu	– CAG	Heart, PN, AN	Sweden
Asp18Asn	– AAT	Heart	US	His56Arg	CAT – CGT	Heart	US
Val20Ile	GTC – ATC	Heart, CTS	Germany, US	Gly57Arg	GGG – AGG	Heart	Sweden
Ser23Asn	AGT – AAT	Heart, PN	US	Leu58His	CTC – CAC	CTS, heart	US (MD) (FAP II)
Pro24Ser	CCT – TCT	Heart, CTS, PN	US	Leu58Arg	– CGC	CTS, AN, eye	Japan
Ala25Ser	GCC – TCC	Heart, CTS, PN	US	Thr59Lys	ACA – AAA	Heart, PN, AN	Italy, US (Chinese)
Ala25Thr	– ACC	LM, PN	Japan	Thr60Ala	ACT – GCT	Heart, CTS	US (Appalachian)
Val28Met	GTG – ATG	PN, AN	Portugal	Glu61Lys	GAG – AAG	PN	Japan
Val30Met	– ATG	PN, AN, eye, LM	Portugal, Japan, Sweden, US (FAP I)	Glu61Gly	– GGG	Heart, PN	US
Val30Ala	– GCG	Heart, AN	US	Glu62Lys	– AAG	PN	Italy
Val30Leu	– CTG	PN, heart	Japan	Phe64Leu	TTT – CTT/TTG	PN, CTS, heart	US, Italy
Val30Gly	– GGG	LM, eye	US	Phe64Ile	– ATT		
Val32Ala	– GCG	PN	Israel	Phe64Ser	– TCT	LM, PN, eye	Canada, UK
Val32Gly	– GGG	PN, AN	France	Gly67Glu	GGG – GAG		
Phe33Ile	TTC – ATC	PN, eye	Israel	Ile68Leu	ATA – TTA	Heart	Germany
Phe33Leu	– CTC	PN, heart	US	Tyr69His	TAC – CAC	Eye, LM	Canada, US, Sweden
Phe33Val	– GTC	PN	UK, Japan, China	Tyr69Ile	– ATC*	Heart, CTS, AN	Japan
Phe33Cys	– TGC	CTS, heart, eye, kidney	US	Lys70Asn	AAA – AAC	Eye, CTS, PN	US
Arg34Ser	AGA – AGC/T	PN, heart	US	Val71Ala	GTG – GCG	PN, eye, CTS	France, Spain
Arg34Thr	– ACA	PN, heart	Italy	Ile73Val	ATA – GTA	PN, AN	Bangladesh
Arg34Gly	– GGA	Eye	UK	Tyr75Ile	ACC – ATC	Heart	France
Lys35Asn	AAG – AAC	PN, AN, heart	France	Ser77Tyr	TCT – TAT	Kidney	US (IL, TX), France
Lys35Thr	– ACG	Eye	US	Ser77Phe	– TTT	PN, AN, heart	France
Ala36Pro	GCT – CCT	Eye, CTS	US	Tyr78Phe	TAC – TTC	PN, CTS, skin	France
Asp38Ala	GAT – GCT	PN, heart	Japan	Ala81Thr	GCA – ACA	Heart	US
Asp38Val	– GTT	PN, heart	Guiana	Ala81Val	– GTA	Heart	UK
Asp39Val	GAC – GTC	Heart	Germany	Ile84Ser	ATC – AGC	Heart, CTS, eye	US (IN), Hungary (FAP II)
Trp41Leu	TGG – TTG	Eye, PN	US	Ile84Asn	– AAC	Heart, eye	US
Glu42Gly	GAG – GGG	PN, AN, heart	Japan, US, Russia	Ile84Thr	– ACC	Heart, PN	Germany, UK
Glu42Asp	– GAT	Heart	France	His88Arg	CAT – CGT	Heart	Sweden
Phe44Ser	TTT – TCT	PN, AN, heart	US	Glu89Gln	GAG – CAG	PN, heart	Italy
Phe44Tyr	– TAT	PN, AN	France	Glu89Lys	– AAG	PN, heart	US
Ala45Thr	GCC – ACC	Heart	US	His90Asp	CAT – GAT	Heart	UK
Ala45Asp	– GAC	Heart, PN	US	Ala91Ser	GCA – TCA	PN, CTS, heart	France
Ala45Ser	– TCC	Heart	Sweden	Glu92Lys	GAG – AAG	Heart	Japan
Gly47Arg	GGG – CGG/AGG	PN, AN	Japan	Val93Met	GTG – ATG		Africa (France) Mali
Gly47Ala	– GCG	Heart, AN	Italy, France	Val94Ala	GTA – GCA	Heart, PN, AN, kidney	Germany, US
Gly47Val	– GTG	CTS, PN, AN, heart	Sri Lanka	Ala97Gly	GCC – GGC	Heart, PN	Japan
Gly47Glu	– GAG	Heart, PN, AN	Turkey, US, Germany	Ala97Ser	– TCC	PN, heart	Taiwan, US
Thr49Ala	ACC – GCC	Heart, CTS	France, Italy	Arg103Ser	GGC – AGC	Heart	US
Thr49Ile	– ATC	PN, heart	Japan, Spain	Ile107Val	ATT – GTT	Heart, CTS, PN	US
Thr49Pro	– CCC	Heart, PN	US	Ile107Met	– ATG	PN, heart	Germany
Thr49Ser	– AGC	PN	Indian	Ile107Phe	– TTT	PN, AN	UK
Ser50Arg	AGT – AGG	AN, PN	Japan, France/Italy, US	Ala109Ser	GCC – TCC	PN, AN	Japan
Ser50Ile	– ATT	Heart, PN, AN	Japan	Leu111Met	CTG – ATG	Heart	Denmark
Glu51Gly	GAG – GGG	Heart	US	Ser112Ile	AGC – ATC	PN, heart	Italy
Ser52Pro	TCT – CCT	PN, AN, heart, kidney	UK	Tyr114Cys	TAC – TGC	PN, AN, eye, LM	Japan, US
Gly53Glu	GGA – GAA	LM, heart	Basque, Sweden	Tyr114His	– CAC	CTS, skin	Japan
Gly53Ala	– ALA	LM	UK	Tyr116Ser	TAT – TCT	PN, CTS, AN	France
Gly53Arg	– AGA	LM	US	Ala120Ser	GCT – TCT	Heart	Afro-Caribbean
Glu54Gly	GAG – GGG	PN, AN, eye	UK	Ala120Thr	– ACT	PN, CTS	Japan
Glu54Lys	– AAG	PN, AN, heart, eye	Japan	Val122Ile	GTC – ATC	Heart	US
Glu54Leu	– CTG	Heart	Belgium	ΔVal122	– ΔΔΔ	Heart, PN	US (Ecuador), Spain
				Val122Ala	– GCC	Heart, eye, PN	US

** Double nucleotide substitution. ** Vitreous deposits. AN = autonomic neuropathy; CTS = carpal tunnel syndrome; LM = leptomeningeal; PN = peripheral neuropathy. Source: courtesy of Merrill Benson, MD.*

rather referred to as familial amyloid cardiomyopathy (FAC).[8,60] Ocular manifestations comprize abnormal conjunctival vessels, keratoconjunctivitis sicca, vitreous opacity and glaucoma.[61] Renal involvement can lead to proteinuria and end-stage renal disease requiring replacement therapy[62] and TTR-FAP patients have been shown to display inappropriately low serum levels of erythropoietin and anemia.[63]

Over the years, progression of amyloid neuropathy and visceral organ involvement is paralleled by significant body weight loss, eventually leading to cachexia. Death occurs on average within 10 years of clinical onset.[7,50,51,64]

Pathology and Pathophysiology

Amyloid deposits, which can be unambiguously detected based on their pathognomonic green birefringence when stained with Congo red and analysed under polarized light, start around endoneurial capillaries and slowly progress. The process is accompanied by degeneration of Schwann cells, destruction of unmyelinated fibers and in late stages, distortion of nerve fibers, demyelination and distal axonal degeneration.[51] The mechanisms underlying the toxic effect of TTR amyloid formation are poorly understood. Mechanical effects, as well as a direct toxic insult from intermediates of the amyloidogenic process, could coincide to drive the neurodegeneration observed in TTR-FAP patients.[65–69] Among Val30Met TTR-FAP patients, the composition of amyloid deposits seems to have some phenotypic and prognostic implications. Presence of TTR fragments in addition to full-length TTR within amyloid deposits is associated with later clinical onset and cardiac involvement.[70] Moreover, patients having both fragmented and intact TTR molecules within the amyloid deposits are more likely to deteriorate pre-existing cardiomyopathy and heart failure after liver transplantation.[71]

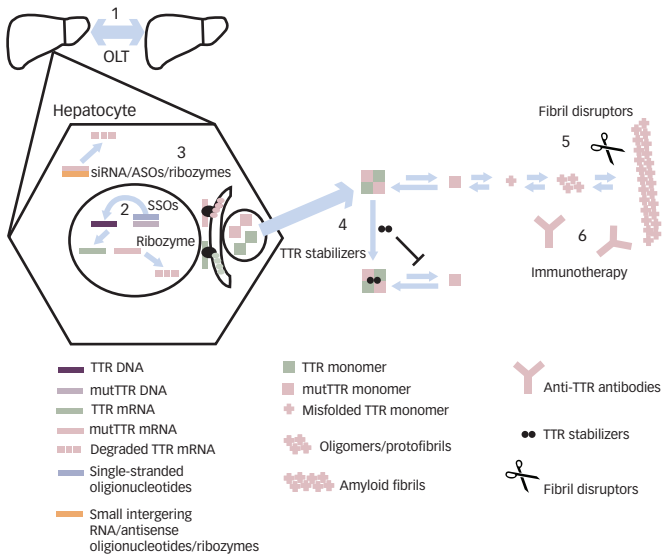
Diagnosis

Physical examination and electrophysiologic tests should point towards a form of length-dependent small-fiber sensorimotor polyneuropathy.[51]

In patients with no family history of neuropathy—not an uncommon occurrence in TTR-FAP due to the late onset and the low penetrance of TTR mutations in some areas[51]—a nerve biopsy may be required to differentiate amyloid polyneuropathy from other conditions, such as chronic inflammatory demyelinating polyneuropathy (CIDP).[51,72,73] In these cases, demonstration of amyloid deposits within the nerve allows diagnosis of an amyloid polyneuropathy. However, to possibly spare such an invasive procedure, abdominal fat aspiration, even if associated with a lower sensitivity compared with amyloid light-chain (AL) amyloidosis, should be performed early in the diagnostic work-up when amyloidosis is suspected, together with a search for mutations in *TTR* by means of direct sequencing. The demonstration of an amyloidogenic variant in the gene is mandatory for the ultimate diagnosis of this disease. Due to its significant allelic heterogeneity, sequencing of the entire coding regions of the *TTR* gene is always recommended.

A differential diagnosis among the various forms of amyloidosis associated with peripheral nerve involvement—that is, immunoglobulin AL amyloidosis and FAP with mutation in apolipoprotein A-I or gelsolin—may be required in some cases.[74]

Figure 1: Current and Emerging Therapies Against Transthyretin Amyloidosis



1: Orthotopic liver transplantation from cadaveric or living donors replaces the main site of transthyretin (TTR) production; 2: Targeted gene repair based on single-stranded oligonucleotides can convert the mutated TTR gene into the wild-type counterpart; 3: Ribozymes, small interfering RNA and antisense oligonucleotides can be used to downregulate the production of the disease-associated TTR; 4: Stabilizers of TTR tetramers, including diflunisal and tafamidis, can prevent their dissociation into monomers, therefore interfering with the rate-limiting step of TTR amyloidogenesis; 5: Disruptors of amyloid fibrils, including 4'-iodo-4'-deoxydoxorubicin and doxycycline, can favor the disaggregation and removal of amyloid fibrils; 6: Antibodies against misfolded TTR, TTR fibrils or serum amyloid P can prevent amyloid formation and/or favor its removal. ASO = antisense oligonucleotide; mRNA = messenger RNA; mutTTR = mutated transthyretin; OLT = orthotopic liver transplantation; siRNA = small interfering RNA; SSO = single-stranded oligonucleotide; TTR = transthyretin.

The demonstration of a monoclonal component in a patient with amyloid neuropathy is compatible with a diagnosis of AL amyloidosis requiring aggressive chemotherapy against the causal plasma cell clone.[75] However, the possibility of an incidental association of a non-AL systemic amyloidosis and an unrelated monoclonal gammopathy (more often a monoclonal gammopathy of undetermined significance [MGUS])[76] should be taken into account.[77,78] In these cases, unambiguous typing of the amyloid deposits with immuno-electron microscopy[79] or mass spectrometry-based techniques[80–82] and DNA testing is decisive.

FAP associated with mutations of apolipoprotein A-I (Gly26Arg, also termed Iowa type) is rare and is generally accompanied by extra-neurologic involvement including renal, hepatic and gastrointestinal involvement.[83] If ATTR and AL amyloidosis have been excluded as causes of a biopsy-proven amyloid polyneuropathy, apolipoprotein A-I Gly26Arg is the most likely candidate to be considered. On the other hand, the epidemiologic and clinical peculiarities of the remaining type of FAP render it unlikely to be considered in the differential diagnosis of an amyloid-associated sensorimotor polyneuropathy. Indeed, the form associated with mutations of gelsolin (also called FAP of Finnish type) is extremely rare, is present almost exclusively in Finland and typically presents with corneal lattice dystrophy followed by cranial neuropathy and cutis laxa.[84]

Alternatively, in the presence of a family history of TTR-FAP and a clear clinical picture, DNA testing is often sufficient to achieve a diagnosis.[51]

However, the possibility of alternative etiologies (CIDP, AL amyloidosis) should be formally considered.

Finally, once a diagnosis of TTR-FAP has been achieved, careful extra-neurologic investigations, including ophthalmologic, cardiologic , and nephrologic assessment, should be performed to determine the level of systemic involvement. Patients and family members are also to be offered adequate genetic counseling.

Treatment—Current and Prospective Therapies

An intensive and multidisciplinary approach – including neurologic, cardiologic, ophthalmologic, nutritional, nephrologic, and rehabilitative interventions – is required to alleviate the symptoms of TTR-FAP patients and to prevent complications associated with disease.[51,85]

However, etiologic treatments are needed to interfere with the cascade of events leading from TTR production to amyloidogenesis and organ dysfunction (see *Figure 1*).

Reduction of the Amyloidogenic Precursor—Liver Transplantation

Akin to other types of systemic amyloidosis, the most effective way to achieve this goal is to eradicate or minimize the synthesis of the amyloidogenic precursor. This was attempted for the first time in 1990 through liver transplantation.[86] Since then, more than 1,900 liver transplantation procedures have been performed, both from cadaveric and living donors (FAP World Transplant Registry, as of 31 December 2010, see www.fapwtr.org). Liver transplantation in TTR-FAP patients results in a sustained and significant reduction in plasma levels of mutated TTR[87–89] and leads to increased survival.[64,90] Non-Val30Met genotypes, a clinical onset after 50 years of age and an advanced clinical stage at the time of surgery adversely influence the outcome of liver transplantation.[64,91] Therefore liver transplantation should ideally be performed in TTR-FAP patients at an early stage of the disease. On the other hand, pre-emptive liver transplantation in individuals at genetic risk of the disease is not feasible due to the incomplete penetrance of pathogenic *TTR* mutations.[51]

Following liver transplantation, visceral amyloid deposits can slowly regress, as documented by whole-body scintigraphy using radiolabeled SAP.[92] More often, amyloid neuropathy does not significantly improve, but its progression can be halted.[93] Liver transplantation does not generally impact the progression or *de novo* appearance of amyloid deposition in the eye or in the CNS, probably due to the unaffected local production of mutated TTR in the retina and in the choroid plexus.[94–96] In the former case, pan-retinal laser photocoagulation aimed at destroying the TTR-producing retinal pigment epithelium could represent a valid therapeutic option,[97] but further investigations are needed.

Remarkably, cardiac amyloidosis also progresses despite almost complete removal of variant TTR from the circulation with liver transplantation, most probably due to the continuous deposition of wild-type TTR in the myocardium.[18,19] The same mechanism could explain the progression of amyloid neuropathy occasionally seen in TTR-FAP patients who underwent liver transplantation,[20] but in these cases the participation of mutated TTR synthesized by the choroid plexus and released in the endoneurial space via the subarachnoid space[98] should be considered. In highly selected patients with severe involvement of the heart or kidney, a combined heart and liver or kidney and liver transplantation is a valid therapeutic option.[99,100]

Explanted livers of TTR-FAP patients, which are typically amyloid-free and do not show any abnormality other than producing a mutated TTR, can be used for so-called 'domino' liver transplantations, which, however, entail the risk of transmitting ATTR to the final recipient.[101,102]

Due to the shortage of liver donors, the requirement for life-long immunosuppressive regimens in recipients, the slight effect on ocular and cerebral deposits and the unfeasibility of liver transplantation for asymptomatic mutation carriers, other therapeutic approaches are urgently needed to halt the production of mutant TTR.[103]

Targeted Gene Repair

The possibility of correcting a pathogenic mutation in the mammalian genome by RNA/DNA oligonucleotides[104] has encouraged researchers to apply this approach in order to repair a mutated *TTR* allele. As a proof of principle, a human hepatic cell line (HepG2) has been used to convert the endogenous wild-type *TTR* gene into the gene encoding the Val30Met mutant of TTR.[105] Single-stranded oligonucleotides (SSOs) proved more efficient than chimeraplasts in achieving the desired gene conversion *in vitro*.[106] The same approach was then applied to transgenic mice expressing murine TTR Val30Met. Intrahepatic, but not intraperitoneal, injection of SSOs led to a gene conversion of 8–9 % at both messenger RNA (mRNA) and protein level.[107]

This strategy requires further methodologic development[103] and the capacity to target extrahepatic sites of TTR production needs to be assessed. Moreover, as observed with liver transplantation, this approach will not halt the progression of amyloid brought about by wild-type TTR if started after disease onset.

However, targeted gene repair could find invaluable applications in the treatment of asymptomatic carriers, especially in areas with high penetrance of pathogenic mutations, provided that safe and efficient protocols for gene conversion can be developed.

Suppression of Mutant Transthyretin Messenger Ribonucleic Acid

Earlier attempts at gene knockdown therapy in ATTR have aimed at achieving specific downregulation of mutant TTR mRNA, without altering the expression of wild-type TTR mRNA. Catalytically active RNAs termed hammerhead ribozymes allowed a significant reduction in wild-type or Val30Met TTR at mRNA and protein levels, in a cell-free system or in cell lines, to be achieved, using different constructs for the normal and mutated TTR.[106,107] Also, hammerhead and hairpin ribozymes designed against another pathogenic TTR mutation, Glu61Lys, resulted in a significant reduction in mutant—but not wild-type—TTR (both mRNA and protein) in cell lines.[108] Likewise, small interfering RNA (siRNA) was shown to downregulate Val30Met, but not wild-type, TTR mRNA and protein expression, even in cell lines expressing both alleles.[109]

Compared with targeted gene repair, which aims at correcting the genetic defect at the genomic level and can, therefore, be permanent, the above-mentioned gene knockdown technologies are transient and rely on continuous administration of the knockdown agent. A Phase I

study for evaluating the toxicity profile, best schedule and effect on plasma TTR concentration of siRNA targeting TTR mRNA has been performed. The results were reported at the recent Eighth International Symposium on Familial Amyloidotic Polyneuropathy held on November 20–22, 2011 at Kumamoto, Japan. Data presented at that time were from 31 patients (eight received placebo and 23 received drug) and showed that administration of ALN-TTR01 resulted in statistically significant reductions in serum TTR protein levels in ATTR patients. Lowering of serum TTR protein was found to be dose-dependent, rapid, and durable after just a single dose.[110]

Alternative knockdown strategies based on the use of antisense oligonucleotides (ASOs) have been developed which aim at silencing both variant and normal TTR. The basis for this strategy relies on the fact that both variant and normal TTR are found in the deposits of TTR-FAP patients and, on the other hand, that TTR expression is dispensable, since *TTR*-null mice are viable, fertile, and do not show any obvious phenotype apart from depressed levels of plasma retinol and thyroid hormone.[111]

Subcutaneous injection of anti-TTR ASOs in transgenic mice homozygous for human Ile84Ser TTR and expressing high levels of the variant TTR resulted in a significant reduction in hepatic TTR mRNA and in circulating levels of TTR.[112] This reduction in TTR levels could be protracted for up to six weeks by repeated applications of ASOs and was not accompanied by significant hepatotoxicity.[112] No reduction in TTR levels could be observed in the choroid plexus, unless anti-TTR ASOs were administered locally via intracerebral ventricular injection.[113] Studies are currently ongoing to assess the safety of anti-TTR ASOs in non-human primates.[114]

Immunization

Based on the enthusiasm accompanying the development of amyloid-β (Aβ) immunotherapy against Alzheimer's disease,[115] an active immunization approach has been tested as a potential treatment of ATTR in a preclinical model. Previous work had identified a TTR variant—TTR Tyr78Phe—which exposes a cryptic epitope present only on highly amyloidogenic TTR variants or on amyloid fibrils.[116] Immunization of transgenic mice expressing human Val30Met TTR[117] with TTR Tyr78Phe reduced or inhibited the formation of non-fibrillary and amyloid TTR as opposed to vehicle-treated or TTR Val30Met-immunized mice.[118] In TTR Tyr78Phe-treated animals, common sites of TTR deposition were characterized by the presence of an inflammatory infiltrate composed mainly of B lymphocytes and macrophages, whereas no histologic modifications were observed at TTR-synthesizing sites.[118] These results are encouraging, but further investigations are needed to extend these observations and develop a safe and effective immunotherapy against ATTR.

Anti-serum Amyloid P Component Therapy

Another strategy to interfere with the formation and persistence of amyloid deposits is directed against SAP. This is based on the observation that SAP prevents proteolysis of amyloid fibrils[119] and accelerates their formation *in vitro*.[120] A palindromic compound, (R)-1-[6-[(R)-2-carboxy-pyrrolidin-1-yl]-6-oxo-hexanoyl]pyrrolidine-2-carboxylic acid (CPHPC) has been developed which inhibits SAP binding to amyloid fibrils, dramatically depletes circulating SAP and eventually removes SAP from amyloid deposits.[121] The safety and biochemical and clinical effects of CPHPC in systemic amyloidoses have been investigated in a recent open-label proof-of-principle study.[122] Overall, the drug appeared to be safe, but amyloid deposits persisted in most patients. The observation that residual SAP was still present inamyloid-laden tissues despite long-term treatment with CPHPC, and might therefore account for the persistence of amyloid deposits in the study subjects, formed the rationale for an integrative approach. Using an experimental mouse model of amyloidosis, the same group has investigated the effect of a combination of CPHPC treatment with the administration of a specific anti-SAP antibody.[123] Mice treated with CPHPC and receiving a single injection of anti-SAP antibody showed a rapid complement-dependent, macrophage-derived giant-cell reaction, which resulted in a striking reduction in amyloid load when compared with untreated animals or animals receiving CPHPC alone.[123] A fully humanized version of an anti-SAP monoclonal antibody is currently under investigation.

Fibril Disruptors

In 1995, we serendipitously discovered the anthracycline 4'-iodo-4'-deoxydoxorubicin (IDOX) as the prototype of a class of compounds able to inhibit protein aggregation *in vitro*, in animal models of systemic amyloidosis[124] and in patients.[125,126] The first study showed that IDOX presented high affinity for all types of amyloid deposits and would have been a candidate for the treatment of all types of amyloidosis.[124] It was demonstrated that IDOX was capable of inducing the destructuration of TTR amyloid fibrils.[127] In consideration of the cytotoxicity of IDOX, in the following years, the tetracycline antibiotics were investigated on the basis of structural homologies with the aglycone moiety of the anthracyclines. Doxycycline was able to interfere with TTR Leu55Pro amyloid formation and to disrupt TTR fibrils.[128] Cardoso and Saraiva assessed the activity of doxycycline *in vivo* in the transgenic mouse model of ATTR and showed that the administration of doxycycline in drinking water resulted in the complete prevention of amyloid deposit formation.[129] More recently, it has been reported that tauroursodeoxycholic acid (TUDCA), a biliary acid, administered to the same transgenic mouse model was effective at lowering deposited non-fibrillar TTR, as well as the levels of markers associated with pre-fibrillar TTR, but only at young ages. Combined cyclic doxycycline and TUDCA administration to mice with amyloid deposition was more effective than either doxycycline or TUDCA individually in significantly lowering TTR deposition and associated tissue markers.[130] The observed synergistic effect of doxycycline plus TUDCA, in the range of quantities tolerable in humans, in the TTR transgenic mice models prompted their application in FAP, particularly in the early stages of disease. Based on these data, a Phase II clinical trial assessing the safety, efficacy and pharmacokinetics of doxycycline plus TUDCA in ATTR was designed and implemented at our institution in Pavia, Italy (NCT01171859 http://clinicaltrials.gov). The preliminary results were reported at the recent Eighth International Symposium on Familial Amyloidotic Polyneuropathy.[131] In the 20 patients treated, no serious adverse events were registered. No clinical progression of cardiac involvement was observed. The neuropathy (Neuropathy Impairment Score in the Lower Limbs [NIS-LL] and Kumamoto score) remained substantially stable over one year. These preliminary data indicate that the combination of doxycycline and TUDCA stabilizes the disease for at least one year in the majority of patients with an acceptable toxicity profile.[131]

Stabilization of Transthyretin Tetramers

The discovery that TTR tetramer dissociation is the required first step in the amyloidogenic cascade has opened the avenue to the discovery of TTR kinetic stabilizers, acting like pharmacologic chaperones which halt the amyloid process by preventing tetramer dissociation.[15,132] Although numerous structurally distinct TTR kinetic stabilizers have been identified,[132] only two drugs, diflunisal (NCT00294671) and tafamidis meglumine (NCT01435655) have entered clinical trials. The results of the double-blind placebo-controlled diflunisal trial are not yet available; preliminary data indicate that the drug is well tolerated.[133] The efficacy and safety of tafamidis (20 mg orally administered once daily) were evaluated in an 18-month randomized double-blind placebo-controlled trial involving 128 patients with TTR-FAP with the Val30Met mutation and primarily stage I disease. After 18 months of treatment, neuropathy did not progress in 60 % of patients who received tafamidis meglumine versus 38 % of the placebo group. Neurologic deterioration was decreased by 52 %; quality of life and modified body mass index were maintained under tafamidis meglumine and worsened under placebo.[134] Of the 91 patients completing the 18-month treatment period, 86 were subsequently enroled in an open-label extension study, in which they all received once-daily 20 mg tafamidis for a further 12 months. The rate of change in the NIS-LL score during the 12 months of treatment in this study was similar to that observed in those patients who had been randomized and treated with tafamidis in the previous double-blind 18-month period.[134]

In November 2011, tafamidis (Vyndaqel®, Pfizer) was granted marketing authorization by the European Commission for the treatment of ATTR in adult patients with stage I symptomatic polyneuropathy to delay peripheral neurologic impairment. The availability of a pharmacologic treatment for TTR-FAP is a milestone in the field of FAPs.

Treatment Strategy

A multidisciplinary approach to treatment of symptoms such as neuropathic pain, orthostatic hypotension, gastrointestinal malfunction, cardiac arrhythmias, cardiac failure, renal insufficiency, and ocular disturbances remains fundamental. In all patients (carrying the Val30Met mutation or other mutations) diagnosed at stage I of the disease, the choice now is between early liver transplantation and tafamidis. Liver transplantation seems to reduce the rate of progression of the neuropathy but does not protect against cardiac involvement, which occurs in about 80 % of cases of TTR-FAP. Cardiac and ocular amyloid depositions progress in patients who have undergone transplants,[135] but whether these manifestations will be controlled by tafamidis remains to be seen. Patients with early disease manifestation should be put on a liver transplant waiting list and tafamidis may be started with mandatory periodic (every six months) evaluation. The patient may stay continuously on tafamidis unless early signs of disease progression should appear. In this case, liver transplantation should be promptly carried out. These indications are subject to modification as more data on the long-term effects of tafamidis treatment are gathered through the ongoing post-marketing evaluation. As the data on the efficacy of diflunisal and doxycycline plus TUDCA mature, more treatment options might become available that will certainly affect treatment strategies.

Concluding Remarks

TTR-FAP has experienced revolutionary progress in our understanding of the mechanisms of this disease, which has translated into an unprecedented blooming of novel medicines. In the near future, the exploitation of these remedies is likely to deeply affect patient outcomes, with the grounded hope of making the invasive liver transplantation procedure no longer necessary. The availability of effective therapies will render the need for early diagnosis even more acute, as the amyloidoses are progressive, devastating diseases. We know now that it is very difficult to recover a damaged nervous system or heart; time is life. For this reason, the role of the neurologist remains fundamental: maintaining a high level of alert towards this rare, but potentially treatable, disease, will allow the timely identification of patients and the prompt institution of therapy. ■

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