Amyloidoses are a heterogeneous group of disorders with a variety of clinical presentations characterized by tissue deposition of insoluble, misassembled fibril proteins (amyloid) that disrupt normal tissue structure and function.\textsuperscript{1,2} Up to 30 different proteins have been identified as causing amyloidoses, including immunoglobulin light chain and transthyretin (TTR).\textsuperscript{1-3} Each disorder differs in presentation and prognosis, and presents challenges to diagnosis and treatment due to heterogeneous organ involvement and indistinct, often vague symptoms.\textsuperscript{3,4}

Hereditary transthyretin-mediated (hATTR) amyloidosis, caused by misfolding of the TTR protein, is a progressive, degenerative, multisystemic, life-threatening disease.\textsuperscript{2,5,6} The best contemporary estimates place the worldwide prevalence at 50,000 individuals, with varying phenotypic presentations.\textsuperscript{4,5,7} Individuals with symptomatic hATTR amyloidosis experience significant impairment to quality of life, regardless of clinical phenotype, when compared with age-matched controls from the general population.\textsuperscript{5,8} hATTR amyloidosis represents an unmet medical need. Therefore, the purpose of this review is to highlight the progressive and multisystemic nature of hATTR amyloidosis, diagnostic challenges associated with the disease, the lack of consistently effective treatments, and emerging therapies.

Pathophysiology

The protein TTR is primarily synthesized and secreted by the liver, and it transports thyroxine (T\textsubscript{4}) and retinol.\textsuperscript{2,9} Mutations in TTR lead to amino acid substitutions in the TTR protein that render the tertiary structure prone to misfolding into a β-pleated sheet configuration,\textsuperscript{4} thereby forming insoluble amyloid fibrils.\textsuperscript{2} This mutation results in an autosomal dominant disorder primarily affecting the nerves and heart.\textsuperscript{2} Clinical manifestations of hATTR amyloidosis are heterogeneous and influenced by TTR genotype, geographic location, and other genetic and environmental factors.\textsuperscript{20,21} Thus, the presenting symptomatology, age of onset, and rate of disease progression varies among the patient population.\textsuperscript{22}

More than 120 amyloidogenic TTR mutations have been identified.\textsuperscript{22} In the United States, the most common mutations, in
order from most to least prevalent, are Val122Ile, Thr60Ala, and Val30Met. Globally, the most common mutations, in order from most to least, are Val30Met, Val122Ile, and Glu89Gln. Val30Met is predominantly found in Portugal, Spain, France, Japan, Sweden, and descendants of these regions. It is often associated with polyneuropathy, whereas Val122Ile is frequently found in African Americans presenting late in life with cardiac symptoms. Other mutations, such as Glu89Gln, have been classically associated with a mixed phenotype.

Despite associations with particular phenotypes, there is considerable variability among patients. The Transthyretin Amyloidosis Outcomes Survey (THAOS) is an ongoing global, multicenter, longitudinal, observational patient registry, owned by Pfizer Inc, designed to characterize differences in disease presentation, diagnosis, and course among the various geographically dispersed patient populations. Data from this registry highlight the heterogeneity and multisystemic nature of hATTR amyloidosis.

**Clinical Features**

hATTR amyloidosis is a debilitating, multisystemic disorder with a heterogeneous clinical presentation. Symptoms may include autonomic dysfunction (eg, orthostatic hypotension or recurrent urinary tract infections), gastrointestinal (GI) dysfunction (eg, alternating symptoms of diarrhea and constipation, and unintentional weight loss), ocular manifestations (eg, vitreous opacity, or glaucoma), cardiac manifestations (eg, conduction block, cardiomyopathy, or arrhythmia), compromised renal function, or carpal tunnel syndrome. Although certain mutations may be associated with specific symptoms, hATTR amyloidosis remains a multisystemic disorder. For example, the Val122Ile mutation is associated with cardiac disease in approximately 97% of patients, but more than half also show signs and symptoms of carpal tunnel syndrome and sensory neuropathy. Similarly, the Val30Met mutation is predominantly associated with polyneuropathy, but a substantial proportion of patients experience cardiac involvement as well as other symptoms, such as GI manifestations.

Generally, symptoms of hATTR amyloidosis are progressive and may include sensory and motor impairment, autonomic and GI symptoms, or cardiac involvement. When polyneuropathy is present, it is a progressive sensorimotor and autonomic polyneuropathy. Polyneuropathy in hATTR amyloidosis may mimic neuropathy observed in other neuropathic conditions, potentially resulting in multiple misdiagnoses prior to identification of the causative condition. If untreated, patients with hATTR amyloidosis experience progressive symptoms until death occurs, typically 3 to 15 years after clinical presentation. When polyneuropathy is present, it is a progressive sensorimotor and autonomic polyneuropathy. Polyneuropathy in hATTR amyloidosis may mimic neuropathy observed in other neuropathic conditions, potentially resulting in multiple misdiagnoses prior to identification of the causative condition. If untreated, patients with hATTR amyloidosis experience progressive symptoms until death occurs, typically 3 to 15 years after clinical presentation.
to as familial amyloid cardiomyopathy (FAC). These clinical presentations are beginning to be referred to as hATTR amyloidosis with polyneuropathy and hATTR amyloidosis with cardiomyopathy, respectively. In practice, a wide range of overlapping clinical phenotypes is observed, and the majority of TTR mutations manifest as a mixed clinical phenotype involving both neurologic and cardiac symptoms. Clinical presentation can even differ among those with the same mutation and among family members.

There is also significant variability regarding age of onset. In an analysis of the worldwide THAOS patient registry, the median age of onset of signs and symptoms among all patients with hATTR amyloidosis in a large patient registry was 39 years of age. However, the average age of onset varies by country, with the median age of symptom onset in the United States estimated at 68.1 years. In other countries, such as Portugal and Brazil, the onset is substantially earlier, with the median age of onset estimated at 32.4 years and 31.4 years, respectively (Figure 2). Globally, the prevalence of hATTR amyloidosis in men and women differs across symptomatic patient populations; the ratio of affected men to women is highest in the United States (4.6) and lowest in Portugal (0.7).

The first signs and symptoms of hATTR amyloidosis with polyneuropathy can be recognized from the second decade of life, but most commonly, clinical manifestations of this disease develop in the third to fifth decade, affecting men and women equally. However, hATTR amyloidosis with cardiomyopathy generally has a later onset and is typically seen in those aged 60 years or more; men are more commonly affected than women. Since the inheritance is autosomal, there are likely gender-specific regulatory factors that result in clinical expression more frequently in males.

Regardless of clinical presentation, hATTR amyloidosis significantly impacts both patients and caregivers. In a survey evaluating the burden of hATTR amyloidosis on patients and caregivers in terms of health-related quality of life (HRQoL), mood, and functional status, researchers collected data on 38 patients with hATTR amyloidosis and 16 caregivers of patients with hATTR amyloidosis. Using the SF-12 instrument, investigators evaluated both physical and mental health in patients with hATTR amyloidosis through a 12-question survey, with possible scores ranging from 0 to 100, with 100 being the best possible score. SF-12 physical health summary scores were substantially lower in patients with hATTR amyloidosis compared with age-matched controls, ranging from 34.5 (SD = 11.0) in patients with hATTR amyloidosis with polyneuropathy who had undergone a liver transplant to 22.3 (SD = 5.8) in patients with hATTR amyloidosis with both polyneuropathy and cardiomyopathy who had not undergone a transplant.

Caregivers of patients with hATTR amyloidosis have moderate to high levels of fatigue. Notably, among caregivers without hATTR amyloidosis, the median amount of time spent per week caring for patients was 144 hours, and was estimated at a median of 100 hours weekly in caregivers who also had hATTR amyloidosis. Findings also indicate a large mental health burden of hATTR amyloidosis both in patients and caregivers.

### Diagnosis and Monitoring

Disease heterogeneity and its rarity make a diagnosis of hATTR amyloidosis challenging. However, making a correct diagnosis is vital to determining prognosis, treatment, and appropriate patient and family counseling. Timely diagnosis is also important because it allows patients the opportunity to receive appropriate care as early as possible in the disease course.

Diagnosis can be confirmed via biopsy of the affected tissue or organ followed by staining with Congo red to confirm the presence of amyloid. Diagnosis can be established less invasively through biopsy of the salivary gland, endoscopic biopsy of the gastric mucosa, or subcutaneous fat aspiration. Western blot analysis, immunohistochemical staining, laser microdissection,
proteomics, and mass spectrometry are subsequently used to characterize amyloid type.\textsuperscript{5,11,15} Limitations of biopsy are due to the often patchy distribution of amyloid deposits, sometimes necessitating multiple biopsies to confirm or exclude the diagnosis.\textsuperscript{3} Additionally, biopsy sensitivity depends on multiple factors, such as pathologist experience and protocol for Congo red staining.\textsuperscript{19}

In patients with a family history of disease and/or evaluation of symptomatic burden (ie, polyneuropathy), genetic testing is a crucial component to confirm a hATTR amyloidosis diagnosis as it identifies the specific TTR mutation present.\textsuperscript{5} Presymptomatic testing is now widely available and may be performed at the request of the patient with appropriate genetic counseling and follow-up.\textsuperscript{21}

The most sensitive test used for diagnosing cardiac involvement in hATTR amyloidosis is endomyocardial biopsy.\textsuperscript{5} However, it is not widely available.\textsuperscript{11} Therefore, other diagnostic tests are used for identifying cardiac involvement in hATTR amyloidosis, including electrocardiography (ECG), echocardiography, and cardiac magnetic resonance imaging (cMRI).\textsuperscript{5,11} Low QRS voltage is considered the most typical ECG finding in patients with cardiac involvement.\textsuperscript{5,11} On echocardiography, cardiac involvement is characterized by ventricular wall thickening with normal ejection fraction and absence of left ventricular dilation.\textsuperscript{5,11} Finally, cMRI can detect cardiac amyloid deposits.\textsuperscript{11} Highly specific findings on cMRI include faster gadolinium washout from the blood and myocardium and late enhancement, often with diffuse, global, and subendocardial distribution.\textsuperscript{5,11}

Nuclear cardiology using scintigraphic tracers can also provide diagnostic value by allowing clinicians to visualize amyloid infiltration throughout the body.\textsuperscript{2,5} These techniques are gaining widespread use because they have been shown to reliably evaluate extent and distribution of amyloid. Available tracers used to detect amyloid deposits include \textsuperscript{123}I-SAP, \textsuperscript{99m}Tc-aprotinin, \textsuperscript{123}I-metaiodobenzylguanidine, \textsuperscript{99m}Tc-3,3-diphospho-1,2-propanodicarboxylic acid (DPD), and \textsuperscript{99m}Tc-lyprophosphate. The pyrophosphate or DPD scan is particularly specific for TTR cardiac amyloidosis and can be helpful in deciding whether to pursue the diagnosis when noninvasive biopsies are negative.\textsuperscript{5,9} The scan cannot differentiate hATTR amyloidosis related to mutated versus wild-type TTR amyloidosis. All patients with suspected mutated TTR amyloidosis should have a pyrophosphate or DPD nuclear scan.\textsuperscript{5,26}

Methods of assessing and monitoring disease severity may include the polyneuropathy disability (PND) score, Portuguese FAP staging system, the Neuropathy Impairment Score (NIS), and Neuropathy Impairment Score + 7 tests (NIS + 7).\textsuperscript{5,18,27} PND scoring and the Portuguese FAP staging system primarily use factors such as a patient’s ability to walk in assessing patient outcomes, among other functional measures.\textsuperscript{5,18} The NIS score focuses on assessment of polyneuropathy. An updated version of the NIS score, known as the Neuropathy Impairment Score + 7 tests (NIS + 7), is more comprehensive in assessing topographical sensation and has emerged as the primary outcome measure in epidemiology surveys and therapeutic trials in patients with hATTR amyloidosis with polyneuropathy.\textsuperscript{5,27} Most recently, a more sensitive modified form of the NIS + 7 scoring system known as mNIS + 7 has been developed, 2 forms of which are currently used in clinical trials to evaluate emerging therapies for patients with hATTR amyloidosis with polyneuropathy.\textsuperscript{27,28}

**Current and Emerging Therapies**

Current treatment options for hATTR amyloidosis are limited, but orthotopic liver transplant (OLT) serves as an established option for patients with early-stage disease.\textsuperscript{2,4,5,18} When performed early in the disease course, OLT has been shown to slow progression of neuropathy and improve survival.\textsuperscript{2,4} However, several limitations exist to using OLT as a strategy for treating hATTR amyloidosis.\textsuperscript{2} OLT does not eliminate progression of amyloid deposition, because wild-type TTR can form deposits on the preexisting template of mutant TTR deposits; there are reports of disease progression and acceleration of amyloid deposition, particularly in patients with non-Val30Met mutations.\textsuperscript{2} In addition, OLT in patients with hATTR amyloidosis may result in decreased long-term HRQoL, although more study is needed in this area.\textsuperscript{29} Other limitations include limited organ availability, surgical morbidity and mortality, need for lifelong immunosuppression, and the high cost of transplant.\textsuperscript{2,4}

Heart transplant with or without OLT can be performed in cases of hATTR amyloidosis with significant cardiac involvement.\textsuperscript{2} However, without OLT, the transplanted heart will remain exposed to amyloid protein, as the native liver continues to produce mutant TTR that has the potential to deposit in the new graft.\textsuperscript{18,30} Heart transplant as a treatment strategy for hATTR amyloidosis has similar limitations to OLT, and many patients are not candidates due to advanced age (≥65 years).\textsuperscript{2,11}

As shown in Figure 3,\textsuperscript{20} there are multiple potential targets for targeted therapies to help correct or mitigate the underlying genetic defect in patients at risk of hATTR amyloidosis. These targets include areas in the liver, eye, and brain where misfolded proteins are produced, as well as TTR tetramers, monomers, misfolded amyloid proteins formed from TTR monomers, and resulting amyloid protein fibrils.\textsuperscript{20} Although not indicated for the treatment of hATTR amyloidosis, the off-label use of the nonsteroidal anti-inflammatory drug (NSAID) diflunisal (Dolobid) may stabilize TTR tetramers, potentially preventing dissociation of misfolded proteins into amyloid fibrils.\textsuperscript{2,52} A small-molecule medication, tafamidis (Vyndaqel), is a TTR kinetic stabilizer that selectively binds to the thyroxine-binding site and stabilizes TTR.\textsuperscript{2,11} Although tafamidis is approved for the treatment of hATTR amyloidosis with polyneuropathy in other countries, it has not yet been approved in the United States.\textsuperscript{2,20} A treatment currently in early clinical development is a monoclonal antibody
that targets serum amyloid P component (SAP). This antibody stimulates macrophage destruction of SAP, an integral structural component of all amyloid deposits in tissues.

Researchers are working to identify novel treatments intended to reduce production of amyloid fibrils or promote clearance of amyloid fibrils. Currently, 2 distinct oligonucleotide-based therapies for hATTR amyloidosis are being evaluated in clinical trials. One agent, a small interfering RNA (siRNA) is an RNA interference (RNAi) therapeutic called patisiran (ALN-TTR02). The other agent is an antisense oligonucleotide (ASO) called IONIS-TTRRx (IONIS-420915). Both agents bind to target RNA to silence genes and suppress the production of mutant and wild-type TTR protein and are in phase III clinical trials. Importantly, although ASO and siRNA therapies are similar in that they inhibit target gene expression through base pairing, ASO therapies differ from siRNA therapies in the intracellular mechanism of gene silencing.

**Conclusion**

hATTR amyloidosis is a progressive disease characterized by deposition of amyloid fibrils in various organs and tissues throughout the body, including the nerves, heart, GI tract, liver, and kidney. This results in a multisystemic disorder with a wide variety of clinical presentations, often leading to misdiagnosis and diagnostic delays. Unfortunately, incorrect or delayed diagnosis can severely impact patient quality of life because hATTR amyloidosis is a progressive disease that leads to significant disability and mortality. Once an accurate diagnosis of hATTR amyloidosis is established, it is key for new patients to undergo appropriate therapy as soon as possible. However, there is currently a need for new treatments, as the only established treatment currently available in the United States is OLT. Given the significant impact of hATTR amyloidosis, it is imperative that development and utilization of new, effective, and safe therapies continues.

**REFERENCES**

32. Diflunisal [prescribing information]. North Wales, PA: Teva Pharmaceuticals USA, Inc; 2016.