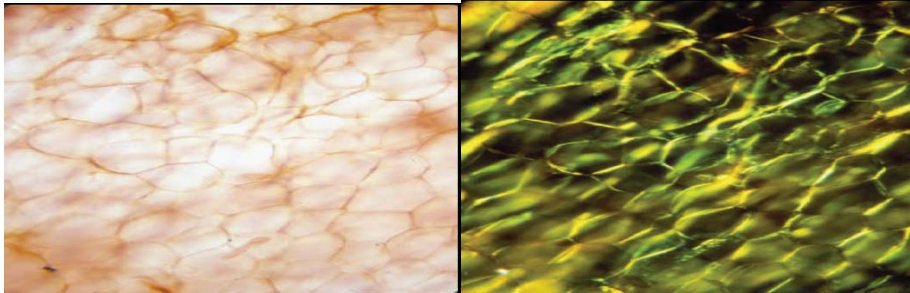


A Physician's Guide  
to  
Transthyretin Amyloidosis



Authored by  
Teresa Coelho, Bo-Göran Ericzon, Rodney Falk, Donna Roy  
Grogan, Shû -ichi Ikeda, Mathew Maurer, Violaine Planté-  
Bordeneuve, Ole B. Suhr, Pedro Trigo

©Amyloidosis Foundation

## What is amyloidosis?

Amyloidosis is a systemic disorder characterized by extra cellular deposition of a protein-derived material, known as amyloid, in multiple organs. Amyloidosis occurs when native or mutant polypeptides misfold and aggregate as fibrils. The amyloid deposits cause local damage to the cells around which they are deposited leading to a variety of clinical symptoms. There are at least 23 different proteins associated with the amyloidoses.

The most common type of amyloidosis is associated with a hematological disorder, in which amyloid fibrils are derived from monoclonal immunoglobulin light-chains (AL amyloidosis). This is associated with a clonal plasma cell disorder, closely related to and co-existing with multiple myeloma in approximately 15% of myeloma patients. Chronic inflammatory conditions such as rheumatoid arthritis or chronic infections such as bronchiectasis are associated with chronically elevated levels of the inflammatory protein, serum amyloid A, which may misfold and cause AA amyloidosis.

The hereditary forms of amyloidosis are autosomal dominant diseases characterized by deposition of variant proteins. The most common hereditary form is transthyretin amyloidosis (ATTR) caused by the misfolding of protein monomers derived from the tetrameric protein transthyretin (TTR). Mutations in the gene for TTR frequently result in instability of TTR and subsequent fibril formation. However, wild-type TTR, particularly in the elderly, can also aggregate and cause non-familial cases of TTR amyloidosis. Other proteins that have been associated with forms of hereditary amyloidosis include apolipoproteins AI and AII, cystatin C, lysozyme, fibrinogen A $\alpha$ -chain and gelsolin.

This pamphlet will concentrate on the most common form of hereditary amyloidosis – ATTR, including a discussion of the non-hereditary wild-type TTR disease.

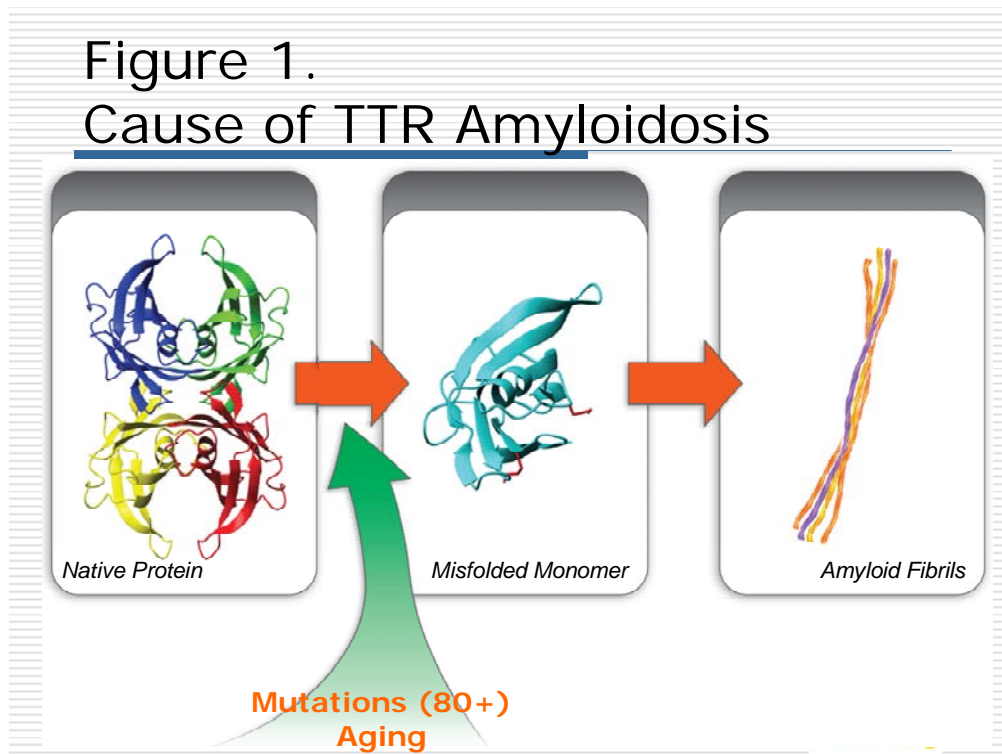
## What is transthyretin (TTR)?

Transthyretin (TTR) is a 127 amino acid, 55 kDa transport protein primarily synthesized in the liver. The protein is a carrier of thyroxine and retinol (vitamin A)-retinol binding protein complex. It is the tertiary carrier of thyroxine in plasma, carrying less T<sub>4</sub> than thyroxine-binding globulin (TBG) and albumin. In its native state TTR is a tetramer, i.e. four single chain TTR monomers form a tetrameric complex.

## What is transthyretin amyloidosis (ATTR)?

ATTR is caused by deposition of TTR amyloid fibrils in various tissues.

The hereditary form of ATTR is caused by autosomal dominant mutations in the TTR gene. The prevailing theory for amyloid formation associated with the amyloidogenic mutations is based on the observations that changes of amino acids are associated with destabilization and dissociation of the TTR tetramer, leading to abnormally folded monomers that ultimately self-assemble to amyloid fibrils (Fig. 1). These TTR amyloid fibrils are then deposited extracellularly in various tissues. There are more than 80 reported TTR single point mutations that have been associated with hereditary ATTR.



Non-hereditary ATTR is caused by spontaneous fibril formation of wild-type TTR, a disease primarily affecting the heart and clinically occurring predominantly in elderly (>65-70 years) men.

## How frequent is hereditary TTR amyloidosis (ATTR)?

ATTR is a rare disorder, with unequal distribution around the world. Certain clusters have been described, mainly in Portugal, Japan and Northern Sweden. The disease was first described from the Porto area in Portugal and is sometimes referred to as the Portuguese type of amyloidosis (this form is primarily related to the V30M mutation). Estimates have been made for the incidence and prevalence of ATTR dominated by polyneuropathy. In Europe, the incidence is estimated as 0.003 cases per 10,000 per year (or 0.3 new cases per year per 1 million inhabitants), with a prevalence estimate of 0.052 per 10,000 (or 5.2 cases per 1 million inhabitants). In the endemic area of Northern Sweden with a population of 600,000 individuals, the frequency of the gene is 1.5%, however, the penetrance is relatively low, and most patients develop the disease after the age of 50 years. The size of the patient population in the US is estimated to not exceed 6,400 patients. In Japan, approximately 400 patients with hereditary ATTR have been reported.

The prevalence of ATTR dominated by cardiomyopathy is unknown, but is almost certainly underdiagnosed, particularly in the African American V122I carrier population who are older than age 60 (approximately 150,000 individuals).

## Clinical aspects of ATTR

ATTR is a systemic disorder resulting in polyneuropathy, autonomic neuropathy and cardiomyopathy.

### *Polyneuropathy*

The main neuropathic feature of ATTR is a progressive sensorimotor and autonomic neuropathy. V30M (valine in position 30 is replaced by methionine) is the most common TTR mutation in patients presenting with neuropathy. The disease onset is usually in the third or fourth decade, but can occur later. The disease initially affects small unmyelinated nerve fibers which mediate pain and temperature sensations, and autonomic nerve functions. Typically, sensory neuropathy with paresthesia (numbness and tingling) and hypesthesia starts in the feet and progresses proximally. By the time the sensory neuropathy has progressed to the knee level, the hands have

usually become involved. With progression of the neuropathy, larger myelinated fibers become involved, impacting position and vibratory sensations, and reflexes. Carpal tunnel syndrome with median nerve compression is common and may be the first presenting symptom.

Motor neuropathy usually follows within a few years. Footdrop, wristdrop and disability of the hands and feet are frequent symptoms of motor neuropathy leading to difficulties in walking and performing fine hand movements.

Autonomic neuropathy often accompanies the sensory and motor deficits and may represent the initial disease presentation. Symptoms include orthostatic hypotension, constipation alternating with diarrhea, nausea, vomiting, delayed gastric emptying, erectile dysfunction, anhidrosis, urinary retention and incontinence. The gastrointestinal involvement results in weight loss and ultimately in cachexia.

Symptoms from the central nervous system (CNS) are rarely found in ATTR except in very rare forms of familial leptomeningeal amyloidosis, in which there may be cerebral hemorrhage with stroke like symptoms.

#### *Cardiomyopathy*

Cardiomyopathy may develop after the onset of neuropathy or may be the predominant feature of ATTR, particularly with certain mutations. ATTR cardiomyopathy occurs when TTR amyloid fibrils infiltrate the myocardium. This initially results in diastolic dysfunction, and may progress to symptomatic heart failure due to restrictive cardiomyopathy.

The mutation V122I (valine in position 122 is replaced by isoleucine), is a common mutation associated with cardiomyopathy, particularly among African-Americans, in whom a carrier frequency as high as 3.5-4% has been reported. The penetrance of this gene is unknown, but the phenotype is characterized by progressive heart failure, often with severe right-sided symptoms. When a low cardiac output supervenes, renal impairment may occur, although autopsy studies have not shown renal involvement by amyloid deposits.

The onset of ATTR cardiomyopathy may occur at any age from the third decade onward, with the typical age of onset > 60 in patients with the V122I mutation. Cardiac involvement can present with conduction system disease

(sinus node or atrioventricular node dysfunction) or congestive heart failure including shortness of breath, peripheral edema, syncope, exertional dyspnea, generalized fatigue or with heart blocks. The echocardiographic findings are indistinguishable from those seen in AL amyloidosis and include thickened ventricular walls (concentric hypertrophy, both right and left) with a normal to small left ventricular cavity, increased myocardial echogenicity, normal or mildly reduced ejection fraction, often with evidence of diastolic dysfunction and severe impairment of contraction along the longitudinal axis, and bi-atrial dilation with impaired atrial contraction (see figure 2).

Unlike in AL amyloidosis, the voltage on the ECG is often normal, although low voltage may be seen despite the increased wall thickness on echocardiography. Marked axis deviation, bundle branch block and AV block is common, as is atrial fibrillation.

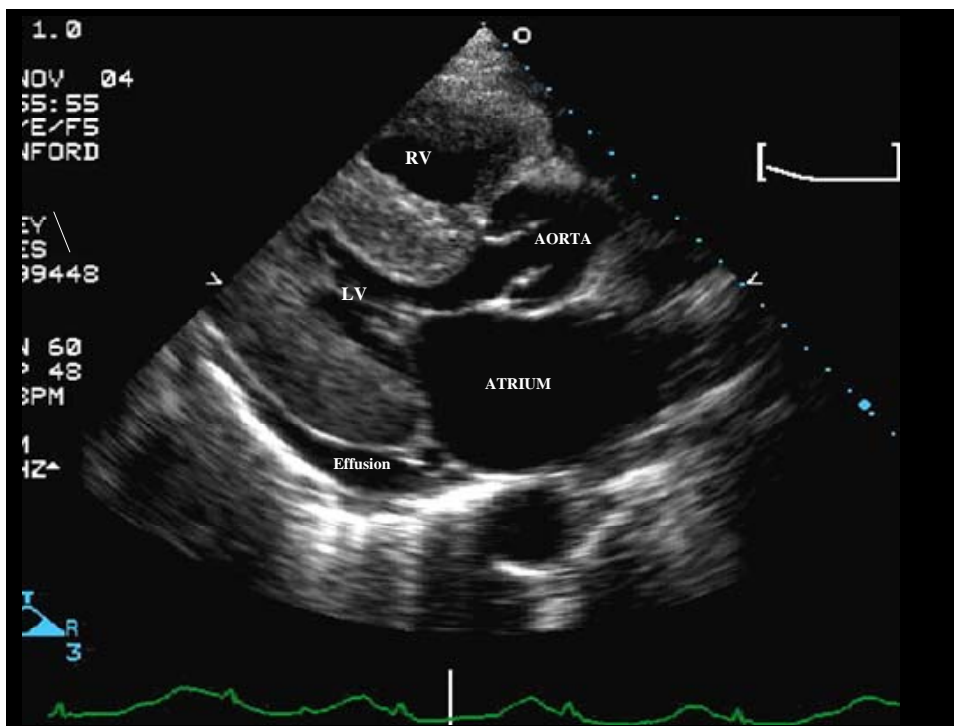


Figure 2. Echocardiographic Findings of Cardiac Amyloid (Courtesy of R. Falk, MD) A parasternal long-axis view in a patient with severe cardiac amyloidosis. The LV walls are thickened and echogenic. The left atrium is enlarged. The aortic valve is particularly well seen due to infiltration.

### *Ocular manifestations*

The TTR gene is expressed in the retinal pigment epithelium of the eye, and approximately 20% of amyloidogenic TTR mutations are associated with vitreous opacities derived from amyloid, and may lead to visual impairment. TTR amyloid can be visualized in the vitreous body with typical cotton wool inclusions (Fig. 3). Demonstration of such deposits is helpful for the diagnosis of ATTR.

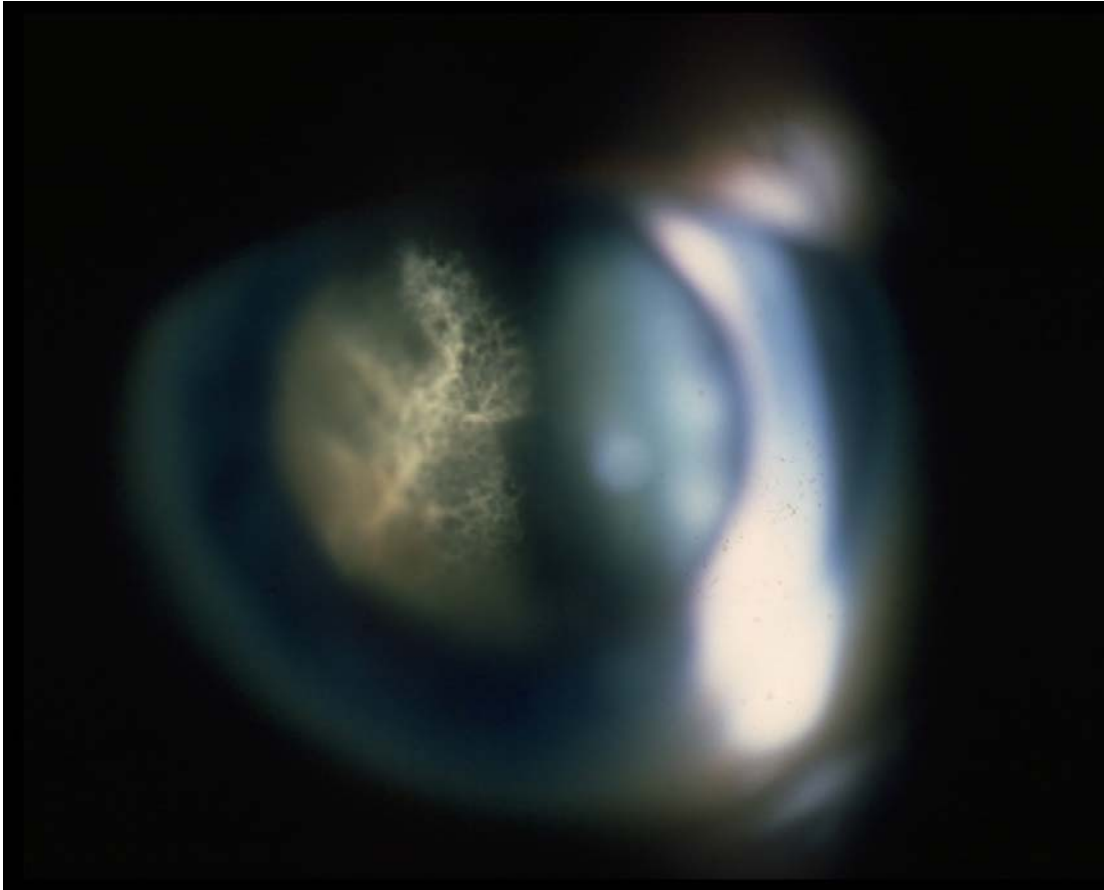


Figure 3. TTR amyloid deposits in vitreous body (“cotton wool inclusions”). Reproduced with permission from R. Andersson & T. Kassmann.

### *Other organs*

Unlike AL amyloidosis, renal involvement is rare in ATTR. When renal involvement is present, proteinuria is the usual manifestation. Very rarely

the renal involvement leads to end-stage renal disease requiring dialysis or renal transplantation.

## Non-hereditary ATTR

Wild-type ATTR cardiomyopathy occurs when wild-type TTR forms fibrils, which then deposit as amyloid, primarily in cardiac tissue. This is a disease, seen predominantly in men older than 65-70 years, and other than carpal tunnel syndrome, deposition is clinically limited to the heart. The clinical presentation is similar to that described for the hereditary form of ATTR cardiomyopathy. The clinical prevalence of wild-type ATTR cardiomyopathy is unknown, but autopsy studies suggest that up to 22-25% of individuals >80 years old have demonstrable TTR amyloid deposits in cardiac tissue, although in most cases the degree of deposition is mild.

## When should a physician suspect ATTR?

The multisystemic involvement in ATTR makes the disease a possible differential diagnosis in many instances. It would be particularly important to consider the diagnosis when one, or especially several of the following symptoms are present:

- when there is a known family history of neuropathic disease, especially if associated with heart failure
- in cases of neuropathic pain or sensory disturbances of unknown etiology
- when there is a history of carpal tunnel syndrome (without obvious cause), particularly if bilateral and requiring decompression
- in patients with gastro-intestinal motility disturbances or autonomic nerve dysfunction of unknown etiology,
- if there is cardiac disease characterized by thickened ventricular walls, diastolic dysfunction, or overt cardiomyopathy, particularly in the presence of normal or preserved ejection fraction and in the absence of hypertension
- when a patient presents with complete heart block of unknown origin and echocardiographic abnormalities
- when there are vitreous body inclusions of cotton wool type

As there are many pitfalls in the diagnosis of ATTR, it is generally recommended that patients with possible ATTR are referred to a specialist center with experience in the diagnosis and management of amyloidosis.

## Diagnosis of TTR amyloidosis

TTR amyloidosis is a systemic disease, in which amyloid deposits can be visualized in most tissues such as skin, fat pad, rectal mucosa, gastric mucosa, nerve tissue or, myocardium.. Tissue biopsy should be performed from an affected organ, if initial biopsies are negative for amyloid. Staining with Congo red, which gives a characteristic apple green color when viewed under polarizing microscopy can confirm amyloidosis, but cannot identify the responsible precursor protein. As the distribution of amyloid may be patchy in certain tissues, a negative biopsy does not rule out the diagnosis of amyloidosis although it is almost universally positive in cardiac amyloidosis if the echocardiogram shows typical findings. Once amyloid is demonstrated, then the type of precursor protein should be identified. ATTR can be confirmed by immunohistochemical staining for TTR. If a tissue biopsy is negative for amyloid staining but the clinical suspicion for hereditary ATTR is high, the investigator may proceed directly with genotyping. However, it should be kept in mind that the penetrance of the underlying trait varies and therefore, before accepting a patient for liver transplantation, demonstration of amyloid deposits is generally required.

Diagnosis of the hereditary form of ATTR requires demonstration of a TTR gene mutation. A history of familial disease is helpful but many patients seen with ATTR do not have a documented family history.

For the diagnosis of non-hereditary ATTR in a patient with suspicious echocardiogram, a cardiac biopsy demonstrating amyloid deposits which are positive for TTR, combined with lack of identifiable mutation in the TTR gene is generally required. Even though non-hereditary ATTR is a systemic disease, it may be difficult to demonstrate amyloid deposits in tissues other than the heart.

## Treatment of ATTR

There is currently no specific pharmacologic therapy available for ATTR; therapy is symptomatic, e.g. analgesics for painful neuropathy, pacemaker implantation for heart conduction problems, diuretics for congestive heart failure etc. Treatment with certain calcium channel blockers and especially digitalis, should be avoided since it may accumulate in the amyloid deposits and increase the risk of heart complications

As TTR is primarily formed in the liver, orthotopic liver transplantation is the only disease modifying treatment available to patients with hereditary ATTR. This procedure will remove approximately 95% of the production of variant TTR and can slow or halt the progression of the disease.

Improvement of nerve function, and in particular of autonomic disturbances, has been reported, but has not been documented in systematic follow-up studies in liver transplant recipients. Cardiac disease may progress after liver transplantation, due to deposition of wild-type TTR-derived amyloid.

Because of this possibility, consideration should be given to combined liver and heart transplantation in patients with TTR amyloid cardiomyopathy. Eye opacities may develop or progress after liver transplantation as they are due to local ocular amyloid formation. It is generally considered that the best outcome of orthotopic liver transplantation is achieved when it is performed before the disease has become too advanced. Overall patient survival rate at 5 years is reported to be above 77%.

New therapies aiming to stabilize TTR tetramers and avoid fibril formation are being tested in clinical trials. Further information about clinical trials, is available at

<http://www.amyloidosisresearchfoundation.org/clinicaltrials/index.html> and <http://www.clinicaltrials.gov/>.

Ongoing trials (June 2008) include studies with the compound diflunisal, a NSAID with TTR stabilization properties and a product with code name Fx-1006A, a specific and potent TTR stabilizing compound with no NSAID activity. Results of the clinical trial with Fx-1006A are expected to become available in 2009.

## Prognosis of ATTR

All forms of ATTR are progressive but the rate of progression is variable and may be dependent on the clinical phenotype and the mutation. Generally patients with the ATTR V30M mutation have a mean life expectancy of 9-11 years from symptom onset. Death is due primarily to malnutrition and cachexia, renal failure, cardiac disease or sudden death. For other more rare mutations, survival is less well documented.

The progression of ATTR when cardiomyopathy is the predominant clinical manifestation is less well documented but the prognosis is often poor with mean life expectancy from symptom onset of 5-6 years, and death due primarily from heart failure or sudden death.

## Relevant reading

Benson MD, Kincaid JC. The molecular biology and clinical features of amyloid neuropathy. *Muscle Nerve* 2007;36(4):411-23.

Falk RH. Diagnosis and management of the cardiac amyloidosis. *Circulation* 2005;112:2047-60.

Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black americans. *NEJM* 1997;336:466-73.

Herlenius G, Wilczek HE, Larsson M et al. Ten years of international experience with liver transplantation for familial amyloidotic polyneuropathy: results from the familial amyloidotic polyneuropathy world transplant registry. *Transplantation* 2004;77(1):64-71.

## Important links and contacts

[www.amyloidosis.org](http://www.amyloidosis.org)

[www.amylidosisresearchfoundation.org](http://www.amylidosisresearchfoundation.org)

[www.fapwtr.org](http://www.fapwtr.org)

[www.clinicaltrials.gov](http://www.clinicaltrials.gov)

## Table I

Disease	Amyloid deposit	Main clinical features	Also Known As
Hereditary ATTR	<p>Mutated TTR.</p> <p>More than 80 different mutations described leading to instability of TTR and amyloid fibril formation and deposit and many organs and tissues</p>	<p><i>Polyneuropathy</i>                      Progressive disease which affects</p> <ul style="list-style-type: none"> <li>• peripheral sensory and motor nerves</li> <li>• autonomic nervous system</li> </ul> <p>causing pain, sensory and motor deficits and autonomic nerve dysfunction like orthostatic hypotension, gastro-intestinal symptoms (obstipation alternating with diarrhea) and anhydrosis.</p> <p>Cardiac and renal manifestations may appear at any time during the course of the disease.</p> <p><i>Cardiomyopathy</i>                      Cardiomyopathy with conduction disturbances and impaired function, mostly restrictive cardiomyopathy.                      Patients may develop neuropathy</p>	<p>Familial Amyloid Polyneuropathy (FAP) when polyneuropathy is the predominant presentation</p> <p>Familial Amyloid Cardiomyopathy (FAC) when cardiac disease is the predominant presentation</p>
Non-hereditary ATTR	Wild type TTR	Cardiomyopathy dominating clinical feature and a disease primarily affecting elderly men (>65-70 years)	Senile Systemic Amyloidosis (SSA)

# Figures

Fig 1

Formation of TTR fibrils from tetrameric TTR

Fig 2

Typical echocardiographic appearance in amyloid cardiomyopathy

Fig. 3

Typical vitreous body inclusions in ATTR

**Author affiliations:**

Teresa Coelho, MD - Neurology  
Hospital Geral de Santo Antonio  
Porto, Portugal

Bo-Göran Ericzon, MD - Transplantation Surgery  
Karolinska Institutet, Karolinska University Hospital  
Stockholm, Sweden

Rodney Falk, MD - Cardiology  
Harvard Vanguard Medical Associates  
Boston, MA, USA

Donna Roy Grogan, MD - Internal Medicine  
CMO, FoldRx Pharmaceuticals  
Cambridge, MA, USA

Shû-ichi Ikeda, MD - Neurology  
Shinshû University School of Medicine  
Matsumoto, Japan

Mathew Maurer, MD - Cardiology  
Columbia-Presbyterian Hospital  
New York, NY, USA

Violaine Planté-Bordeneuve, MD, PhD - Neurology  
CHU Henri Mondor  
Créteil, France

Ole B. Suhr, MD, PhD - Gastroenterology  
Umeå University Hospital  
Umeå, Sweden

Pedro Trigo, MD - Hepatology  
FLENI  
Buenos Aires, Argentina

Supported by an unrestricted educational grant from FoldRx Inc.



This publication is supported by an unrestricted educational grant  
from FoldRx Pharmaceuticals, Inc.  
August 2008