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# Consultations in Molecular Diagnostics

## Homozygous Transthyretin Mutation in an African American Male

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**Cardiac amyloidosis of transthyretin type in the elderly may be senile or familial. The senile form is not typically associated with specific genetic changes. However, the familial form is and also occurs more frequently in African Americans than in the general population. One transthyretin mutation, V122I, is common in the African-American population, has a carrier frequency of 4%, and has marked cardiac specificity. Symptoms generally develop in the eighth and ninth decades. Here, we report the case of a 60-year-old African-American man who had a 2-year history of dyspnea and diffuse left ventricular wall thickening. Endomyocardial biopsy showed interstitial deposits of amorphous material confirmed as amyloid by Congo red staining and electron microscopy. Mass spectrometry showed a shift in protein mass of 14 d, indicative of transthyretin and confirming the production of abnormal protein. Bidirectional whole gene sequencing showed a homozygous mutation leading to a valine 122 isoleucine substitution (V122I). The 14-d mass shift observed using mass spectrometry is consistent with the V122I mutation. Homozygosity for the V122I mutation may be associated with earlier onset of cardiac disease. Transthyretin analysis should be considered for older African Americans with amyloid heart disease of transthyretin type.** ***(J Mol Diagn 2007, 9:127–131; DOI: 10.2353/jmoldx.2007.060061)***

Amyloidosis refers to a group of disorders that share in common the deposition in one or various organs of abnormal fibrillar proteins that are characterized by a β-pleated sheet conformation. These amyloid protein deposits have an amorphous pale eosinophilic appearance with standard hematoxylin and eosin staining, and they display a distinctive apple-green birefringence when stained with Congo red and viewed with cross-polarized light.

Investigators have characterized more than 20 different proteins that can be deposited as amyloid.[1,2] The processes that lead to amyloid formation vary widely and include plasma cell neoplasms that produce immunoglobulin light chains, hepatic production of abnormal transthyretin, chronic inflammation causing serum amyloid A deposition, chronic renal dialysis with deposition of β-2-microglobulin, or hereditary disorders. These various amyloidogenic proteins can then be deposited locally or systemically. The morphological appearance of the amyloid deposits does not allow accurate distinction between the various types of amyloid.[3]

In addition, familial transthyretin amyloid may occur in patients who do not have a known family history. This can be particularly troublesome in those who also have coincidental monoclonal protein identified in their serum or urine, which can mislead clinicians to assume that the amyloid is likely to be primary light chain type.[4] Because the prognosis and treatment vary widely for the various types of amyloid, misdiagnosis can be devastating for appropriate patient management.

Wild-type transthyretin (TTR) has been identified as the deposited protein in senile amyloidosis, which does not have a known hereditary component. Mutations in the transthyretin gene can greatly destabilize the TTR molecule and are responsible for the most frequent form of hereditary autosomal dominant amyloidosis.[5] One such mutation (V122I) is common in the African-American population and causes amyloid deposition specifically in the heart.[6] This is the subject of the current case report.

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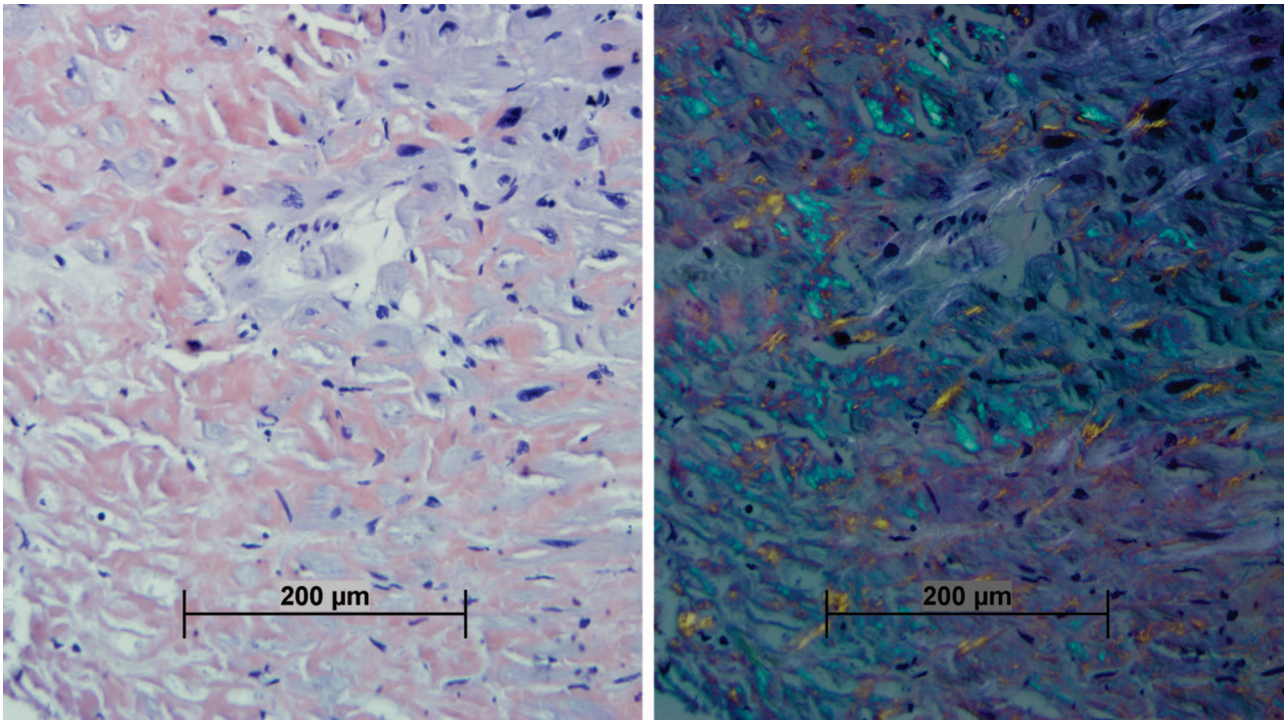


**Figure 1.** The left panel demonstrates the typical red-orange appearance of amyloid when stained with Congo red. The image on the right panel shows the classic apple-green birefringence of amyloid when viewed with cross-polarized filters.

## *Case Reports*

### *Clinical Summary*

Over a 2-year period a hypertensive 60-year-old African-American man developed shortness of breath, swelling of the extremities, and syncope. The initially diagnosis was dilated cardiomyopathy, and his exertional dyspnea progressively worsened. An echocardiogram showed left ventricular hypertrophy with severe global hypokinesia, consistent with hypertensive heart disease or an infiltrative cardiomyopathy. To distinguish between the two, an endocardial biopsy was performed.

### *Endomyocardial Biopsy*

Biopsy material from the apex of the right ventricle was fixed in formalin, processed and embedded in paraffin, and cut and stained with hematoxylin and eosin, as well as with Congo red. Immunohistochemical staining was performed using antibody to serum amyloid A protein. A portion of the biopsy specimen was also processed for transmission electron microscopy.

The endomyocardial biopsy tissues showed amorphous eosinophilic extracellular deposits that were confirmed as amyloid using Congo red staining and transmission electron microscopy (Figures 1 and 2). On electron microscopy, focal myocardial interstitial accumulation of fine fibrillar material, with a random arrangement, solid or tubular cross-section, ranging from 8 to 12 nanometers in diameter, confirmed the presence of amyloid fibrils. Adjacent muscle fibers showed mild atrophic change. Immunohistochemical studies showed that the amyloid did not stain for serum amyloid A protein and therefore suggested either the light chain or transthyretin type of amyloid. Because of the patient's history and ethnicity and the absence of known factors causing primary or secondary amyloidosis, transthyretin analysis was undertaken.

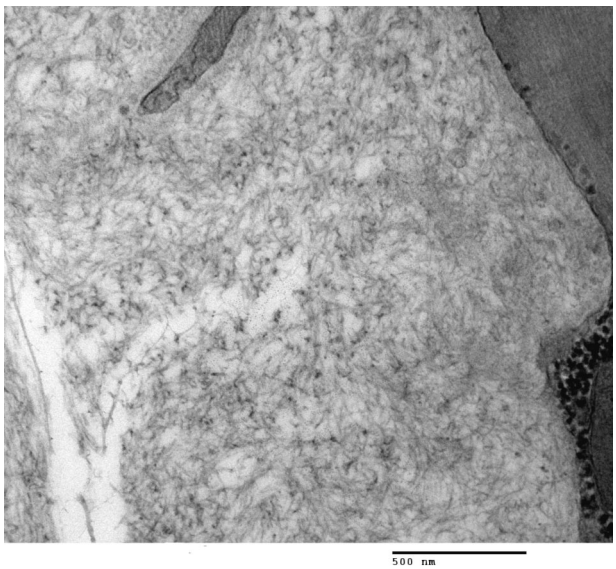


**Figure 2.** Transmission electron microscopy of endomyocardial biopsy tissue. Fine fibrillar deposits arranged are in a haphazard manner with cross-sections ranging in size from 8 to 12 nm (×77,500).

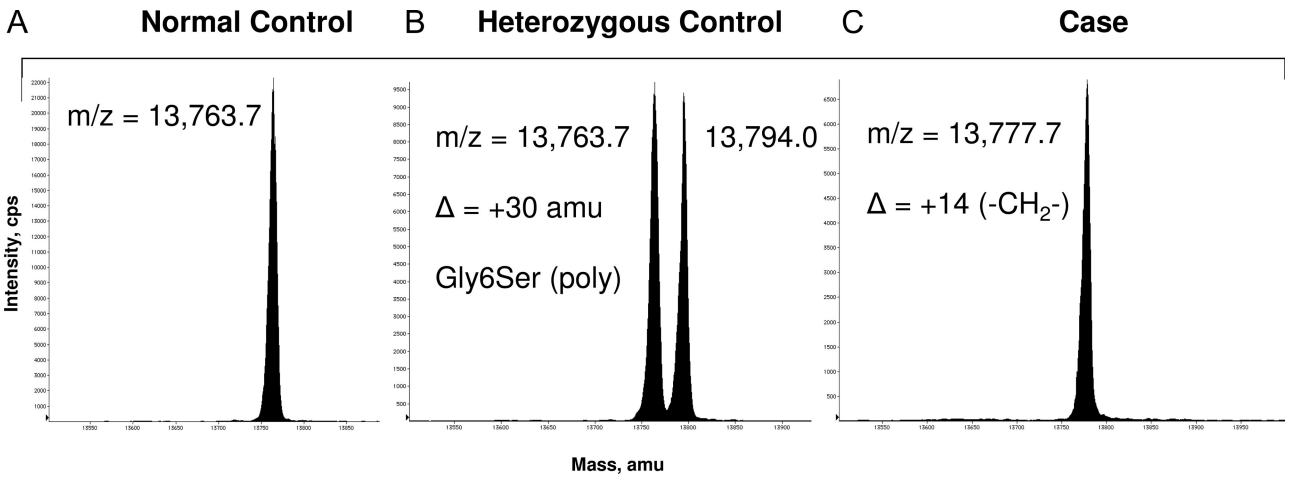


**Figure 3.** Mass spectroscopy data. **A:** Normal transthyretin control. **B:** Heterozygous transthyretin control, Gly6Ser. **C:** Patient sample.

## *Transthyretin Analysis by Mass Spectroscopy*

Mass spectroscopy was performed to confirm the presence of a mutant transthyretin protein, as previously described.[7,8] In brief, serum was incubated with anti-TTR antibodies coupled to resin beads. After incubation, the beads are washed, then the immunocaptured TTR eluted with pH 2.5 glycine buffer. After elution, the TTR was reduced with Tris-(2-carboxyethyl)-phosphine and introduced into an electrospray ionization (ESI) source of a quadrupole mass spectrometer (Applied Biosystems API 150; Foster City, CA) via a short $C_4$ (butyl) cartridge (Phenomenex, Torrance, CA).

Figure 3A shows the signal resulting from the ESI-MS analysis of a normal control. The mass observed, molecular mass = 13,763.7 d, corresponds to the molecular weight of the wild-type version of TTR (serum from an individual without TTR mutations or polymorphisms). Figure 3B shows the signal resulting from the analysis of an abnormal control (serum from a normal individual heterozygous for the G6S variant). This shows two peaks whose masses differ by 30 d. This is the result of the glycine to serine amino acid change. This is a benign polymorphism with allele frequencies of approximately 6% in Caucasians and 1% in African Americans.[9]

Any mutation that is heterozygous will result in the presence of two different masses due to the different molecular weights of the two TTR molecules produced. This method is capable of distinguishing between wild type and modifications that produce changes in the TTR molecule that cause a molecular weight shift of 10 d or more.

A rare occurrence is a homozygous mutation where both parents provide DNA that produces a mutated TTR. In the present case of the sample reported in Figure 3C, a single mass was observed at 13,777.7 d. This mass is shifted 14 d from the normal wild-type mass. The fact that no wild-type mass was observed indicates that this is a homozygous mutation. The measured mass of 13,777.7 d is consistent with a V122I mutation and was confirmed through DNA sequence analysis.

## *DNA Sequence Analysis*

Genetic analysis was performed on DNA obtained from the patient's whole blood. Bidirectional whole gene sequencing was performed using the Sanger dideoxy-terminator method and analyzed on an ABI 3100 automated sequencer. To verify that the observed homozygosity was not due to a rare polymorphism or sequence variation at one of the exon 4 primers, an alternate set of primers were synthesized and the PCR and sequencing repeated. The standard primers used in our laboratory for amplification of exon 4 of the TTR gene were forward, 5′-GGACTTCCGGTGGTCAGT-3′; and reverse, 5′-TGCCTGGACTTCTAACATAGC-3′. The alternate primers were forward, 5′-CTTCCGGTGGTCAGTCATGTG-3′; and reverse, 5′-TCTAACATAGCATATGAGGTGAAAAC-3′.

Sequencing of the entire TTR coding region revealed a point mutation at codon 122 that results in a valine to isoleucine substitution at amino acid 122 (V122I). Additional primer sets were used to confirm that the patient was homozygous for this mutation (Figure 4). This mutation corresponds to the 14-d shift (one additional —$CH_2$— group) in protein size found by mass spectrometry.

## *Discussion*

Hereditary amyloidosis can be caused by mutations in one of several genes. The most common of these encodes for transthyretin. Transthyretin is a serum protein produced in the liver that transports thyroxine and retinol-binding protein, hence the name.[6] Approximately 100 mutations in transthyretin are known, most of which can lead to amyloidosis.[10]

Studies indicate that 3.9% of African Americans are heterozygous carriers of the V122I mutation associated with cardiac deposition of amyloid. Cardiac amyloidosis has been noted to be more common in African Americans than other ethnic groups.[6] The V122I mutation is apparently a major cause of the overabundance of cardiac

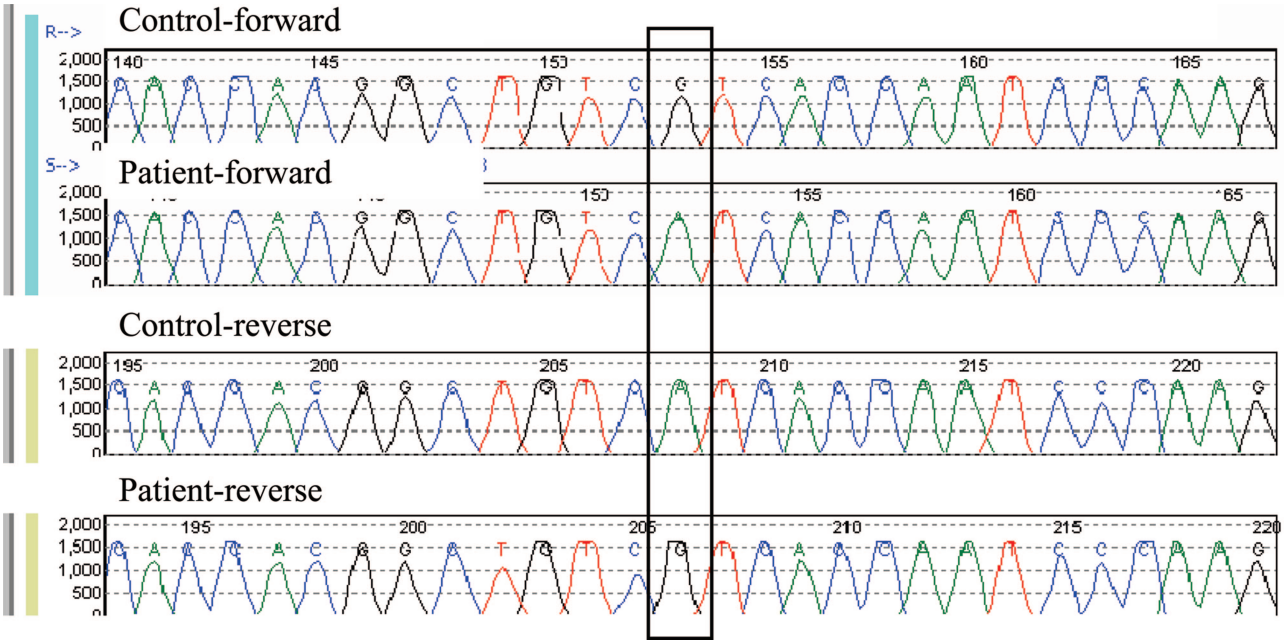


**Figure 4.** Bidirectional sequencing of transthyretin exon 4 shows a point mutation at codon 122, leading to isoleucine replacing valine.

amyloidosis in this ethnic group. In addition, it has been proposed by others that the increase in cardiac amyloidosis may be one contributing factor to the increased number of cardiac deaths in older African Americans.[6] Not known, however, is the risk of amyloid formation among heterozygous and homozygous carriers of the mutation.

Although the heterozygous state is a relatively common finding in the African-American population, homozygosity has been reported only rarely. This is despite the fact that the predicted frequency of homozygosity for V112I would be approximately 1 in 2500 (4% × 4% × 25%), or approximately the same frequency as cystic fibrosis in the Caucasian population. The V122I mutation was discovered by protein sequencing of TTR amyloid isolated from the heart of an African-American male who died at age 71 of cardiac amyloidosis after a 10-year history of congestive heart failure.[11,12] Subsequent DNA testing demonstrated homozygosity for the V122I mutation.[13] Nichols et al[14] described a 72-year-old African-American male who died with restrictive cardiomyopathy. This individual also had a 10-year history of congestive heart failure. A 2000 report describes a 70-year-old African-American male homozygous for the V122I mutation who had a 7-year history of congestive heart failure and a progressive limb muscle weakness diagnosed as sporadic inclusion body myositis. The inclusion bodies were shown by immunohistochemistry and electron microscopy to consist of TTR amyloid.[15] Together, these reports describe three individuals homozygous for the V122I mutation. The onset of symptoms occurred in the early 60s with death in the early 70s. In the autopsy study of Jacobson et al, one of the six V122I heterozygotes with cardiac amyloidosis died at age 71. Three patients died in their 80s, and two in their 90s.[6] Thus, although the number of reported cases is small, and there is overlap in the ages at death between the reported heterozygotes and homozygotes, it seems that the group of individuals homozygous for the V122I mutation tends to expire of their disease at an earlier age than heterozygotes and, presumably, have an earlier onset of symptoms. The patient reported here became symptomatic in his late 50s, consistent with the hypothesis that homozygosity for the V122I mutation is associated with early onset. Further research is clearly needed to determine the role of hetero- versus homozygosity and to address issues of penetrance and genetic modifiers in age of onset and severity of disease.

Currently, treatments for hereditary cardiac amyloidosis are limited. Liver transplantation may be of some benefit, especially if performed early in the course of disease. In addition, in experimental models, amyloidogenic mutants of TTR have been stabilized by nonsteroidal anti-inflammatory drugs.[16] For the present patient, the amount of amyloid deposition was so advanced that decreasing or even stopping the progression of disease by liver transplantation would have been unlikely to appreciably relieve symptoms.

Because amyloid deposition with the V122I mutation occurs solely in the heart, cardiac transplantation would remove essentially the entire body's burden of amyloid, in contrast to other types of amyloid that deposit in various organs. Although further amyloid production by the liver could still occur, it would probably take 40 to 50 years to become symptomatic in the new donor heart.

Transthyretin analysis should be considered in African-American patients with cardiac amyloidosis. Compared with heterozygotes, the homozygotes for V122I may be predisposed to relatively early onset of disease. This information can be used to counsel other family members, to guide potential screening procedures, and to initiate appropriate management/treatment. Larger and

long-term studies may uncover the actual incidence of this disease and certain triggering factors to form amyloid protein in the homozygous individuals.

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