2014 JMD CME Program

The Journal of Molecular Diagnostics, Vol. 16, No. 1, January 2014

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

Homozygosity for the V122I Mutation in Transthyretin Is Associated with Earlier Onset of Cardiac Amyloidosis in the African American Population in the Seventh Decade of Life

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CME Accreditation Statement: This activity ("JMD 2014 CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical Pathology (ASCP) and the American Society for Investigative Pathology (ASIP). ASCP is accredited by the ACCME to provide continuing medical education for physicians.

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CME Disclosures: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial interests to disclose.

Accepted for publication August 15, 2013.

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Individuals heterozygous for the V122I mutation in transthyretin (*TTR*) tend to develop cardiac amyloidosis, often after the seventh decade of life. Although homozygotes have been reported, these have typically been single case reports. We report a cohort of 13 V122I homozygotes. *TTR* gene sequencing results from the Mayo Clinic Molecular Genetics Laboratory between September 2004 and January 2013 were reviewed; 177 heterozygotes and 13 homozygotes for the V122I alteration were identified. Detailed clinical history was available for the 24 heterozygotes seen at Mayo Clinic. We compared age at onset of disease for this group to homozygotes, both alone and pooled with the 11 homozygotes from the literature. Individuals with homozygous V122I manifested symptoms a mean of 10 years earlier than heterozygotes (63.8 $\pm$ 5.7 versus 72 $\pm$ 8.1 yrs, $P = 0.0002$). Further, males were significantly overrepresented in both heterozygous and homozygous individuals. There was a trend for an even higher male bias in the homozygous group. All 24 homozygotes were African American, whereas four of the heterozygotes were reported as white. Two novel V122I compound heterozygotes were also identified, with clinical presentation in the late fifth or early sixth decade of life. This study is the largest homozygous V122I cohort reported and demonstrates association with earlier age at onset. It also highlights the uncertain penetrance, particularly with respect to sex. *(J Mol Diagn 2014, 16: 68—74; http://dx.doi.org/10.1016/j.jmoldx.2013.08.001)*

Amyloidosis is characterized by the abnormal deposition of one of a variety of plasma proteins as insoluble β-pleated sheet aggregates, resulting in disruption of organ and tissue function. Hereditary amyloidosis is one of the major subtypes of this disorder and occurs due to mutations in several genes, including, but not limited to, *APOAI*, *APOAII*, *FGA*, *GSN*, *LYZ*, and *TTR*.[1] Mutations in the *TTR* gene are the most common, accounting for approximately 90% of hereditary (familial) amyloidosis.

Transthyretin amyloidosis (ATTR) is a systemic disease that involves extracellular deposition of amyloid fibrils that accumulate in various organs and tissues,[2] causing

Supported by a Mayo Clinic Molecular Genetics Fellowship.

http://dx.doi.org/10.1016/j.jmoldx.2013.08.001

progressive dysfunction and potentially fatal disease. There is a substantial range of symptoms associated with ATTR. It can be characterized by sensory, motor, and autonomic neuropathy (polyneuropathy) with or without cardiomyopathy or cardiomyopathy alone. ATTR also includes an age-related form known as senile systemic amyloidosis, an acquired disorder that mainly affects men, exhibiting an approximately 25 to 50:1 male/female ratio[3] after the age of 75 years that results from the deposition of wild-type ATTR, primarily in the heart.[4] Given its phenotypic unpredictability and variability, ATTR can be difficult to recognize and manage. Misdiagnosis is common, and patients may wait several years before accurate diagnosis, risking additional significant irreversible deterioration.

Diagnosis of amyloidosis involves Congo Red staining of biopsied tissues to confirm amyloid deposition, which demonstrates a characteristic apple-green birefringence under polarized light. Amyloid protein typing is performed using immunohistochemical (IHC) staining of an affected tissue biopsy with antisera to *TTR*, κ and λ light chains, and amyloid A[5,6] or liquid chromatography and tandem mass spectrometry of tryptic digests of microdissected amyloid plaques.[7,8] Detection of one of the proteins involved in the hereditary forms of amyloidosis requires follow-up genetic testing to confirm the diagnosis.[1,5] Currently, the only treatment for familial amyloidosis with proven efficacy is orthotropic liver transplantation. However, other forms of therapy are being pursued, including development of small molecules that stabilize the *TTR* tetramer.[9,10]

ATTR is an autosomal dominant disorder, and there are >80 mutations that are known to be causative.[1] There is a strong genotype/phenotype correlation, with specific *TTR* mutations being associated with purely neurologic disease, purely cardiac disease, or both.[3] The V30M mutation is primarily associated with neuropathy, the T60A mutation demonstrates both neuropathy and cardiomyopathy, and the V122I variant is associated exclusively with a cardiac phenotype.[11] The V122I variant is a common mutation in African Americans, appearing to have originated in West Africa.[12] Heterozygosity for this mutation is associated with cardiac amyloidosis, congestive heart failure, and mortality in African Americans, typically after the age of 70 years.[13] The heterozygote frequency is approximately 4% in African Americans. Jacobson et al[14] calculate that there should be approximately 13,000 individuals homozygous for the V122I mutation in the United States. Curiously, only 11 homozygotes have been reported, almost all as single case reports,[15–20] with one study reporting on five cases.[21]

The current study presents findings from a large cohort of 13 homozygous V122I cases. We also reviewed previously reported homozygous cases and integrated the 11 reported cases with our group of 13 to attempt to understand the effect of the homozygous V122I mutation on age at onset. In addition, we report two compound heterozygotes, individuals with one V122I mutation and another pathogenic *TTR* mutation. We found that there is an approximately 10-year decrease in the age at onset of cardiac symptoms, moving from V122I heterozygotes to homozygotes to compound heterozygotes with other pathogenic mutations.

Materials and Methods

Identification of V122I Mutation Carriers

Sequencing results for all four exons of the *TTR* gene from September 2004 through January 2013 at the Mayo Clinic Molecular Genetics Laboratory were evaluated to identify cases with genetic alterations. For the 24 cases with V122I heterozygous mutations, three with homozygous V122I, and one compound heterozygote that were Mayo Clinic patients, the electronic medical records were reviewed to determine age at onset of symptoms or age at diagnosis and confirmation of amyloid deposits by IHC or tissue mass spectrometry. Clinical information on the other 10 V122I homozygous mutation cases and two cases of V122I compound heterozygotes was obtained from the attending physicians at other institutions. For Mayo Clinic patients, ethnicity was found in the self-reported demographic information in the electronic medical record. For non-Mayo or literature cases, ethnicity was as reported by the attending physician or published information, respectively. Reporting of the *TTR* alterations is based on historical nomenclature.[1] This study was approved by the Mayo Clinic institutional review board (number 12-007376).

Published Reviews of V122I Homozygotes

PubMed (*http://www.ncbi.nlm.nih.gov/pubmed*) was queried to find all studies published to date that elucidated V122I mutations. Seven articles with information on V122I homozygotes were identified and parsed to determine number of cases and age at onset of symptoms or age at diagnosis and ethnicity of reported individuals.

Statistical Analysis

Age at onset is reported as means ± SD and was compared between groups (ie, heterozygotes versus homozygotes) with two-tailed *t*-tests. Sex and race/ethnicity were compared between groups with χ^2 tests or Fisher's exact tests (where appropriate). Further, χ^2 goodness-of-fit tests were used to assess whether the sex distribution was different from a 50/50 split within groups. All analyses were performed using JMP software version 9 (SAS Institute, Cary, NC). $P < 0.05$ was considered statistically significant.

Results

TTR Full-Gene Sequencing Results

A total of 2787 samples were sequenced across all four exons of the *TTR* gene, with 2261 cases having no detectable genetic alterations. Of the genetic alterations identified, 72 were benign polymorphisms, 23 were variants of

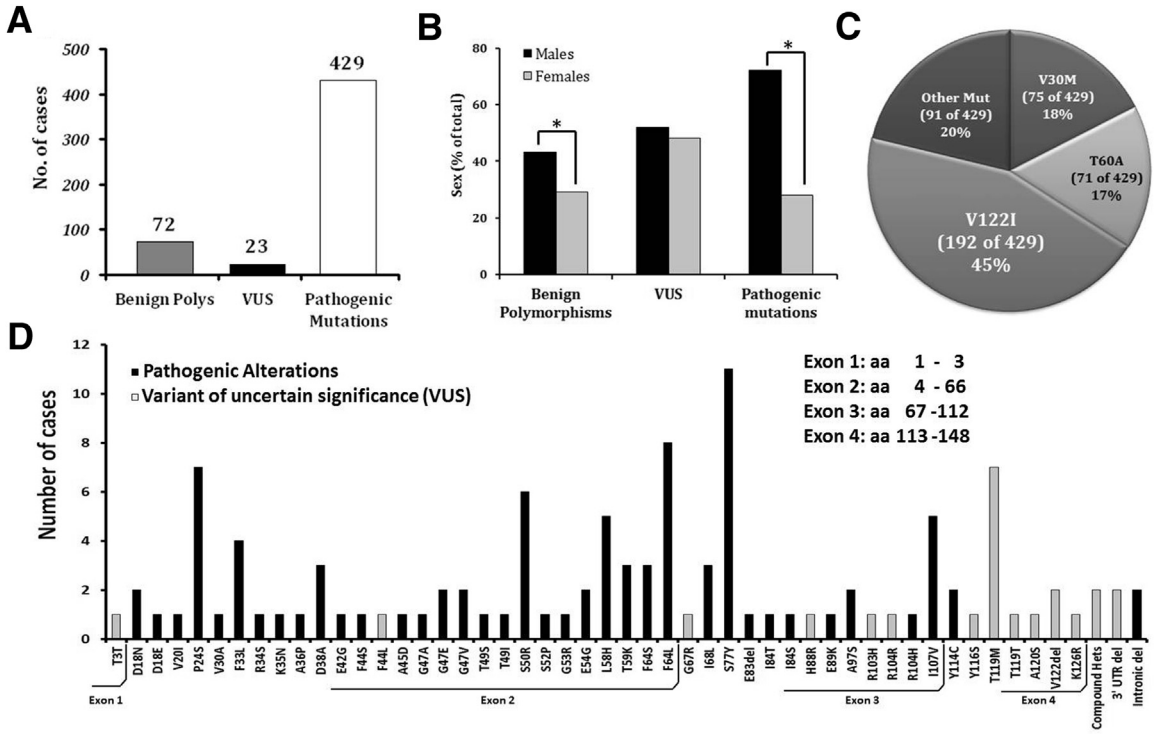

Figure 1 *TTR* gene sequencing results. **A:** Genetic alterations observed: benign polymorphisms, VUSs, and pathogenic mutations. **B:** Sex distribution of all cases with genetic alterations, indicating a significantly higher number of males in cases with benign polymorphisms and pathogenic alteration. **C:** Number of cases observed with different variants associated with ATTR, common mutations (V30M, T60A, and V122I), and other mutations (Mut). **D:** Number of cases seen with the rarer pathogenic mutations and VUSs observed in *TTR* outlined to the different exons within the *TTR* gene. $*P < 0.05$, $**P < 0.005$.

uncertain significance (VUSs), and 429 samples were pathogenic mutations (Figure 1A). The VUSs were evenly split between sexes ($P = 0.83$); however, 60% and 72% of the cases with benign polymorphisms and pathogenic alterations, respectively ($P < 0.0001$), were male (Figure 1B). Three mutations accounted for 80% of the pathogenic alterations identified: V122I (194 samples, 45%), T60A (71 samples, 17%), and V30M (75 samples, 18%) (Figure 1C). All but two (heterozygous codon 122 deletions) pathogenic alterations were missense mutations (Figure 1D). VUSs included seven cases of Thr119Met (Figure 1D).

Evaluation of V122I Cases

Of the 194 cases with mutations at codon 122, two were heterozygous deletions (c.424_426delGTC, amino acid change: p.Val122del), 177 (92%) were V122I heterozygotes, 13 (7%) were V122I homozygotes, and two were V122I compound heterozygotes (V122I + I68L and V122I + T60A). Most V122I cases were male [heterozygotes, 146 of 177 (75%), $P < 0.001$; and homozygotes, 11 of 13 (85%) $P < 0.0006$] (Figure 2A).

Association of Homozygous V122I with Earlier Age of Onset in African Americans

The 13 homozygotes had a significantly earlier age at onset as defined by the age of diagnosis (62 ± 5.75 years) than did the 24 heterozygotes (72 ± 8.14 years) ($P < 0.005$) (Figure 2B). Review of the literature identified 11 cases of V122I homozygotes.[15–21] Analysis of these 11 cases demonstrated all to be of African American ethnicity, with a means ± SD age at onset of 64.8 ± 4.33 years (Table 1). We integrated these 11 cases into our homozygote cohort (total $n = 24$) for a comprehensive comparison with the 24 heterozygotes with clinical information available in our study. Using the two-tailed *t*-test, age at diagnosis was significantly different ($P = 0.0002$), with a means ± SD age of 72 ± 8.1 years versus 63.8 ± 5.7 years for heterozygotes versus homozygotes (Figure 2C). Similar to our initial analysis, the analysis of the combined literature and current cases demonstrated a higher percentage of males in the homozygotes versus heterozygotes (92% versus 75%); however, this finding did not reach statistical significance ($P = 0.07$) (Figure 2D). All 24 homozygotes were African American, whereas, of the 24 heterozygotes with clinical information available (Mayo Clinic patients), four were white (Figure 2E).

Symptom Manifestation of Compound Heterozygotes with Two Cardiomyopathy-Associated Mutations in the Sixth Decade of Life

Compound heterozygotes for mutations in *TTR* are extremely rare. We report the finding of two novel V122I compound heterozygotes in the current cohort and provide additional information on the case previously reported by Connors et al.[21]

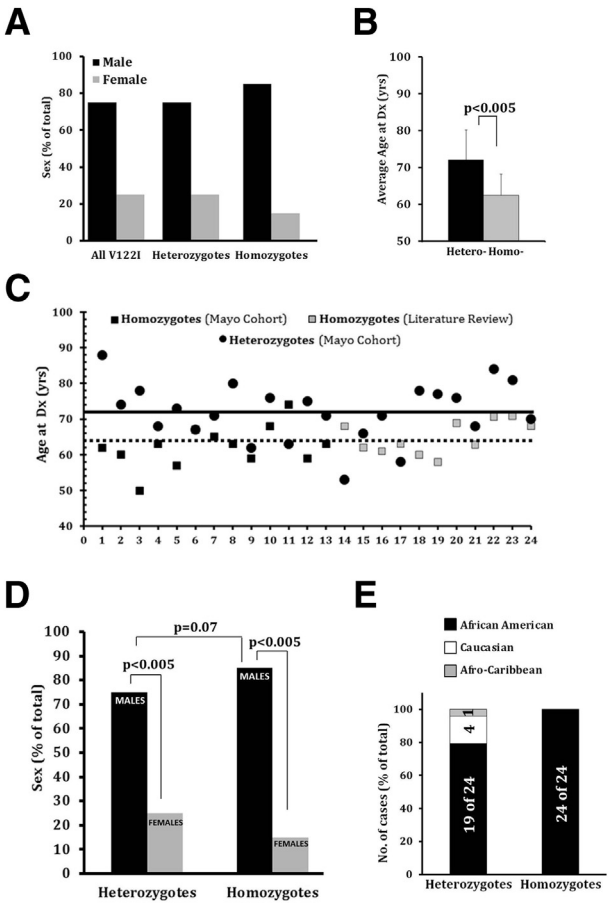

Figure 2 Evaluation of V122I cases. **A:** Sex of all V122I cases (Mayo cohort) versus those with a single copy change (heterozygotes) and those with two copy changes (homozygotes). **B:** Significant difference in age at diagnosis ($P < 0.005$) between heterozygotes (black bar) versus homozygotes (gray bar) in the Mayo cohort in the eighth versus seventh decade of life. **C:** Age at diagnosis (Dx) in years for each of the 24 V122I heterozygotes (black circles) and homozygotes, with a means ± age at diagnosis of 72 ± 8.14 years for heterozygotes (solid line) and 63.7 ± 5.71 years for homozygotes (dashed line). **D:** Significantly higher number of males ($P < 0.001$) were observed in both groups, with a trend toward a higher number of homozygote males ($P = 0.07$). **E:** Homozygotes were all African American (24 of 24) compared with 79% of heterozygotes (19 of 24) ($P = 0.046$).

Case 1 had both V122I and I68L alterations. The individual was an African American male diagnosed as having ATTR at 58 years of age but whose symptoms were reported to have started by 52 years of age. Endomyocardial biopsy result was positive for amyloid deposits, prompting evaluation of serum *TTR* (Figure 3A) and follow up by Sanger sequencing (Figure 3, D and G). Clinical history indicated bilateral carpel tunnel syndrome, peripheral neuropathy, no renal involvement, and shortness of breath related to heart failure.

Case 2 had the V122I and T60A mutation. The individual was an African American female diagnosed as having ATTR at 49 years of age. Tissue liquid chromatography and tandem mass spectrometry result was positive for the presence of two *TTR* mutations (Figure 3B), which was confirmed by Sanger sequencing (Figure 3, E and H). The patient did not have any polyneuropathy but had significant cardiac failure. A more detailed description of this case is published elsewhere (Lui et al, unpublished data).

The V122I/F44L compound heterozygote has been previously reported.[21] We expand on the clinical phenotype to determine differences, if any, between compound heterozygotes, where both mutations are associated with a cardiac phenotype (V122I, T60A, L111M, and I68L),[11] and an individual with one common cardiac mutation (V122I) and one VUS (F44L). The individual with V122I/F44L mutations was a 74-year-old Nigerian man hospitalized with decompensated heart failure with a reported diagnosis of nonischemic cardiomyopathy and atrial fibrillation 2 years previously. At the time of hospitalization, a diagnosis of amyloidosis was pursued. The result of Congo Red testing of an abdominal fat aspirate sample at the time was negative, as were laboratory studies for amyloid light chain amyloidosis. Although there was no apparent family history, ATTR was considered. Screening for a mutant *TTR* by serum isoelectric focusing suggested that there were two *TTR* variants (ie, no wild-type protein) (Figure 3C). This observation was confirmed by *TTR* genotyping, which revealed point mutations in codons 122 and 44 (Figure 3I). An additional fat aspirate obtained nearly 3 years later at a follow-up visit stained positively with Congo Red and provided biopsy proof of amyloidosis (Figure 3F). Cardiac symptoms were the major manifestation of amyloidosis in this patient; there was no bilateral carpal tunnel syndrome, peripheral neuropathy, or renal involvement. The patient died of heart failure at 78 years of age.

Discussion

This study is a presentation of the clinical manifestations of the largest cohort of individuals with homozygous *TTR*

Table 1 *TTR* V122I Homozygous Cases Reported in the Literature (1990 to the Present)

Case No.	Ethnicity	Sex	Reported age at onset of symptoms (years)	Study reference
1	AA	Male	61	15
2	AA	Male	62	16
3	AA	Male	68	17
4	AA	Male	63	18
5	AA	Male	60	19
6	AA	Male	58	20
7	AA	Male	68.9	21*
8	AA	Male	62.8	21*
9	AA	Male	70.6	21
10	AA	Male	70.9	21*
11	AA	Male	68.0	21*

Mean ± SD age of the 11 patients was 64 ± 4.33 years.

*Additional information provided via personal communication by L.C., the first author of Reference 21. Only the mean age for the 5 V122I homozygotes were reported in Reference 21. L.C. provided the age of onset for the individual patients reported in this table.

AA, African American.

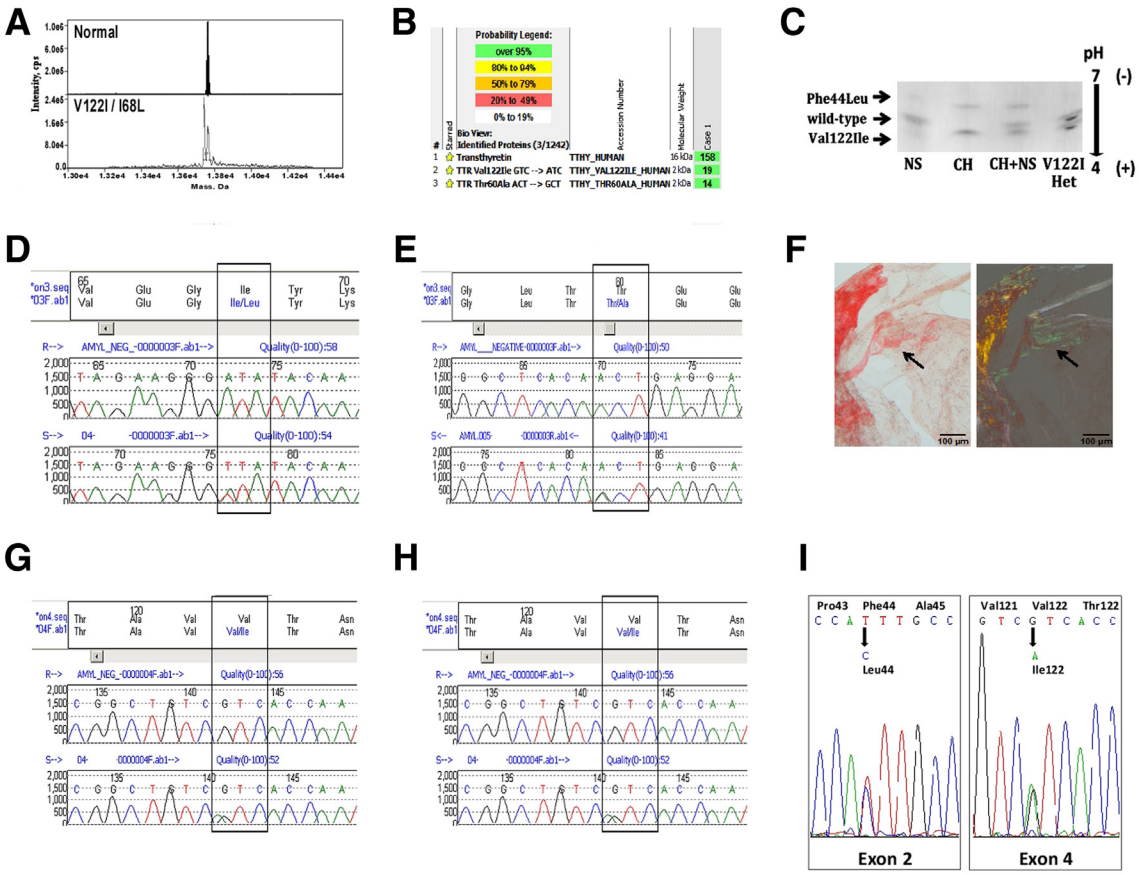

Figure 3 Novel V122I compound heterozygotes. Two cases of novel compound heterozygosity were identified in our cohort. **A**: Mass spectrometry of serum *TTR* identified abnormal structure in the case compared with a normal profile. Sanger sequencing identified I68L (**D**) and V122I (**G**) in an African 52-year-old American man with bilateral carpel tunnel syndrome, peripheral neuropathy, and cardiac disease. Tissue mass spectrometry of the endomyocardial biopsy specimen identified two abnormal peptides, T60A and V122I (**B**), both of which were confirmed by Sanger sequencing (**E** and **H**), in a 49-year-old African American women with cardiac failure and without polyneuropathy. The third case is an expansion of an earlier report[21] describing the clinical manifestations of a V122I/F44L compound heterozygote with a positive isoelectric focusing result (**C**), a positive Congo Red stain result confirmatory for amyloidosis. **Arrows** indicate the amyloid deposits (**F**), and molecular confirmation of compound heterozygosity by Sanger sequencing (**G**) in a 72-year-old African American man with primary symptoms of cardiac failure. **I**: *TTR* genotyping reveals point mutations in codons 122 and 44.

V122I alterations reported to date. We confirm the onset of symptoms a decade earlier than individuals with a single copy mutation (62 ± 5.75 years versus 72 ± 8.14 years) (Figure 2B). In addition, we found that all homozygotes were of African American ethnicity, with a significant sex predilection to males at a ratio of 6:1. Meta-analysis of the 11 homozygous cases from the literature (Table 1), when integrated with our cohort, modified the age at onset slightly to 63.8 ± 5.7 years (Figure 2C). In addition, it increased the sex bias, shifting it to 11.5:1 ($P < 0.005$) for males versus females (Figure 2D), specifically in individuals of African American ethnicity (Figure 2E). We also report two novel V122I compound heterozygotes, one with the well-known cardiomyopathy associated T60A and another with the less common I68L mutation (Figure 3), that manifested symptoms in the late fifth or early sixth decade of life. The age at onset and clinical course of the individual with the F44I/V122I genotype was more similar to the current group of V122I heterozygotes than that of the two compound heterozygotes, arguing that F44I, which is currently reported as a VUS, is likely to be a rare benign polymorphism rather than a pathogenic mutation.

Although *TTR* mutations were first reported primarily in familial amyloid neuropathy, recent studies have elucidated much clearer genotype-phenotype correlations that range from largely neurologic mutations (V30M) to those that are primarily associated with late-onset cardiac amyloidosis: I68L, T60A (Appalachian and Irish Donegal), L111M (Denmark), and V122I (African American) mutations.[9] The clinical profile of individuals with a cardiac phenotype is relatively well defined, with nearly all cases being men 65 years or older. The V122I heterozygotes reported here do not differ from the reported phenotype. However, we clearly demonstrate that homozygotes manifest symptoms a decade earlier (in their 60s) and individuals compound heterozygote for two cardiomyopathy-associated mutations present with symptoms even earlier in their 50s.

TTR functions as a homotetrameric protein. Mutations in *TTR* destabilize the tetramer, dissociating it into monomers that assemble into *TTR* fibrils, resulting in systemic amyloid

deposition.[12] Denaturation studies demonstrate that the V122I mutation destabilizes the *TTR* quaternary structure much faster than other mutations, increasing its rate of tetramer dissociation and consequently increasing monomer accumulation,[22] which is less stable than wild-type monomers.[23] Kinetic and thermodynamic studies therefore provide some insight into understanding the effect of specific mutations in *TTR* and the associated disease phenotypes that help rationalize our observations that homozygous V122I results in manifestation of symptoms a decade earlier than the heterozygous mutation.

The V122I mutation occurs with an incidence of 4% in the African American population and is associated with late-onset cardiac amyloidosis with a predilection for males. Homozygotes have been extremely rare, with only 11 cases reported in literature (Table 1) since the mutation was first reported in 1989.[24] This report brings the total number of reported cases to 24. This rarity is curious given the expected frequency of approximately 1 in 2500 (or 1 in 4000 to 5000 if symptoms were largely limited to males). Whether this discrepancy represents a lack of ascertainment of cardiac disease in the elderly African American population or whether it represents a penetrance that is substantially $<100\%$ is an important question for future research. Our observation of the male predilection, both in the homozygotes and the heterozygotes, is similar to earlier studies in individuals with a predominant cardiac phenotype, including senile systemic amyloidosis,[3,11] providing support for the hypothesis that the female sex confers some degree of protection against myocardial amyloid deposition.[25]

Individuals with two mutations in *TTR* are extremely rare. Compound heterozygotes currently reported in literature include six cases with the following genotypes: V30M/R104H,[26] V30M/T119M,[27] V30M/K90N,[28] T59L/R104H,[29] K90N/T119M,[30] and V122I/F44L.[21] In most cases the clinical phenotype appears to be a combinatorial effect of both mutations. The T119M seems to be protective with delayed tetramer dissociation and increased stability.[30] Both V122I compound heterozygotes described here manifested symptoms in the fifth to sixth decades of life, significantly earlier than observed with single *TTR* mutations. The I68L mutation is associated primarily with cardiac disease but has also been found to be associated with carpel tunnel in most cases.[11] Bilateral carpel tunnel syndrome is present in our V122I/I68L case, as well as cardiomyopathy, demonstrating a cumulative effect of both alterations. However, although the T60A variant is associated with progressive neuropathy and cardiomyopathy,[4] only cardiac disease was observed in the V122I/T60A case. Further studies are therefore needed to understand the effect of the presence of two alterations within *TTR* on tetramer stability, disease onset, and progression.

In summary, our observations indicate that the clinical effect of the homozygous V122I genotype is high, with significant cardiac symptoms beginning in the early seventh decade of life. Further, based on the clinical history of the two V122I compound heterozygotes within our cohort, it appears that manifestation of symptoms in these cases occurs even earlier, in the late fifth to early sixth decade of life. This observation highlights that genetic *TTR* testing should not be reserved for only the very elderly but that it may prove useful in much younger patients, facilitating the initiation of appropriate treatment and management strategies.

Acknowledgments

We thank Keri Kruckerberg for providing the sequencing results of *TTR* testing within the Mayo Clinic Molecular Genetics Laboratory for this study.

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