

## VARIANT-SEQUENCE TRANSTHYRETIN (ISOLEUCINE 122) IN LATE-ONSET CARDIAC AMYLOIDOSIS IN BLACK AMERICANS

DANIEL R. JACOBSON, M.D., RAYMOND D. PASTORE, M.D., ROBERT YAGHOUBIAN, M.D., IMMACULATA KANE, M.S., GLORIA GALLO, M.D., FRANCIS S. BUCK, M.D., AND JOEL N. BUXBAUM, M.D.

### ABSTRACT

**Background** After the age of 60, isolated cardiac amyloidosis is four times more common among blacks than whites in the United States; 3.9 percent of blacks are heterozygous for an amyloidogenic allele of the normal serum carrier protein transthyretin in which isoleucine is substituted for valine at position 122 (Ile 122). We hypothesized that the high prevalence of transthyretin Ile 122 is at least partially responsible for the increased frequency of senile cardiac amyloidosis among blacks.

**Methods** Paraffin blocks of cardiac tissue were obtained from an earlier study of 52,370 autopsies in Los Angeles and were examined by immunohistochemical and DNA analyses. Samples were available from 32 of 55 blacks and 20 of 78 whites over 60 years of age with isolated cardiac amyloidosis and from two control groups (228 cases).

**Results** Transthyretin amyloidosis was identified in 31 of the 32 cardiac-tissue samples from the black patients and in 19 of the 20 samples from the white patients. Six of the 26 analyzable DNA samples (23 percent) from the black patients and none of the 19 samples from the white patients were heterozygous for the Ile 122 variant. Four of 125 DNA samples obtained at autopsy (3.2 percent) from a second, more recent, age-matched cohort of blacks without amyloidosis at the same institution were heterozygous for the transthyretin Ile 122 allele. On reexamination the cardiac tissue from these four patients contained small amounts of amyloid not detected at the initial autopsies. All subjects with the Ile 122 variant had ventricular amyloid.

**Conclusions** The assessment of elderly black patients with unexplained heart disease should include a consideration of transthyretin amyloidosis, particularly that related to the Ile 122 allele. (N Engl J Med 1997;336:466-73.)

©1997, Massachusetts Medical Society.

**I**SOLATED cardiac amyloidosis appearing late in life (senile cardiac amyloidosis) was first described in the 19th and early 20th centuries.<sup>1,2</sup> Pathological studies established its greater prevalence with increasing age, but the distinction between atrial and ventricular deposits was usually ignored.<sup>3-6</sup> The amyloid was thought to be a coincidental finding of limited importance. Later studies documented the clinical significance of the ventricular deposits in producing congestive heart failure, atrial fibrillation, and death from cardiac causes.<sup>7,8</sup>

The subsequent detection of small vascular deposits in other tissues prompted the suggestion that this disorder be renamed senile systemic amyloidosis.

The deposited fibrils contain transthyretin, a serum protein that normally transports retinol-binding protein and 25 percent of circulating thyroxine.<sup>9,10</sup> In some elderly patients, the transthyretin amyloid has a normal amino acid sequence<sup>11-13</sup>; the cause of amyloid formation in these patients is unknown. In contrast, patients with autosomal dominant familial transthyretin amyloidosis produce a more fibrillogenic mutant protein.<sup>14</sup> More than 50 amyloidogenic transthyretin mutations are known, most of which cause deposition in midadult life, primarily in the heart and peripheral nerves (designated familial amyloid cardiomyopathy or familial amyloid polyneuropathy, depending on the primary site of deposition).<sup>14,15</sup>

Senile cardiac amyloidosis and familial amyloid cardiomyopathy are clinically similar; thus, amyloidogenic transthyretin variants may be unrecognized, common causes of cardiac amyloidosis in some populations. One such candidate is transthyretin isoleucine 122 (Ile 122), which results from a change from A to C in codon 122, leading to the substitution of isoleucine for valine. This variant was discovered in 1988, in transthyretin isolated from the fibrils of a 68-year-old black man with no known family history of amyloidosis who died of massive cardiac amyloidosis.<sup>16</sup> DNA analysis revealed that the patient was homozygous for the amyloidogenic allele.<sup>17</sup> Three unrelated patients of black ancestry with cardiac transthyretin amyloidosis and the Ile 122 substitution were subsequently described.<sup>18-20</sup> The presence of transthyretin Ile 122 in several patients of black ancestry with cardiac amyloidosis suggested that the variant may be a common cause of heart disease in blacks. If so, it would contribute to the 28 percent of deaths that are due to cardiovascular disease among blacks and a rate of death from cardiovascular disease that is 1.5 times that of the total population of the United States.<sup>21</sup>

From the Research Service, New York Veterans Affairs Medical Center, New York (D.R.J., R.D.P., R.Y., I.K., J.N.B.); the Departments of Medicine (D.R.J., J.N.B.) and Pathology (G.G., J.N.B.), New York University School of Medicine, New York; and the Department of Pathology, Los Angeles County-University of Southern California Medical Center, Los Angeles (F.S.B.). Address reprint requests to Dr. Buxbaum at the Research Service, New York Veterans Affairs Medical Center, 423 East 23rd St., New York, NY 10010.

In a molecular epidemiologic analysis, we found 66 transthyretin Ile 122 alleles in DNA samples from 65 of 1688 black Americans. The calculated allele frequency of 0.020 was similar for all geographic areas in the United States, indicating that 1.3 million U.S. blacks carry the Ile 122 allele, including 13,000 homozygotes.<sup>22</sup> To date the variant has been reported only in persons of African ancestry, despite extensive screening of other populations.<sup>23-25</sup>

In a review of 52,370 autopsies performed at the Los Angeles County–University of Southern California Medical Center from 1949 to 1982, 136 cases of senile cardiac amyloidosis were identified on the basis of the patient’s age (over 60 years), the heart as the main organ involved, and the confinement of amyloid in other organs to small blood vessels. After the age of 60, the prevalence of senile cardiac amyloidosis among U.S. blacks (55 of 3334, or 1.6 percent) was significantly greater than among either non-Hispanic whites (78 of 18,470, or 0.42 percent) or Hispanics of Mexican origin (3 of 2354, or 0.13 percent), even though all other types of amyloidosis were less prevalent among blacks.<sup>26</sup> These data suggested that the higher prevalence of senile cardiac amyloidosis among blacks reflected a factor increasing the likelihood of cardiac amyloid deposition that was specific to this group, perhaps the transthyretin Ile 122 allele.

The present study was designed to analyze the molecular genetics of late-onset cardiac transthyretin amyloidosis, in particular to assess the role of transthyretin Ile 122.

**METHODS**

**Pathological Analysis**

Cardiac-tissue blocks were available from 32 of the 55 blacks and 20 of the 78 non-Hispanic whites given a diagnosis of senile cardiac amyloidosis on the basis of conventional pathological criteria in an earlier review of autopsies performed at the Los Angeles County–University of Southern California Medical Center.<sup>26</sup> They were confirmed to be positive on the basis of Congo red staining, and the degree of deposition was assessed according to established standards: a score of 1+ indicates replacement of <10 percent of myocardium with amyloid; a score of 2+, replacement of 10 to 25 percent of myocardium; a score of 3+, replacement of 26 to 50 percent of myocardium; and a score of 4+, replacement of more than 50 percent of myocardium.<sup>27</sup> An additional score of 0.5+ (trace) was used to identify barely detectable deposits. The slides were processed for immunoperoxidase staining and examined with antiserum specific for transthyretin, immunoglobulin kappa and lambda light chains, amyloid A, and amyloid P component.<sup>28</sup> Control specimens for the immunohistologic studies included cardiac tissues from New York patients known to have AL (amyloid light chain) amyloid, patients with familial amyloid polyneuropathy with cardiac involvement, and patients with AL amyloid from the Los Angeles autopsy series. The pathologist performing the immunohistochemical analyses did not know the genetic results.

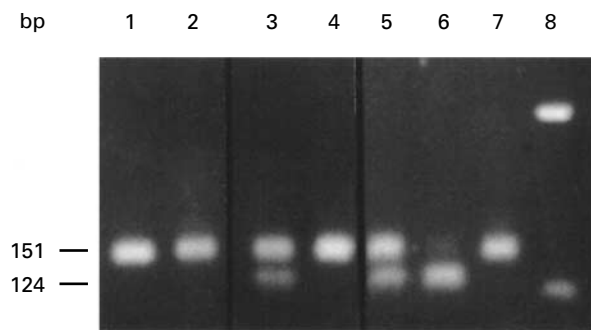
The original study, which was the source of the archival material,<sup>26</sup> was a retrospective analysis of all autopsies in which amyloidosis had been diagnosed. The cases that could not be confirmed on histologic reexamination were excluded. The inves-

tigators did not ascertain how often histologically evident cardiac amyloidosis was missed in the original autopsies. To establish the frequency of false negative results in the original series, we reexamined cardiac tissue from 103 randomly chosen black patients over the age of 65, in whom amyloidosis was not diagnosed at the time of the original autopsy, for histologic evidence of ventricular amyloid.

**DNA Analysis**

DNA was extracted from the paraffin blocks as described previously.<sup>29</sup> The polymerase chain reaction (PCR) was used to amplify exon 4 of the transthyretin gene.<sup>30</sup> Because very little DNA was extractable from the paraffinized tissue blocks, two rounds of PCR were required; an aliquot of the initial PCR product was used as the template for the second reaction, in which both primers bound to the product of the initial amplification and one primer contained a mismatch that introduced a *FokI* restriction site into the PCR products derived from the transthyretin Ile 122 allele.<sup>31</sup> Digested PCR products were subjected to electrophoresis on agarose gels and stained with ethidium bromide to identify bands representing the digested (transthyretin Ile 122) and undigested (transthyretin Val 122) alleles (Fig. 1).

To minimize the risk of false positive results,<sup>32</sup> PCR was performed in laminar-flow tissue-culture hoods exposed to ultraviolet light between experiments, aerosol-resistant pipette tips were used, and DNA isolation and PCR analyses were performed in separate laboratories. PCR controls included DNA samples from a normal subject, a subject who was heterozygous for the transthyretin Ile 122 allele, a subject who was homozygous for the allele, and multiple negative controls (no DNA) in each experiment. Because of variations in the age of the samples and the method of fixation, the amount of intact DNA in the samples varied considerably, with some yielding PCR products only sporadically; thus, any PCR product seen in a single experiment could have represented a contaminant or may have been derived from only one allele in the original sample.<sup>33,34</sup> To ensure that all assays detected the true genotype, results were considered reliable only if they were confirmed during at least two separate determinations of each of two independent DNA extractions (each result had to be confirmed in quadruplicate). Samples for which these criteria were not fulfilled were considered technically inadequate. As population controls we assayed DNA isolated from tissue



**Figure 1.** The Transthyretin Ile 122 and Val 122 Alleles. PCR products were digested with *FokI*. Lanes 1 and 2 show samples from blacks with cardiac transthyretin amyloidosis and the normal allele at position 122 (Val 122); lane 3 shows a sample from a black patient with cardiac transthyretin amyloidosis who was heterozygous for the transthyretin Ile 122 allele. Lanes 4, 5, 6, and 7 show control specimens from subjects with the normal allele (lanes 4 and 7), a subject heterozygous for the transthyretin Ile 122 allele (lane 5), and a subject homozygous for the transthyretin Ile 122 allele (lane 6). Lane 8 shows the DNA size markers, with sizes of 383 and 121 bp.

blocks from 19 whites with senile cardiac amyloidosis from the same autopsy series and from autopsies of 125 age-matched blacks without a pathological diagnosis of any form of amyloidosis from a later period (1984 to 1989) at the same institution.

**Clinical Data**

Clinical summaries contained in pathology files were reviewed for all patients given a diagnosis of senile cardiac amyloidosis. Electrocardiograms or information revealing the presence or absence of congestive heart failure, atrial fibrillation or other arrhythmias, hypertension, and a history of treatment with digitalis glycosides was available in 59 of 136 cases. Among the 31 blacks with isolated cardiac amyloidosis, the clinical cardiac data sets were complete for only 7. The original hospital charts were not available for any patient.

**RESULTS**

Fifty of the 52 available cardiac blocks (31 of 32 from black patients and 19 of 20 from white patients) were positive for transthyretin (Table 1); 49 of the 50 did not react with any of the other precursor antiserum. One sample (from Patient 9 in Table 1) was positive for both transthyretin and immunoglobulin light chains, presumably reflecting nonspecific binding due to variability in tissue processing. The patient met all other criteria for senile cardiac amyloidosis. All samples were positive for amyloid P component, confirming that they contained amyloid.<sup>35</sup> The transthyretin-negative specimen (from Patient 27), although positive for anti-amyloid P component, did not react with any other antiserum. These results validated the original pathological diagnosis of senile cardiac amyloidosis in 96 percent of the patients.

Among the cardiac-tissue samples from black patients, 6 of the 31 that were positive for transthyretin had amyloid-deposition scores of 4+; 5 had scores of 3+; 1 had a score of 2+ to 3+; 6 had scores of 2+; and 13 had scores of 1+. One patient had coronary arterial amyloidosis, and two had deposits in intramural vessels. Apart from minimal-to-mild coronary atherosclerosis, amyloidosis was the only cardiac abnormality in 10 of 26 patients (38 percent). The remainder had more extensive coronary artery disease or hypertensive cardiovascular disease. When the cardiac-tissue slides from 103 black patients over the age of 60 that were presumed to be amyloid-negative (obtained during the same period as the original study) were reexamined for the presence of amyloid, 1 contained trace amounts of transthyretin amyloid. Thus, the false negative rate in the original sample was less than 1 percent. DNA was not available from that sample.

Molecular analysis was successful on DNA isolated from cardiac tissue from 26 of 31 blacks (Table 1). Six of the 26 (23 percent; 95 percent confidence interval, 11 to 35 percent) were heterozygous for the transthyretin Ile 122 allele, a value that is significantly higher than the value of 3.9 percent for the U.S. black population at large<sup>22</sup> (P<0.001). The sample

**TABLE 1. CLINICAL, PATHOLOGICAL, AND MOLECULAR CHARACTERISTICS OF 37 BLACK PATIENTS WITH LATE-ONSET CARDIAC AMYLOIDOSIS.\***

PATIENT No.	AGE (YR)/SEX	CONGESTIVE HEART FAILURE†	ATRIAL FIBRILLATION‡	EXTENT OF AMYLOID DEPOSITION§	TRANSTHYRETIN¶	Ile 122
1	71/M	-	-	1+	+	-
2	69/F	-	-	4+	+	-
3	81/M	-	+	1+	+	-
4	78/F	-	-	2+	+	-
5	77/M	-	-	2+	+	-
6	84/F	+	+	4+	+	+
7	71/M	+	+	4+	+	+
8	83/M	-	-	3+	+	+
9	91/F	NA	NA	3+	***	+
10	85/F	+	-	2+	+	+
11	74/F	+	+	1+	+	-
12	79/M	+	-	2+	+	-
13	91/M	NA	NA	1+	+	-
14	>65/F††	-	-	2+	+	-
15	71/F	NA	NA	2+	+	-
16	82/M	NA	NA	1+	+	-
17	85/M	-	-	4+	+	TI
18	77/M	+	-	2+ to 3+	+	-
19	76/F	+	-	3+	+	-
20	83/F	+	-	1+	+	TI
21	72/M	-	-	1+	+	TI
22	72/M	NA	NA	1+	+	TI
23	93/M	+	-	3+	+	+
24	73/M	NA	NA	1+	+	-
25	67/F	NA	NA	1+	+	-
26	85/F	+	NA	4+	+	-
27	84/M	NA	NA	1+	-	-
28	86/M	+	-	4+	+	TI
29	97/M	-	-	1+	+	-
30	71/F	NA	NA	1+	+	-
31	86/M	+	-	3+	+	TI
32	80/M	NA	NA	1+	+	-
33††	64/F	-	-	0.5+	+	+
34††	72/M	+	-	0.5+	+	+
35††	70/M	-	-	0.5+	+	+
36††	60/M	-	-	0.5+	-	+
37††	79/F	+	+	2+	+	-

\*A plus sign indicates the presence of the variable, and a minus sign its absence. NA denotes information not available, and TI technically inadequate (samples in which DNA could not be amplified from the archival material).

†The presence or absence of congestive heart failure was determined from information in the clinical abstract.

‡The presence or absence of atrial fibrillation was determined only in patients for whom electrocardiograms were available.

§The extent of amyloid deposition was determined by Congo red staining. A score of 1+ indicates replacement of <10 percent of myocardium with amyloid; a score of 2+, replacement of 10 to 25 percent of myocardium; a score of 3+, replacement of 26 to 50 percent of myocardium; and a score of 4+, replacement of more than 50 percent of myocardium.<sup>27</sup> An additional score of 0.5+ (trace) was used to identify barely detectable deposits.

¶Transthyretin status was determined by immunohistochemical analysis.

||Transthyretin Ile 122 status was determined by PCR and restriction-enzyme analysis.

\*\*This sample was also positive with an antiserum against immunoglobulin light chains.

††The patient's exact age was not known.

‡‡Patients 33 through 37 were part of a cohort from whom samples were obtained between 1982 and 1985 (see the Results section).

that could not be classified immunohistochemically was negative for transthyretin Ile 122. None of the samples from the 19 white patients with amyloidosis were positive for transthyretin Ile 122 (Table 2).

Of the 6 black heterozygotes, 5 (83 percent) had amyloid-deposition scores of 3+ or 4+, whereas only 3 of the 20 black patients who were negative for the transthyretin Ile 122 allele (15 percent) had heavy deposits ( $P < 0.005$ ). The clinical summaries provided pertinent data on five of the six heterozygotes: four had congestive heart failure, and two had atrial fibrillation.

To control for geographic, temporal, and selection biases inherent in autopsy studies, we assayed DNA obtained from tissue blocks from 125 randomly chosen U.S. blacks over the age of 60 in whom no amyloid was identified at the original autopsy, performed at the same institution from 1984 to 1989. Four patients (Patients 33, 34, 35, and 36 in Table 1) were heterozygous for the transthyretin Ile 122 allele (3.2 percent; allele frequency, 0.016); this prevalence is statistically identical to that in the general black population in the United States,<sup>22</sup> indicating that the autopsy population was not biased with respect to the frequency of the allele. Histologic examination of cardiac ventricles from 115 of the 125 controls by an observer who was unaware of the molecular analysis revealed myocardial amyloid in 5 (additional tissue was not available from 10 samples obtained at autopsy, all of which were negative for transthyretin Ile 122). Four were those identified as heterozygous for the transthyretin Ile 122 allele (Patients 33, 34, 35, and 36 in Table 1). Review of the fifth case (Patient 37 in Table 1) confirmed the presence of moderate amyloidosis, which was misdiagnosed at the time of the original routine autopsy. The prevalence of detectable amyloid was significantly greater in the patients with transthyretin Ile 122 than in those without (Table 3).

When the prevalence of transthyretin amyloid deposition was analyzed according to the age at death, a greater proportion of blacks than whites was affected in every age group, with a ratio of blacks to whites of approximately 8:1 for the ages 60 to 69. The ratio was greatest (13:1) for the ages 70 to 79 (Table 4). The ratio decreased in later decades because of an increase in cardiac amyloidosis among whites (Fig. 2). Homozygosity for the transthyretin Val 122 allele predominated in blacks with cardiac transthyretin amyloidosis into the ninth decade, whereas among blacks over 90 years of age, the majority were heterozygous for the transthyretin Ile 122 allele.

**DISCUSSION**

These data, obtained with the use of tissue from a large, racially and ethnically diverse autopsy study of amyloid in which specific precursors were identified, confirm earlier observations that the prevalence

**TABLE 2. FREQUENCY OF HETEROZYGOSITY FOR TRANSTHYRETIN Ile 122.**

POPULATION EXAMINED	NO. WITH TRANSTHYRETIN Ile 122/TOTAL (%)	P VALUE*
Blacks over 60 yr with cardiac amyloidosis	6/26 (23)	
All blacks	65/1688 (3.9)	<0.001
Whites over 60 yr with cardiac amyloidosis	0/19	0.03

\*The P value is for the comparison with blacks over 60 years of age with cardiac amyloidosis, by Fisher's exact test.

**TABLE 3. FREQUENCY OF LATE-ONSET TRANSTHYRETIN AMYLOIDOSIS IN AUTOPSIES OF PATIENTS OVER 60 YEARS OF AGE, ACCORDING TO THE TRANSTHYRETIN Ile 122 STATUS.**

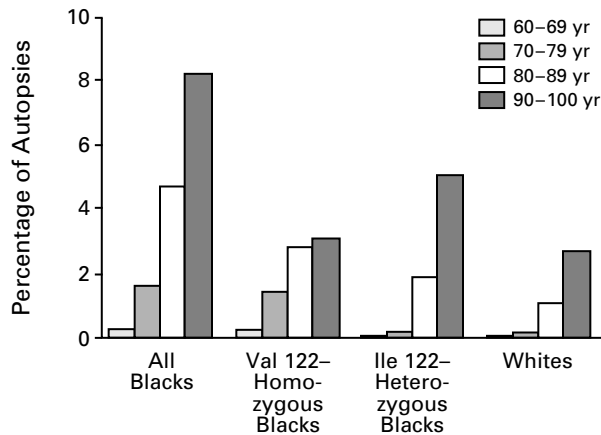
TRANSTHYRETIN Ile 122 STATUS	NO. WITH CARDIAC AMYLOIDOSIS/TOTAL (%)
Positive	4/4 (100)*
Negative	1/111 (0.9)

\* $P < 0.001$  for the comparison between the two groups, by Fisher's exact test.

**TABLE 4. DEMOGRAPHIC FEATURES OF PATIENTS WITH SENILE CARDIAC AMYLOIDOSIS, ACCORDING TO THE AGE AT DEATH.**

AGE (YR)	BLACKS		WHITES		BLACK: WHITE*
	NO. OF AUTOPSIES	NO. IN WHICH AMYLOIDOSIS DIAGNOSED (%)	NO. OF AUTOPSIES	NO. IN WHICH AMYLOIDOSIS DIAGNOSED (%)	
60-69	1652	4 (0.2)	6563	2 (0.03)	8:1
70-79	1118	18 (1.6)	7209	9 (0.1)	13:1
80-89	464	22 (4.7)	4145	46 (1.1)	4:1
90-99	98	8 (8.2)	551	15 (2.7)	3:1
>99	2	2 (100)	2	1 (50)	2:1

\*The ratio was calculated as (the number of blacks given a diagnosis of amyloidosis ÷ the number of blacks autopsied) ÷ (the number of whites given a diagnosis of amyloidosis ÷ the number of whites autopsied). The Hispanic cohort was of Mexican, not Caribbean, origin and was not included in the analysis since the number was too small (3 of 2354, 0.13 percent) for meaningful comparison by decade; the 3 cases of amyloidosis were in patients 80 to 89 years of age.



**Figure 2.** Percentage of Autopsies among All Blacks, Blacks Homozygous for the Transthyretin Val 122 Allele, Blacks with the Transthyretin Ile 122 Allele, and Whites in Which Transthyretin Cardiac Amyloidosis Was Identified, According to the Age at Death.

Only 3 of 2354 Mexican Americans (0.13 percent) had this form of amyloidosis, all of whom were 80 to 89 years of age.

of cardiac amyloid increases with age and demonstrate that, after the age of 60, the risk among blacks is four times greater than that among whites in the United States. Among blacks, the prevalence of the transthyretin Ile 122 allele was higher among those with cardiac amyloidosis at autopsy than among age-matched controls or in the general black population in the United States. Transthyretin Ile 122 was responsible for approximately 25 percent of the cases among blacks. Despite extensive screening in several centers, transthyretin Ile 122 has not yet been reported in persons who are not of African descent.<sup>23-25</sup>

Prior autopsy studies suggested that isolated ventricular amyloidosis (i.e., transthyretin-related amyloidosis) occurs in up to 25 percent of persons over the age of 90.<sup>8</sup> In the present study, 2.7 percent of whites and 8.2 percent of blacks who were 90 to 99 years of age had ventricular amyloidosis. Since both the current data and prior prevalence data were derived from autopsies, it is unlikely that the difference between them reflects the systematic underestimation of disease prevalence known to occur in autopsy series. The documentation of a false negative rate of 1 percent in the detection of cardiac amyloid at autopsy also makes a systematic error in diagnosis unlikely. It is not known why the prevalence was lower than in previous reports, but the discrepancy may reflect population differences or a disparity in autopsy rates.<sup>6</sup>

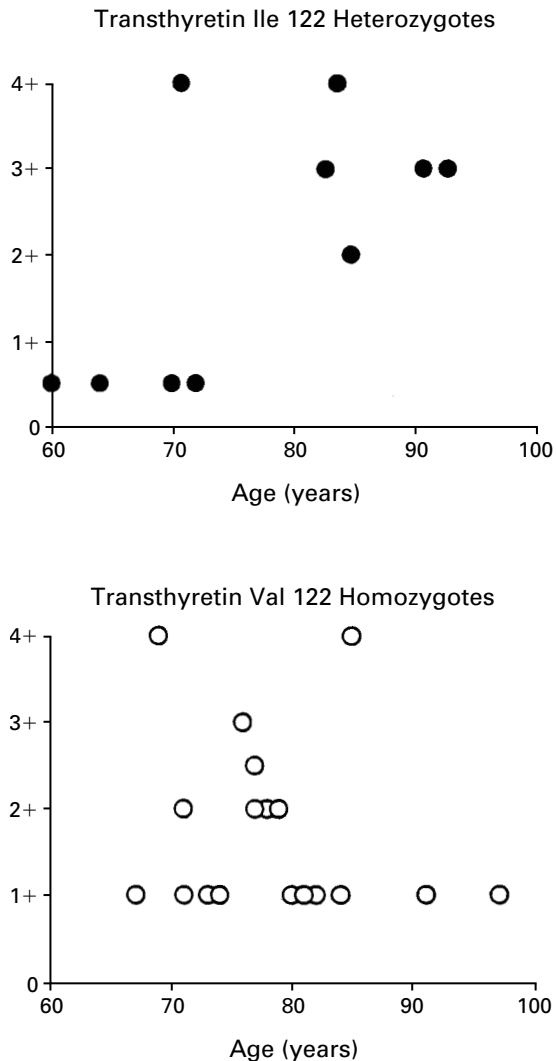
Our data raise the question of whether other investigators have found an increased risk of cardiac amyloidosis among blacks. Most series do not address ethnic or racial status, but two studies, both conducted before specific amyloid antiserum became

available, suggested a high risk in this group. In a Tennessee hospital, cardiac amyloidosis was identified in 15 of 600 of consecutive autopsies (2.5 percent) of patients over the age of 50<sup>36</sup>; 14 of the 15 patients were black. The reporting institution had a predominantly black population, making racial or ethnic comparisons impossible. Another study, which included both blacks and whites in the cohort of 1958 subjects over the age of 60, noted 42 cases of cardiac amyloidosis.<sup>3</sup> There was a trend toward a greater prevalence in blacks, but the sample size was too small to detect less than a threefold increase.

Of four patients with cardiac transthyretin Ile 122 amyloidosis described in prior case reports, two were homozygous for the variant allele,<sup>17-20</sup> despite the heterozygote-to-homozygote ratio of 100:1 in the black population in the United States,<sup>22</sup> suggesting that the risk of clinical disease is further increased in homozygotes. The absence of homozygotes in the present study is reasonable because of the size of the autopsy population: 0.038 percent of U.S. blacks are predicted to be homozygous for the variant allele. If there is no early increase in mortality among homozygotes, 1.3 homozygotes (95 percent confidence interval, 0.67 to 2.2) would be expected among 3334 subjects.

The examination of tissue obtained at autopsy from ethnically and age-matched patients without amyloidosis from a subsequent period (1984 to 1989) permitted us to evaluate the hypothesis that transthyretin Ile 122 confers an absolute risk for some degree of cardiac amyloidosis. The availability of a genetic marker, identifying persons potentially at risk, enabled us to study the genome and cardiac tissues independently (Table 3). The 3.2 percent prevalence of transthyretin Ile 122 in patients in whom amyloid deposition was missed at the initial autopsy did not differ statistically from that (3.9 percent) in our population survey.<sup>22</sup> The fact that the quantities of amyloid found in these patients (all under the age of 72) were very small supports a relation between increasing age and the degree of deposition (Fig. 3). The transthyretin Ile 122 allele appears to behave as an autosomal dominant gene with age-dependent penetrance. Our studies indicate that there is a threshold for detection of amyloidosis by a pathologist during a routine autopsy and that the observer must be both assiduous and suspicious.

Although the degree of deposition of transthyretin Ile 122 defined pathologically is semiquantitative, it is at least partially related to age (Fig. 3); a similar association was not seen among whites or blacks without the transthyretin Ile 122 allele. Additional factors (different environments, other gene products, or both) must affect the extent of deposition and explain the portion of the increased risk in blacks that is not related to the transthyretin Ile 122 allele. Some persons who are negative for the trans-



**Figure 3.** Degree of Amyloid Deposition According to the Age of the Patient at the Time of Death and the Type of Transthyretin Present.

Each point represents one patient. Since the degree of deposition was not measured as a continuous variable, the relation between age and amyloid deposition was assessed with a non-parametric analysis (Spearman's rank order). For patients who had one transthyretin Ile 122 allele, the *r* value was 0.5938, suggesting that approximately one third of the variance could be attributed to age. The analysis of the patients who were homozygous for transthyretin Val 122 revealed no relation between age and the degree of amyloid deposition. A score of 1+ indicates replacement of <10 percent of myocardium with amyloid; a score of 2+, replacement of 10 to 25 percent of myocardium; a score of 3+, replacement of 26 to 50 percent of myocardium; and a score of 4+, replacement of more than 50 percent of myocardium.

thyretin Ile 122 allele but have amyloidosis may have other mutations. The proposed model of an autosomal dominant disease with age-dependent penetrance is similar to that for disease associated with other transthyretin variants, such as transthyretin Met 30, although the latter usually has an earlier age of onset.<sup>37,38</sup>

What are the clinical implications of these findings? Almost 3 million blacks in the United States are over the age of 65, and 107,000 carry at least one transthyretin Ile 122 allele.<sup>21,22</sup> In this age group, a score of 3+ or 4+ for cardiac ventricular amyloid deposition is associated with an increased frequency of atrial fibrillation and congestive heart failure.<sup>27</sup> Among persons over the age of 90 in whom cardiac amyloidosis was found at autopsy, amyloid was the cause of death in half.<sup>27</sup>

Fragmentary information gleaned from the available hospital records of 59 of the 136 patients with senile cardiac amyloidosis included in the original autopsy study allowed some tentative judgments concerning the clinical relevance of the finding in those patients. Almost one third of the patients with congestive heart failure, atrial fibrillation, other conduction disturbances, or some combination thereof had no serious pathological evidence of heart disease other than amyloidosis.

With recent advances in the treatment of amyloidosis, the specific type of amyloid in each patient must be determined, because chemotherapy is now the standard approach to the treatment of patients with AL amyloid deposition<sup>39,40</sup> and liver transplantation is useful for young patients with familial transthyretin amyloidosis.<sup>41,42</sup> Although there is no specific therapy for patients with deposition of normal-sequence transthyretin amyloid and liver transplantation is problematic in elderly patients, our observations indicate that molecular diagnosis is important. Two thirds of the patients with amyloidosis had other types of heart disease. Since cardiac amyloidosis is known to increase sensitivity to digoxin and calcium-channel-blocking drugs,<sup>43-45</sup> the identification of amyloid deposition may affect the treatment of coexisting heart disease. Echocardiography and endomyocardial biopsy should make possible precise diagnosis in living patients whose transthyretin genotype is known.

In the absence of a known family history, patients with cardiac transthyretin Ile 122 amyloidosis may be given a clinical diagnosis of senile cardiac amyloidosis. Their disease is, by molecular definition, more accurately termed familial amyloid cardiomyopathy; thus, the term senile cardiac (systemic) amyloidosis should be reserved for patients confirmed to have deposition of normal-sequence transthyretin amyloid. Elderly patients with cardiac transthyretin amyloidosis who are not further evaluated are best given a diagnosis of late-onset cardiac transthyretin amyloidosis, indi-

cating the biochemical identity of the amyloid protein and the major clinical features of the disease.<sup>46,47</sup>

Except for some areas of Portugal, Sweden, and Japan in which there is a high prevalence of the transthyretin Met 30 allele, familial amyloidosis resulting from deposition of variant-sequence transthyretin has been considered rare. The data suggest that in the United States, transthyretin Ile 122 is a common, unrecognized genetic cause of late-onset heart disease among blacks. Our study was performed on autopsy samples collected from 1949 to 1982, when the average life expectancy was several years less than it is today.<sup>48</sup> The findings are even more relevant now when life expectancy, even among the medically underserved, has increased. The high frequency of the transthyretin Ile 122 allele among blacks and its age-dependent penetrance require that it be considered in any assessment of cardiac disease in black patients over the age of 60.

Supported by a grant (34900) from the National Institute of Diabetes and Digestive and Kidney Diseases, Veterans Affairs Merit Review funds (to Dr. Buxbaum), and an Established Scientist Award and grants-in-aid from the New York City Division of the American Heart Association (to Dr. Jacobson).

*We are indebted to Susan Hedayati, Helen Jordan, and Susan Schechter Booda for technical assistance.*

## REFERENCES

1. Soyka J. Ueber amyloide Degeneration. *Prag Med Wochenschr* 1876;1:165-71.
2. Beneke R, Bönning F. Ein Fall von lokaler Amyloidose des Herzens. *Beitr Pathol Anat* 1908;44:362-85.
3. Buerger L, Braunstein H. Senile cardiac amyloidosis. *Am J Med* 1960;28:357-67.
4. Wright JR, Calkins E. Amyloid in the aged heart: frequency and clinical significance. *J Am Geriatr Soc* 1975;23:97-103.
5. Wright JR, Calkins E, Breen WJ, Stolte G, Schultz RT. Relationship of amyloid to aging: review of the literature and systematic study of 83 patients derived from a general hospital population. *Medicine (Baltimore)* 1969;48:39-60.
6. Pomerance A. Age-related cardiovascular changes and mechanically induced endocardial pathology. In: Silver MD, ed. *Cardiovascular pathology*. 2nd ed. New York: Churchill Livingstone, 1991:155-62.
7. Hodkinson HM, Pomerance A. The clinical significance of senile cardiac amyloidosis: a prospective clinico-pathological study. *Q J Med* 1977;46:381-7.
8. Lie JT, Hammond PI. Pathology of the senescent heart: anatomic observations on 237 autopsy studies of patients 90 to 105 years old. *Mayo Clin Proc* 1988;63:552-64.
9. Kanai M, Raz A, Goodman DS. Retinol-binding protein: the transport protein for vitamin A in human plasma. *J Clin Invest* 1968;47:2025-44.
10. Bartalena L, Robbins J. Variations in thyroid hormone transport proteins and their clinical implications. *Thyroid* 1992;2:237-45.
11. Gustavsson Å, Jahr H, Tobiasson R, Jacobson DR, Sletten K, Westermarck P. Amyloid fibril composition and transthyretin gene structure in senile systemic amyloidosis. *Lab Invest* 1995;73:703-8.
12. Christmansson L, Betsholtz C, Gustavsson Å, Johansson B, Sletten K, Westermarck P. The transthyretin cDNA sequence is normal in transthyretin-derived senile systemic amyloidosis. *FEBS Lett* 1991;281:177-80.
13. Jacobson DR, Gertz MA, Kane I, Buxbaum JN. Genetic analysis of 9 unrelated patients with transthyretin (TTR)-cardiac amyloidosis: correlation of clinical and genetic findings and description of 2 new TTR variants. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. *Amyloid and amyloidosis 1993*. New York: Parthenon Publishing, 1994:474-6.
14. Jacobson DR, Buxbaum JN. Genetic aspects of amyloidosis. *Adv Hum Genet* 1991;20:69-123, 309-11.
15. Benson MD, Uemichi T. Transthyretin amyloidosis. *Amyloid Int J Exp Clin Invest* 1996;3:44-56.
16. Gorevic PD, Prelli FC, Wright J, Pras M, Frangione B. Systemic senile amyloidosis: identification of a new prealbumin (transthyretin) variant in cardiac tissue: immunologic and biochemical similarity to one form of familial amyloidotic polyneuropathy. *J Clin Invest* 1989;83:836-43.
17. Jacobson DR, Gorevic PD, Buxbaum JN. A homozygous transthyretin variant associated with senile systemic amyloidosis: evidence for a late-onset disease of genetic etiology. *Am J Hum Genet* 1990;47:127-36.
18. Nichols WC, Liepnieks JJ, Snyder EL, Benson MD. Senile cardiac amyloidosis associated with homozygosity for a transthyretin variant (ILE-122). *J Lab Clin Med* 1991;117:175-80.
19. Saraiva MJM, Sherman W, Marboe C, et al. Cardiac amyloidosis: report of a patient heterozygous for the transthyretin-isoleucine 122 variant. *Scand J Immunol* 1990;32:341-6.
20. Jacobson DR, Irtmann M, Buxbaum JN, Wiecezorek R, Gorevic PD. Cardiac amyloidosis resulting from transthyretin Ile 122 deposition in African-Americans: two case reports. *Texas Heart Inst J* (in press).
21. Blacks. In: Russell C. *The official guide to racial and ethnic diversity*. Ithaca, N.Y.: New Strategist, 1996:71-188.
22. Jacobson DR, Pastore R, Pool S, et al. Revised transthyretin Ile 122 allele frequency in African-Americans. *Hum Genet* 1996;98:236-8.
23. Jacobson DR, Reveille JD, Buxbaum JN. Frequency and genetic background of the position 122 (Val→Ile) variant transthyretin gene in the black population. *Am J Hum Genet* 1991;49:192-8.
24. Almeida MR, Altland K, Rauh S, et al. Characterization of a basic transthyretin variant — TTR Arg 102 — in the German population. *Biochim Biophys Acta* 1991;1097:224-6.
25. Alves IL, Altland K, Almeida MR, Becher P, Costa PP, Saraiva MJM. Screening of TTR variants in the Portuguese population by HIEF. *J Rheumatol* 1993;20:185. abstract.
26. Buck FS, Koss MN, Sherrod AE, Wu A, Takahashi M. Ethnic distribution of amyloidosis: an autopsy study. *Mod Pathol* 1989;2:372-7.
27. Smith TJ, Kyle RA, Lie JT. Clinical significance of histopathologic patterns of cardiac amyloidosis. *Mayo Clin Proc* 1984;59:547-55.
28. Gallo GR, Feiner HD, Chuba JV, Beneck D, Marion P, Cohen DH. Characterization of tissue amyloid by immunofluorescence microscopy. *Clin Immunol Immunopathol* 1986;39:479-90.
29. Mills NE, Fishman CL, Scholes J, Anderson SE, Rom WN, Jacobson DR. Detection of K-ras oncogene mutations in bronchoalveolar lavage fluid for lung cancer diagnosis. *J Natl Cancer Inst* 1995;87:1056-60. [Erratum, *J Natl Cancer Inst* 1995;87:1643.]
30. Jacobson DR, Buxbaum JN. A double-variant transthyretin allele (Ser 6, Ile 33) in the Israeli patient "SKO" with familial amyloidotic polyneuropathy. *Hum Mutat* 1994;3:254-60.
31. Jacobson DR. A specific test for transthyretin 122 (Val→Ile), based on PCR-primer-introduced restriction analysis (PCR-PIRA): confirmation of the gene frequency in blacks. *Am J Hum Genet* 1992;50:195-8.
32. Kwok S. Procedures to minimize PCR-product carry-over. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press, 1990:142-5.
33. Greer CE, Lund JK, Manos MM. PCR amplification from paraffin-embedded tissues: recommendations on fixatives for long-term storage and prospective studies. *PCR Methods Appl* 1991;1:46-50.
34. Navidi W, Arnheim N, Waterman MS. A multiple-tubes approach for accurate genotyping of very small DNA samples by using PCR: statistical considerations. *Am J Hum Genet* 1992;50:347-59.
35. Gallo G, Picken M, Frangione B, Buxbaum J. Nonamyloidotic monoclonal immunoglobulin deposits lack amyloid P component. *Mod Pathol* 1988;1:453-6.
36. Jones RS, Frazier DB. Primary cardiovascular amyloidosis: its clinical manifestations, pathology and histogenesis. *Arch Pathol* 1950;50:366-84.
37. Coelho T, Sousa A, Lourenco E, Ramalheira J. A study of 159 Portuguese patients with familial amyloidotic polyneuropathy (FAP) whose parents were both unaffected. *J Med Genet* 1994;31:293-9.
38. Grateau G, Adams D, Malapert D, Viemont M, Delpech M, Said G. Late-onset familial amyloid polyneuropathy with the TTR Met 30 mutation in France. *Clin Genet* 1993;43:143-5.
39. Skinner M, Anderson JJ, Simms R, et al. Treatment of 100 patients with primary amyloidosis: a randomized trial of melphalan, prednisone, and colchicine versus colchicine only. *Am J Med* 1996;100:290-8.
40. Kyle RA, Gertz MA, Garton JP, Greipp PR, Witzig TE, Lust JA. Primary systemic amyloidosis (AL): randomized trial of colchicine vs. melphalan and prednisone vs. melphalan, prednisone, and colchicine. In: Kisilevsky R, Benson MD, Frangione B, Gauldie J, Muckle TJ, Young ID, eds. *Amyloid and amyloidosis 1993*. New York: Parthenon Publishing, 1994:648-50.
41. Suhr OB, Holmgren G, Steen L, et al. Liver transplantation in familial amyloidotic polyneuropathy: follow-up of the first 20 Swedish patients. *Transplantation* 1995;60:933-8.

42. Skinner M, Lewis WD, Jones LA, et al. Liver transplantation as a treatment for familial amyloidotic polyneuropathy. *Ann Intern Med* 1994;120:133-4.
43. Rubinow A, Skinner M, Cohen AS. Digoxin sensitivity in amyloid cardiomyopathy. *Circulation* 1981;63:1285-8.
44. Griffiths BE, Hughes P, Dowdle R, Stephens MR. Cardiac amyloidosis with asymmetrical septal hypertrophy and deterioration after nifedipine. *Thorax* 1982;37:711-2.
45. Gertz MA, Falk RH, Skinner M, Cohen AS, Kyle RA. Worsening of congestive heart failure in amyloid heart disease treated by calcium channel-blocking agents. *Am J Cardiol* 1985;55:1645.
46. WHO-IUIS Nomenclature Sub-Committee. Nomenclature of amyloid and amyloidosis. *Bull World Health Organ* 1993;71:105-8.
47. Husby G. Nomenclature and classification of amyloid and amyloidoses. *J Intern Med* 1992;232:511-2.
48. Public Health Service. Healthy People 2000: national health promotion and disease prevention objectives. Washington, D.C.: Government Printing Office, 1991:46. (DHHS publication no. (PHS) 91-50212.)