# Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Quarta CC, Buxbaum JN, Shah AM, et al. The amyloidogenic V122I transthyretin variant in elderly black Americans. N Engl J Med 2015;372:21-9. DOI: 10.1056/NEJMoa1404852

**The Amyloidogenic V122I Transthyretin Variant in Elderly Black Americans**

**Supplementary Appendix.**

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## Methods

### Statistical analysis

Summary statistics were expressed as mean±standard deviation (SD), median [$25^{th}$-$75^{th}$ percentiles], or numbers (percentages). Comparisons were made between carriers and noncarriers of the *TTR* variant. In contingency tables, independence of categorical variables was tested using Fisher's exact test or Pearson's chi-square test. Independence of continuous variables was tested using Mann-Whitney U test or t-test to compare groups at visit 1 as well as visit 5. Adjusted comparisons were made using linear or logistic regression models, as appropriate.

Clinical characteristics, including hypertension, obesity, diabetes mellitus, coronary heart disease, smoking status, estimated glomerular filtration rate, lipid and glucose values and electrocardiographic criteria for left ventricular hypertrophy were assessed as described previously.[1,2] Survival estimates as a function of time from visit 1 were obtained according to the Kaplan-Meier method. Age- and gender-stratified hazard ratios were estimated to investigate the association between the *TTR* variant and the risk of death and incident heart failure using Cox proportional hazards regression. The stratified model effectively compares carriers with noncarriers of the same age and gender. We then obtained Kaplan-Meier estimates of survival using age as the underlying time variable. The areas under these curves were used to estimate the restricted mean survival time for mortality and for incident heart failure among patients alive and free of heart failure at age 53 over the subsequent 30 years. We excluded from the analyses of survival free from incident heart failure those patients with prevalent or missing heart failure status at the time of visit 1.

To address the issue of missing data on patients who were alive but didn't attend visit 5, we performed a multiple imputation analysis. We used all visit 1 variables featured in Table 1, the same variables obtained at visit 4, carrier status, and echocardiography data obtained from a subset of patients at visit 3. We attempted to impute all post-baseline missing values (including visit 5 echo data) using multiple imputation by chained equations.

We assessed whether age above or below 80 modified the relationship between carrier status and mortality.

Finally, a meta-analysis of the effect of the variant allele on the incidence of HF was conducted by combining our results with those from the Cardiovascular Health Study (CHS),[3] which is an observational study that, in 1989-1990, included 5201 subjects of all races, 931 of whom were black Americans; an additional 687 self-identified black Americans were added between 1992 and 1993. The age of CHS population at the time of enrolment ranged from 65 to 93. All had detailed medical histories recorded, complete physical examinations, electrocardiography and echocardiography performed according to standardized protocols. In 2010 Buxbaum et al analysed the prevalence and significance of the V122I *TTR* variant in this population.[3] Seventeen (11 females and 6 males) of the 805 consenting African-American participants in CHS carried the amyloidogenic V122I *TTR* allele (prevalence 2.12%). The frequency of congestive heart failure (38% vs 15%, RR 2.62, p = 0.04) and mortality (76% vs 53 %, RR 1.46, p =0.08) were higher in V122I allele carriers than in age, gender and ethnically matched controls, with more echocardiographic evidence suggestive of cardiac amyloidosis, findings consistent with age dependent clinical penetrance of this autosomal dominant gene.[3]

Two-sided p-values <0.05 were considered significant.

## Results

### Clinical and echocardiographic profiles at visit 5

We compared the group of 410 individuals with unknown genetic data with the 3856 participants with available DNA sequencing who were enrolled in the present analysis. We observed some minor differences in 3 of 16 baseline characteristics (Table S2).

## Results

### Clinical and echocardiographic profiles at visit 5

After imputation (table S3), we were able to analyze data for 2154 noncarriers and 76 carriers who were alive at the beginning of visit 5. Our conclusions remain generally consistent: our most significant differences (the ratio of early mitral inflow velocity to late mitral inflow velocity and the ratio of early mitral inflow velocity to lateral mitral early relaxation velocity) remain significant (p=0.02 and p=0.004, respectively), differences in left ventricular global longitudinal strain and left ventricular end-diastolic diameter become more significant (p=0.03 and p=0.02 in imputation analysis), while lateral mitral systolic velocity and relative wall thickness become non-significant (p=0.16 and p=0.37).

**Figure S1.** Study population recruitment and study flow chart starting from the enrolment of black Americans in the ARIC study. Of the 3732 non-carriers and the 124 carriers, respectively, recruited in the study and followed over time, 1194 (32%) and 46 (37%) underwent echocardiography during visit 5 and were compared for clinical, laboratory and echocardiographic data.

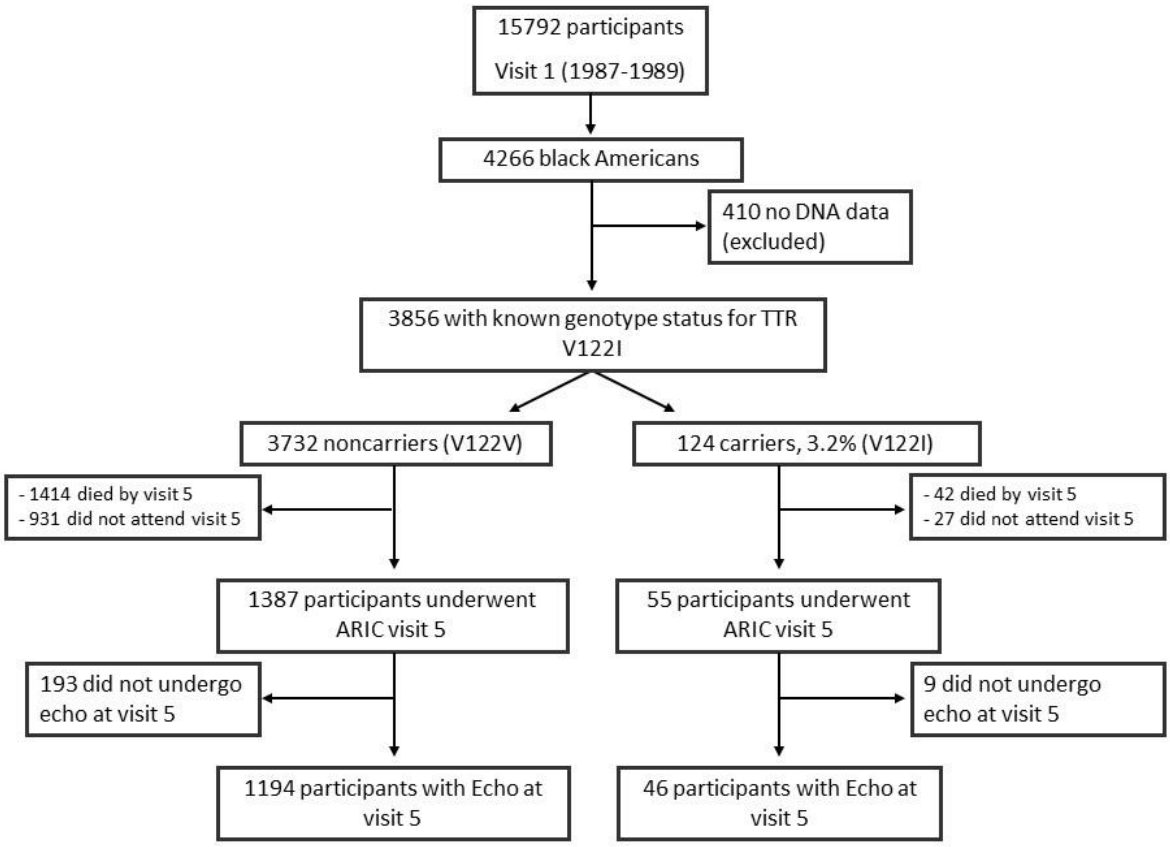
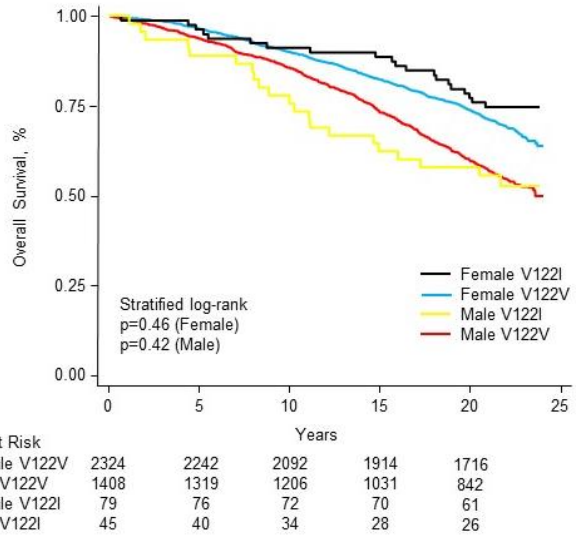


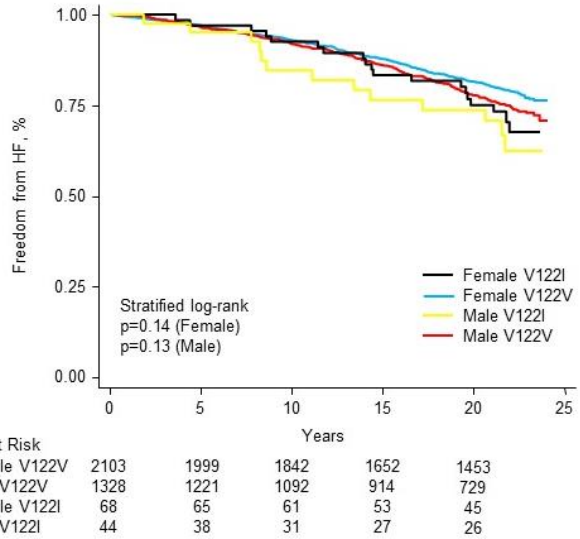
**Figure S2.** Kaplan-Meier curves for (a) overall survival and (b) freedom from heart failure according to the genotype status (carriers vs. noncarriers). V122I identifies carriers of the V122I *TTR* variant. V122V identifies the carriers of the wild-type (i.e. non mutant) allele.

a)




| No. at Risk | | | | | |
|---|---|---|---|---|---|
| Female V122V | 2324 | 2242 | 2092 | 1914 | 1716 |
| Male V122V | 1408 | 1319 | 1206 | 1031 | 842 |
| Female V122I | 79 | 76 | 72 | 70 | 61 |
| Male V122I | 45 | 40 | 34 | 28 | 26 |

b)




| No. at Risk | | | | | |
|---|---|---|---|---|---|
| Female V122V | 2103 | 1999 | 1842 | 1652 | 1453 |
| Male V122V | 1328 | 1221 | 1092 | 914 | 729 |
| Female V122I | 68 | 65 | 61 | 53 | 45 |
| Male V122I | 44 | 38 | 31 | 27 | 26 |

**Table S1.** Clinical characteristics in noncarriers vs. carriers of the V122I *TTR* allele at visit 5.*****

| | Noncarriers (N=1194) | Carriers (N=46) | P value |
|---|---|---|---|
| Age | | | |
| Median (IQR) — yr | 74 (71-78) | 74 (71-77) | 0.38 |
| Body Mass Index† | | | |
| Mean | 31 ± 7 | 29 ± 5 | 0.06 |
| Systolic blood pressure | | | |
| Mean — mm Hg | 134 ± 19 | 139 ± 16 | 0.08 |
| Diastolic blood pressure | | | |
| Mean — mm Hg | 70 ± 10 | 72 ± 10 | 0.18 |
| Hypertension — no./total no. (%) | 1046/1187 (88) | 38 (83) | 0.26 |
| Antihypertensive medication — no./total no. (%) | 718/1120 (64) | 27/43 (63) | 0.86 |
| Lipid-lowering medication — no./total no. (%) | 588/1114 (53) | 21/43 (49) | 0.61 |
| Diabetes — no. (%) | 571 (48) | 18 (39) | 0.25 |
| Smoking — no./total no. (%) | 80/1180 (7) | 4 (9) | 0.55 |
| Creatinine | | | |
| Median (IQR) — mg/dl | 1.0 (0.8-1.2) | 0.9 (0.8-1.2) | 0.79 |
| Estimated GFR | | | |
| Median (IQR) — ml/min | 81 (65-96) | 80 (66-90) | 0.78 |
| Heart rate | | | |
| Mean — beats/min | 64 ± 11 | 68 ± 10 | 0.06 |
| Atrial fibrillation — no./total no. (%) | 13/1168 (1) | 0 (0) | 1.00 |
| N-terminal pro–brain natriuretic peptide, Median (IQR) — pg/ml | 92 (44-188) | 127 (69-349) | 0.03 |
| Pacemaker — no./total no. (%) | 24/1168 (2) | 1 (2) | 1.00 |
| Increased PR interval on ECG — no./total no. (%) | 39/1168 (3) | 1 (2) | 1.00 |
| Low QRS voltage on ECG — no. (%) | 24 (2) | 1 (2) | 0.62 |

*Plus-minus values are means ± SD. Between-group differences were analyzed with the use of Fisher's exact test or Pearson's chi-square test for categorical variables and the Mann-Whitney U test or t-test for continuous variables. To convert values for creatinine values to micromoles per liter, multiply by 88.4. GFR denotes glomerular filtration rate, and IQR interquartile range.
†The body-mass index is the weight in kilograms divided by the square of the height in meters.
Low QRS voltage is defined as the presence of a QRS amplitude ≤0.5 mV in all limb leads or ≤1 mV in all precordial leads.

**Table S2.** Clinical characteristics at visit 1 in individuals without vs. those with known genotype status.*

| | Unknown genotype status (n=410) | Known genotype status (n=3856) | P value |
|---|---|---|---|
| Age | | | |
| Median (IQR) — yr | 53 (49-59] | 53 (48-58) | 0.31 |
| Male gender — no. (%) | 178 (43) | 1453 (38) | 0.023 |
| Body Mass Index† | | | |
| Mean | 29 ± 6 | 30 ± 6 | 0.12 |
| Systolic blood pressure | | | |
| Mean — mm Hg | 131 ± 25 | 129 ± 21 | 0.022 |
| Diastolic blood pressure | | | |
| Mean — mm Hg | 80 ± 13 | 80 ± 12 | 0.96 |
| Heart rate (bpm) | 67 ± 12 | 67 ± 11 | 0.95 |
| Systolic blood pressure ≥ 140 mmHg — no. (%) | 122 (30) | 975 (25) | 0.04 |
| Diastolic blood pressure ≥ 90 mmHg — no. (%) | 72 (18) | 678 (18) | 0.96 |
| Hypertension — no. (%) | 234 (57) | 2140 (56) | 0.54 |
| Antihypertensive medication — no. (%) | 170 (42) | 1557 (41) | 0.68 |
| Diabetes — no. (%) | 81 (21) | 740 (20) | 0.58 |
| Lipid-lowering medication — no. (%) | 7 (2) | 55 (1) | 0.64 |
| Smoking — no. (%) | 136 (33) | 1137 (30) | 0.12 |
| Prevalent heart failure — no. (%) | 41 (10) | 255 (7) | 0.009 |
| Creatinine | | | |
| Median (IQR) — mg/dl | 0.9 (0.8, 1.1) | 0.9 (0.8-1.0) | 0.08 |
| Estimated GFR | | | |
| Median (IQR) — ml/min | 101.7 (87-119] | 102 (87-119) | 0.72 |

*Plus-minus values are means ± SD. Between-group differences were analyzed with the use of Fisher's exact test or Pearson's chi-square test for categorical variables and the Mann-Whitney U test or t-test for continuous variables. To convert values for creatinine values to micromoles per liter, multiply by 88.4. GFR denotes glomerular filtration rate, and IQR interquartile range.
†The body-mass index is the weight in kilograms divided by the square of the height in meters.

**Table S3.** Imputation analysis using echocardiographic findings in noncarriers vs. carriers of the V122I *TTR* allele.*

| | Noncarriers (N=2154) | | Carriers (N=76) | | P value |
|---|---|---|---|---|---|
| | Mean | Std. Err. | Mean | Std. Err. | |
| Interventricular septum thickness — cm | 1.08 | 0.005 | 1.10 | 0.028 | 0.58 |
| Posterior wall thickness — cm | 0.96 | 0.005 | 0.99 | 0.026 | 0.15 |
| Relative wall thickness‡ | 0.45 | 0.003 | 0.46 | 0.018 | 0.37 |
| LV mass — g | 150.18 | 1.494 | 158.39 | 7.337 | 0.21 |
| LV mass index | | | | | |
| Calculated as g/$m^2$ of body-surface area | 79.23 | 0.785 | 84.08 | 3.385 | 0.11 |
| LV end-diastolic diameter — cm | 4.31 | 0.019 | 4.38 | 0.092 | 0.35 |
| LV end-systolic diameter — cm | 2.58 | 0.015 | 2.78 | 0.090 | 0.017 |
| LV end-diastolic volume — ml | 84.00 | 0.752 | 90.48 | 4.587 | 0.13 |
| LV end-systolic volume — ml | 30.77 | 0.484 | 34.06 | 2.945 | 0.23 |
| LV ejection fraction — % | 64.34 | 0.198 | 63.56 | 1.213 | 0.44 |
| Left atrial volume index | | | | | |
| Calculated as ml/m2 of body-surface area | 26.85 | 0.238 | 28.55 | 1.366 | 0.12 |
| E-wave Deceleration Time — msec | 198.81 | 1.545 | 190.18 | 7.102 | 0.23 |
| Ratio of early mitral inflow velocity to late mitral inflow velocity | 0.83 | 0.010 | 0.93 | 0.055 | 0.017 |
| Lateral mitral early relaxation velocity — cm/sec | 6.93 | 0.089 | 6.41 | 0.310 | 0.11 |
| Ratio of early mitral inflow velocity to lateral mitral early relaxation velocity | 10.39 | 0.147 | 11.81 | 0.510 | 0.004 |
| Lateral mitral systolic velocity — cm/sec | 7.18 | 0.046 | 6.83 | 0.255 | 0.16 |
| Tricuspid-regurgitation velocity — cm/sec | 240.42 | 1.649 | 245.64 | 9.577 | 0.60 |
| LV global longitudinal strain — % | -17.31 | 0.088 | -16.35 | 0.410 | 0.026 |
| Basal LV longitudinal strain — % | -15.98 | 0.125 | -15.05 | 0.612 | 0.16 |
| Mid LV ventricular longitudinal strain — % | -16.53 | 0.098 | -15.65 | 0.490 | 0.06 |
| Apical LV longitudinal strain — % | -19.41 | 0.198 | -18.35 | 0.838 | 0.22 |
| LV global radial strain — % | 24.13 | 0.279 | 22.40 | 1.085 | 0.11 |
| LV global circumferential strain — % | -26.89 | 0.200 | -26.12 | 0.810 | 0.41 |

*Values are expressed as means and standard errors. LV denotes left ventricular. ‡Relative wall thickness is calculated as (2 × the posterior wall thickness measured at end diastole in centimeters) ÷ the end-diastolic diameter of the left ventricle in centimeters.

**References**

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[2] Kottgen A, Russell SD, Loehr LR, et al. Reduced kidney function as a risk factor for incident heart failure: the Atherosclerosis Risk in Communities (ARIC) study. J Am Soc Nephrol 2007;18:1307–15.

[3] Buxbaum J, Alexander A, Koziol J, Tagoe C, Fox E, Kitzman D. Significance of the amyloidogenic transthyretin Val 122 Ile allele in African Americans in the Arteriosclerosis Risk in Communities (ARIC) and Cardiovascular Health (CHS) Studies. Am Heart J 2010;159:864–70.