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Clinical Implications of the Amyloidogenic V122I Transthyretin Variant in the General Population

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Abstract

AIMS.—The V122I variant in transthyretin (TTR) is the most common amyloidogenic mutation worldwide. The aim of this study is to describe the cardiac phenotype and risk for adverse cardiovascular outcomes of young V122I *TTR* carriers in the general population.

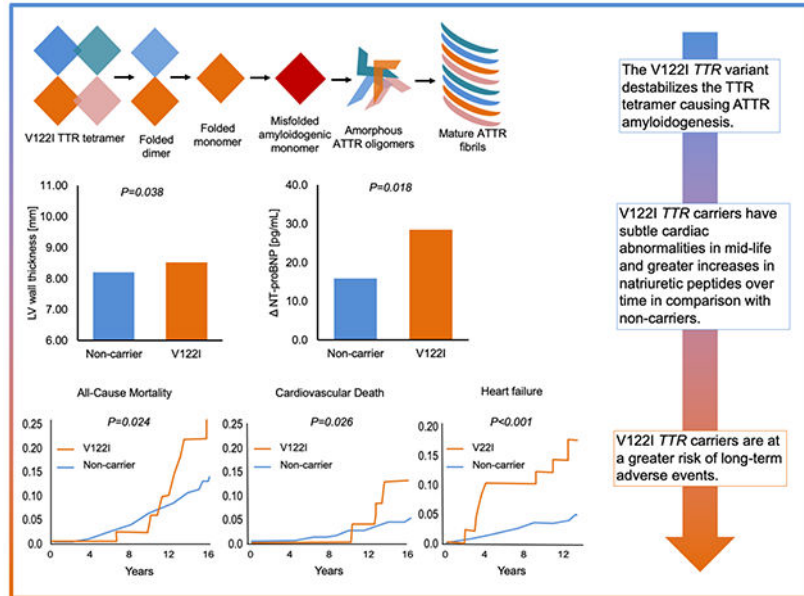
METHODS AND RESULTS.—*TTR* genotypes were extracted from whole-exome sequence data in participants of the Dallas Heart Study. Participants with African ancestry, available V122I *TTR* genotypes (N=1,818), and either cardiac magnetic resonance imaging (CMR) (n=1,364), or long-term follow-up (n=1,532) were included. The prevalence of V122I *TTR* carriers (45±10 years) was 3.2% (n/N=59/1,818). V122I *TTR* carriers had higher baseline LV wall thickness (LVWT, 8.52±1.82 vs. 8.21±1.62 mm; adjusted P=0.038) than non-carriers, but no differences in other CMR measures (P>0.05 for all). Although carrier status was not associated with amino terminal pro-B-type natriuretic peptide (NT-proBNP) at baseline (P=0.79), V122I *TTR* carriers had a greater increase in NT-proBNP on follow-up than non-carriers (median [interquartile range] 28.5 [11.4–104.1] vs. 15.9 [0.0–43.0] pg/mL; adjusted P=0.018). V122I *TTR* carriers were at a higher adjusted risk of heart failure (HF) (HR 3.82, 95% CI 1.80–8.13, P<0.001), cardiovascular death (HR 2.65, 95% CI 1.14–6.15, P=0.023), and all-cause mortality (HR 1.95, 95% CI 1.08–3.51, P=0.026) in comparison with non-carriers.

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CONCLUSION.—V122I *TTR* carrier status was associated with a greater increase in NT-proBNP, slightly greater LVWT, and a higher risk for HF, cardiovascular death, and all-cause mortality. These findings suggest the need to develop amyloidosis screening strategies for V122I *TTR* carriers.

Graphical Abstract



ONE-SENTENCE SUMMARY

Carriers of the V122I *TTR* mutation may have subtle differences in cardiac structure, greater changes in natriuretic peptides over time, and are at a higher risk of long-term events including heart failure and death.

LAY SUMMARY

The V122I variant in transthyretin (TTR) is the most common cause of hereditary cardiac amyloidosis worldwide – a disease where abnormal protein is deposited in the heart muscle, eventually leading to heart failure. However, early phases of the disease are not well known. 3.2% of individuals (45 ± 10 years) with African ancestry in the Dallas Heart Study carried the V122I *TTR* variant. In comparison with non-carriers, V122I *TTR* carriers had subtle differences in cardiac structure, greater increases in biomarkers that indicate cardiac stress, and a higher risk of heart failure and death. This study highlights the need to develop amyloidosis screening strategies.

Keywords

Cardiac amyloidosis; transthyretin; heart failure

INTRODUCTION

Cardiac ATTR, whether hereditary due to a *TTR* genetic variant (ATTR_v) or acquired (i.e. wild-type ATTR), is a progressive, infiltrative cardiomyopathy that leads to limitations in functional capacity, declines in quality of life, increased healthcare utilization, and higher risk of death.¹ Fortunately, therapeutic options that have the potential to improve the outcomes of patients with either form of the disease are emerging rapidly.^{2, 3} In parallel, developments in noninvasive imaging techniques such as CMR imaging and bone scintigraphy have facilitated an increased recognition of ATTR.^{1, 4, 5}

The valine-to-isoleucine substitution at position 122 (V122I; p.V142I) in the TTR protein causes tetrameric instability leading to amyloidogenesis,⁶ is the most common cause of ATTR_v cardiomyopathy in the United States, and is nearly exclusive to individuals with African ancestry.⁷ Observations from epidemiologic studies have highlighted that carriers of the V122I TTR allele are at higher lifetime risk for HF than their non-carrier counterparts.^{8, 9}

Although estimates suggest that penetrance of the V122I ATTR_v phenotype increases with age,⁸ the overall penetrance of the V122I TTR allele remains unknown and may be related to other genetic and environmental interactions.¹⁰ As a result, the pre-symptomatic natural history is uncertain, and there is no consensus on age-appropriate screening strategies for asymptomatic V122I *TTR* carriers.¹¹ The DHS, a large, multi-ethnic, population-based study with extensive clinical, genetic, imaging, and biomarker data, afforded the opportunity to test the hypotheses that young V122I *TTR* carriers, as compared with their non-carrier counterparts, may have both a distinct cardiac phenotype and increased risk for adverse cardiovascular outcomes.

METHODS

Study population

The DHS is a multiethnic probability-based population cohort study of adults in Dallas County, Texas that had intentional oversampling of self-identified Black individuals. Phase 1 of the DHS (DHS-1) was conducted between 2000 and 2002 and comprised of 3 visits: 1) initial home visit for collection of demographic, medical history, blood pressure, and anthropometric data (N=6,101); 2) second home visit for collection of blood and urine biospecimens (N=3,557); and 3) a final visit to the University of Texas Southwestern Medical Center for completion of detailed, protocolized, core lab imaging studies (N=2,971). Phase 2 of the DHS (DHS-2) was conducted between 2007 and 2009 and included alive and willing participants who underwent repeat cardiac imaging and fasting blood sample collection as previously described.¹²

The present analysis was restricted to DHS participants of genetically inferred African ancestry (defined below), who completed visit 2 of DHS-1 and had whole-exome sequencing data available through a collaboration with the Regeneron Genetics Center (Tarrytown, New York). The association between *TTR* V122I genotype and incident clinical outcomes was assessed in participants with long-term follow-up data (n=1,532, Figure 1).

The association between *TTR* V122I genotype and cardiac phenotype was assessed in n=1,364 participants with CMR imaging data at baseline for DHS-1, and the association between *TTR* V122I genotype and changes in cardiac biomarkers, electrocardiogram parameters, and CMR data was assessed for n=872 participants with paired DHS-1 and DHS-2 data. This study complies with the Declaration of Helsinki, was approved by the University of Texas Southwestern Medical Center Institutional Review Board, and informed consent was obtained from all study subjects.

Imaging methods

CMR imaging was performed with a 1.5 Tesla system (Intera; Philips Medical Systems, Best, The Netherlands) at DHS-1. Follow-up imaging was performed a median of 7 years after baseline imaging at DHS-2. In DHS-2, CMR imaging was obtained using a 3.0 Tesla system (Achieva; Phillips Medical Systems, Best, The Netherlands). An accreditation phantom was imaged on the 1.5-T and 3-T systems, allowing baseline and follow-up mass and volume measurements to be normalized to this control. QMass software (Medis Medical Imaging Systems, Leiden, The Netherlands; version 6.2.3) was used to analyze the data. Short-axis scans were performed with breath hold and electrocardiographic-gated cine as previously described.^{12, 13} LV measurements, LV mass, and LV wall thickness were then determined. Briefly, left ventricle LV measurements were determined by manually traced endocardial and epicardial contours. LV mass was calculated by multiplication of myocardial specific gravity (1.05 g/mL) with the difference between end-diastolic endocardial and epicardial contour. LV wall thickness was acquired from the short-axis images, with the exclusion of the most apical and basal slices. LV concentricity was defined as LV mass/LV end diastolic volume^{0.67}. Myocardial contraction fraction was calculated as stroke volume/myocardial volume where myocardial volume was calculated as LV mass/1.05 g/mL.¹⁴ The interobserver difference, intraobserver difference, and interscan variability were previously described.^{12, 13} Some individuals failed to complete the CMR study for the following reasons: equipment failure (N=2), contraindication (N=48), refusal (N=6), claustrophobia (N=184), or scheduling conflicts (N=28). The comparison of characteristics of DHS-1 participants and *TTR* V122I carriers stratified by whether they had the CMR assessment is shown in Supplement 1.

ECG Acquisition

A twelve-lead ECG was performed for both DHS-1 and DHS-2 and measured using a Marquette Medical System (General Electric) with MAC 5000 hardware and software configuration, which measured rate, rhythm, intervals (PR, QRS, and QT), and voltages. Limb lead voltage was calculated as the sum of the entire QRS voltage of leads I, II, and III. The Sokolow voltage was calculated as the sum of the S wave in V1 plus the highest voltage R wave in V5 or V6.

Genetic sequencing

Genomic DNA was extracted from circulating leukocytes. DNA sample preparation and whole-exome sequencing were performed using standard methods, as previously described.¹⁵

Principal components of genetic ancestry were estimated in PLINK version 1.9b.¹⁶ African ancestry was inferred from genetic principal components based on a linear model trained on PC estimates from the 1000 genomes project known ancestry groups, as described previously.¹⁵ *TTR* genotypes were extracted from whole-exome sequence data. All V122I *TTR* carriers were of African ancestry based on principal component analysis. Genotype frequencies of the *TTR* V122I (rs76992529) variant were in Hardy-Weinberg proportions ($P>0.05$).

Measurement of Biomarkers

For both DHS-1 and DHS-2, blood samples were collected by venipuncture, centrifuged and the plasma component was stored in EDTA tubes. Samples were maintained at 4°C for less than 4 hours before undergoing centrifugation (1430 g for 15 minutes). Plasma was extracted and frozen at -70°C and stored until measurement was performed. Hs-cTnT and NT-proBNP measurements were obtained by previously established protocols^{17, 18}.

Variable definition

Participants self-reported their age, sex, and ethnicity. Hypertension was determined as mean systolic blood pressure ≥ 140 mmHg, mean diastolic pressure ≥ 90 mmHg, or use of antihypertensive medications. Estimated glomerular filtration rate was calculated with the Modification of Diet and Renal Disease equation. Diabetes was defined as fasting glucose ≥ 126 mg/dL, non-fasting glucose ≥ 200 , or use of antihyperglycemics.

Long-term Outcomes

The National Death Index was queried to determine participant mortality through December 31, 2016. Deaths were classified as cardiovascular according to *International Classification of Diseases, Revision 10* codes 100-199. Global cardiovascular disease, atherosclerotic cardiovascular disease, HF, myocardial infarction, and stroke were adjudicated through December 31, 2013. In the DHS, two overlapping approaches were used to capture nonfatal events: 1) a detailed health survey interval cardiovascular events was administered by the Data Coordinating Center during annual calls to study subjects; and 2) for subjects providing informed consent ($>90\%$), quarterly tracking was performed for hospital admissions using the Dallas-Fort Worth Hospital Council Data Initiative Database, which includes all hospital admission data for 70 out of 72 hospitals in the Dallas-Fort Worth area. The registry captures claims data from 96% of hospitals within a 75-mile radius in the metroplex (<https://dfwhc.org>).¹⁹ Two cardiologists, blinded to all study variables, separately adjudicated the data and hospital records. Follow-up data were available for $n=1,532$ individuals.

Statistical Analysis

Baseline demographics, clinical characteristics, CMR, and ECG were presented as mean \pm standard deviation or median (interquartile range, IQR) for continuous variables, where appropriate, and as number (percentage) for categorical variables. Statistical comparisons of these characteristics stratified by presence of the V122I *TTR* variant (non-carriers vs. carriers) were completed using the Wilcoxon rank-sum test for continuous variables,

Fisher's exact test for categorical variables, or Cochran-Armitage trend test for ordinal categorical variables. Changes in paired values of NT-proBNP, hs-cTnT, and ECG intervals were calculated by subtracting the DHS-1 value from the DHS-2 value of that parameter. A natural logarithm or an inverse normal transformation was applied to right-skewed variables prior to regression analysis. Multivariable linear regression models adjusted for age, sex, 2 principal components of ancestry, systolic blood pressure, height, and weight were performed to determine the independent association of the V122I *TTR* genotype (non-carriers vs. carriers) with CMR and ECG parameters at baseline and at follow-up. Logistic regression models adjusted for the same covariates were used to determine the association with categorical outcomes. To assess the association of *TTR* V122I genotype with changes in NT-proBNP, hs-cTnT, and ECG intervals, we modeled the DHS-2 value as the response variable, with adjustment for baseline value of each parameter, ancestry, age, gender, and systolic blood pressure. The Kaplan-Meier method was used to estimate cumulative incidence of fatal and non-fatal events stratified by V122I *TTR* carrier status, and log-rank test was used to compare the groups. Follow-up data for participants who did not experience the event of interest prior to the end of follow-up or those who died from other causes without experiencing the event of interest, were censored. Cox proportional hazards models were used to test the association between V122I *TTR* carrier status and incident outcomes. These models were adjusted for age, sex, systolic blood pressure, estimated glomerular filtration rate, and 2 principal components of ancestry. The proportional hazards assumption was checked by examining Schoenfeld residuals. In order to determine the influence of different baseline risk factors on the association between V122I *TTR* carrier status and outcomes, we repeated the analyses in subgroups stratified by sex, hypertension, and diabetes, and tested for interaction between V122I *TTR* genotype and these risk factors. All covariates in the multivariable models were selected *a priori* based on their capacity to influence the carrier status-risk relationship. In addition, four separate sensitivity analyses were performed to clarify the association between V122I *TTR* carrier status and HF: 1) additionally adjusting for a prior self-reported history of HF; 2) additionally adjusting for a prior self-reported history of cardiovascular disease; 3) excluding participants with a prior reported history of HF; and 4) excluding participants with a prior reported history of cardiovascular disease. Two-sided P-values <0.05 were considered statistically significant. P-values and 95% confidence intervals presented in this report have not been adjusted for multiplicity, and therefore inferences drawn from these statistics may not be reproducible. All statistical analyses were performed using R statistical software, version 3.6.0.

RESULTS

Among the 1,818 DHS participants of African ancestry, a total of 59 carried at least one copy of the *TTR* V122I variant (carrier frequency 3.2% [59/1,818]). Only one of the 59 individuals with the variant was homozygous for the 122I allele (allele frequency 1.65% [n/2N=60/3,636]). Similar estimates were obtained in the subset with CMR data (n=1,364, Table 1). The study consort diagram is highlighted in Figure 1.

The mean baseline age of the cohort was 45 ± 10 years. The baseline characteristics of participants (DHS-1) stratified by V122I *TTR* carrier status are shown in Table 1. There were no statistically significant differences in age, sex, prevalent hypertension,

prevalent diabetes mellitus, or smoking status. In addition, there were no differences in anthropomorphic measures (weight and body mass index), serologic markers of renal function (creatinine and estimated glomerular filtration rate), or levels of natriuretic peptides (NTproBNP) and cardiac troponin (hs-cTnT). The follow-up characteristics of participants (DHS-2) stratified by V122I *TTR* carrier status are shown in Supplement 2.

The cardiac phenotypes of participants by CMR and ECG in DHS-1 and DHS-2, stratified by V122I *TTR* carrier status, are shown in Table 2 and Supplement 3, respectively. No differences were observed in these parameters by V122I *TTR* carrier status in unadjusted analyses for both DHS phases. After multivariable adjustment, carriers of the V122I *TTR* variant had higher LV wall thickness by approximately 0.3 mm ($P=0.038$) in DHS-1 and by approximately 0.6 mm in DHS-2 ($P=0.009$). However, this was not seen either with LV mass or LV concentricity. No other differences were apparent in markers of LV cavity size, left atrial size, and LV mass. The changes in CMR parameters from DHS-1 to DHS-2, stratified by V122I *TTR* carrier status are shown in Table 3. Carriers of the V122I *TTR* variant had a small increase in LV ejection fraction ($P=0.047$) and decrease in LV end systolic volume ($P=0.035$). No other differences were apparent for changes in markers of LV cavity size, left atrial size, and LV mass from DHS-1 to DHS-2.

Similarly, there were no differences in ECG intervals, QRS voltage amplitude, or the ratios of QRS voltage amplitudes by LV mass by V122I *TTR* carrier status in unadjusted or adjusted analyses in DHS-1.

Of the study cohort, there were 872 participants with paired cardiac biomarker and ECG data. The changes in cardiac biomarkers and ECG parameters from DHS-1 to DHS-2 stratified by V122I *TTR* carrier status are shown in Table 3. Relative to non-carriers, V122I *TTR* carriers had nearly a 2-fold larger median increase in levels of NT-proBNP over time in both unadjusted and adjusted analyses ($P=0.04$ and $P=0.01$, respectively). After additional adjustment for body mass index, eGFR, and time between DHS-1 and DHS-2, the associations between V122I *TTR* carrier status and increased NT-proBNP persisted ($\beta=0.35$, $P=0.026$). In contrast, there were no differences in change in hs-cTnT or the ECG intervals, QRS voltage amplitudes, or the ratios of QRS voltage amplitudes by LV mass from DHS-1 and DHS-2 in both unadjusted and adjusted analyses ($P>0.05$ for all).

After 22,603 person-years of follow-up (through 2016, median duration of follow-up 15.3 years, IQR: 14.8-15.8 years), there were 195 all-cause deaths, and 77 cardiovascular deaths. After 18,495 person-years of follow-up for incident events (through 2013, median duration of follow-up 12.4 years, IQR: 11.9-12.8 years), there were 151 incident atherosclerotic cardiovascular disease events, 67 incident HF events, and 58 myocardial infarctions. Kaplan-Meier estimates of cumulative incidence of fatal and non-fatal events are shown in the Figure 2. Of these, there was a significantly higher incidence of HF (Log-rank $P<0.001$), higher all-cause mortality (Log-rank $P=0.026$) and cardiovascular death (Log-rank $P=0.024$) for V122I *TTR* carriers in comparison with non-carriers. In the Cox proportional hazards models, there was no association between V122I *TTR* carrier status and atherosclerotic vascular disease, myocardial infarction or stroke in both unadjusted and adjusted analyses ($P>0.05$ for all). After multivariable adjustment, V122I *TTR* carriers were at a 3.8-fold

higher risk of HF events (HR 3.82, 95% CI 1.80-8.13, $P<0.001$), 2.7 fold higher risk of cardiovascular death (HR 2.65, 95% CI 1.14-6.15, $P=0.023$), and 2-fold higher risk of all-cause mortality (HR 1.95, 95% CI 1.08-3.51, $P=0.026$) in comparison with non-carriers (Table 4). Additional adjustment for income and education did not alter the results (data not shown). In the four sensitivity analyses (Supplement 4), the independent association between V122I *TTR* carrier status and HF events remained largely consistent when additionally adjusting for prior HF (n events/N total=66/1,528) or prevalent cardiovascular disease (n events/N total=66/1,528) or excluding individuals with prior HF (n events/N total=51/1,463) or prior cardiovascular disease (n events/N total=42/1,382). In a sensitivity analysis restricted to participants who had complete longitudinal data with biomarkers, ECG, and CMR, results were qualitatively similar (Supplement 5).

At baseline, V122I *TTR* carriers who experienced HF were older and had lower eGFR, higher NT-proBNP and hs-cTnT, larger LV volumes and mass, and longer QRS intervals, with a trend towards lower indexed ECG voltages to LV mass, as compared with non-carriers (Supplement 6).

In a separate analysis, no heterogeneity was observed for the association between V122I carrier status and incident HF when stratified by sex, prevalent hypertension, or prevalent diabetes (P -interaction >0.05 for all, Supplement 7).

DISCUSSION

This analysis from the DHS - a young, population-based cohort with detailed imaging and biomarker phenotyping - has several important observations that may clarify the natural history of V122I *TTR* carriers. First, V122I *TTR* carrier status was associated with increased risk for incident HF, even though carriers only had subtle cardiac phenotypic differences versus non-carriers at baseline. This was coupled with a higher risk for all-cause mortality and cardiovascular death. Second, V122I carrier status was associated with a larger increase in NT-proBNP levels over time than was seen in non-carriers. In addition, V122I *TTR* carriers both at DHS-1 and DHS-2 had a slightly increased LV wall thickness, but not LV mass or concentricity. Carrier status was also associated with a small increase in LV ejection fraction and decrease in LV end systolic volume from DHS-1 to DHS-2, but no other differences in cardiac phenotype by CMR or ECG were observed compared with non-carriers in this cohort. These observations highlight a clear unmet need to better understand the natural history of pre-symptomatic amyloid progression in V122I *TTR* carriers.

V122I *TTR* carrier status is associated with a lifelong risk for incident HF – suggestive of downstream effects of progressive ATTRv amyloid cardiomyopathy. Observations from DHS are consistent with data from other cohorts and confirm the genotypic prevalence of the V122I *TTR* genotype (~3-4% of all African Americans) and the association between V122I carrier status and HF risk.^{8, 9, 20} In particular, data from the Arteriosclerosis Risk in Communities and Cardiovascular Health Studies (ARIC),⁸ highlighted the association of V122I *TTR* carrier status and higher long-term HF risk. Findings from the present study may add to those observations in 4 ways: 1) the V122I *TTR* variant was associated with an increased risk for not only HF but also all-cause mortality and cardiovascular death; 2) there

was a suggestion of a subtle increase in LV wall thickness at baseline, also seen at DHS-2, but otherwise no obvious baseline subclinical ATTRv phenotype; 3) over time, there was a small but significantly greater increase in natriuretic peptide level, decline in end-systolic volume and increase in LV ejection fraction; and 4) the association with incident HF was observed among participants at a younger average age, and did not appear to vary according to sex, hypertension, or diabetes.

At the same time, we noted some differences from previous studies. The estimated relative hazard for incident HF was numerically higher in DHS (>3 in DHS vs 1.5 in ARIC) and we observed a higher risk of all-cause and cardiovascular death among *TTR* V122I carriers. While the reasons for these differences are uncertain, in comparison with data from ARIC,⁸ V122I *TTR* carriers in the DHS were younger and had less prevalent diabetes and hypertension. In lower risk populations with fewer competing comorbidities, the relative risk of both incident HF and death associated with V122I *TTR* carrier status may be larger, due to lower rates of HF and death among non-carriers. Indeed, the absolute risk difference for HF between carriers and non-carriers was comparable between the two studies. It is also possible the higher point estimates for incident HF in the DHS may result from sampling variation since the confidence intervals overlap between the two cohorts, highlighting the need for additional studies with larger samples sizes to derive more precise estimates. Nevertheless, the strong pathological phenotype for carriers who developed subsequent HF in the DHS provides some evidence that midlife structural and biomarker abnormalities precede clinical HF in V122I *TTR* carriers and underscore this variant's importance over an individual's lifespan.

Data from the DHS may inform the potential screening strategies for ATTR in V122I *TTR* carriers. Similar to prior observations in ARIC,⁸ we observed that V122I carriers had a subtle increase in LV wall thickness compared with non-carriers. A potentially important difference between the two studies was that the mean age of DHS subjects at baseline was 45 years, whereas the subset of ARIC participants in which this observation was made was approximately 60-80 years old. One hypothesis for this observation is that the increased LV wall thickening represents early ATTRv amyloid deposition. Indeed, CMR studies have demonstrated associations of quantifiable amyloid deposition with increased LV wall thickness.⁵ though gadolinium was not administered in DHS-1 or DHS-2. However, additional study of this important issue is needed before accepting this hypothesis, especially since the excess wall thickening was evident only after adjustment in multivariable analysis, was of small magnitude, and neither LV mass nor LV concentricity was associated with carrier status. V122I *TTR* carriers had a slight increase in LV ejection fraction and decrease in LV end systolic volume from DHS-1 to DHS-2. These findings were unexpected, but directionally consistent with greater LV end diastolic volumes observed in similarly aged V122I *TTR* carriers in the Coronary Artery Risk Development in Young Adults (CARDIA) study.²¹ The implications of these observations are limited by small sample size, however data from the DHS and CARDIA suggest the subclinical cardiac ATTR phenotype may experience a phase of eccentric remodeling. Whether contemporary CMR studies with gadolinium,⁵ bone scintigraphy,²² or histologic assessments could detect evidence of ATTR deposition at this degree of LV thickening is also unknown. Nevertheless, the similarities between V122I *TTR* carriers and non-carriers in this age range emphasize the importance

of genetic testing and argue for the inclusion of V122I *TTR* into genome first screening programs.

In comparison with wild-type ATTR, V122I ATTRv is more aggressive with shorter time from symptom onset until clinical presentation, increased natriuretic peptides over time, worse functional capacity, and increased risk of mortality.¹ Therefore, efforts to investigate optimal timing for implementing novel ATTR treatments are critical to alter the natural history of the disease.^{2, 3} In parallel with individuals with manifest V122I ATTRv,¹ V122I *TTR* carriers in the DHS were observed to have a modest increase in natriuretic peptides over time (from DHS-1 to DHS-2). Because natriuretic peptides are associated with ATTRv disease progression and prognosis,^{23, 24} this observation may support the potential utility of performing serial measurements of NT-proBNP levels in V122I *TTR* carriers. In contrast, there were no such trends with hs-cTnT or ECG parameters over time that distinguished V122I *TTR* carriers from non-carriers. Two potential explanations for this include that ECG abnormalities may inconsistently distinguish those with clinical ATTRv from individuals without ATTR and that elevations in cardiac troponins are later manifestations of the disease.^{25, 26}

This study had several limitations. First, the number of participants with V122I *TTR* in our study was small (n=59) and, although the groups overlapped, the individuals with CMR imaging were not an exact subset of those with follow-up data. We cannot exclude the potential for bias related to incomplete MRI and biomarker follow up measures. Apart from body mass index and weight, however, there were no differences in characteristics at DHS-1 for those who had a CMR assessment and those who did not (Supplement 1). Furthermore, the duration of time between MRI assessments was shorter (median duration of follow-up 7.0 years, IQR 6.5-7.5 years) than the duration of follow-up from DHS-1 for non-fatal events (median duration of follow-up 12.4 years, IQR 11.9-12.8 years) and fatal events (median duration of follow-up 15.3 years, IQR 14.8-15.8 years) which may explain why there were more subtle differences in cardiac and biomarkers phenotypes when compared with the high relative risk of long-term outcomes. Second, there were no amyloid-specific diagnostic modalities, such as tissue biopsy, contrast enhanced CMR with parametric mapping, or bone scintigraphy employed in the DHS protocols – a limitation shared with ARIC and CARDIA.^{8, 21} As such, we can only hypothesize that the observed association between the V122I *TTR* genotype with HF and fatal outcomes is mediated by ATTRv infiltration. Furthermore, musculoskeletal syndromes which can occur years prior to ATTRv disease onset, such as carpal tunnel syndrome, lumbar canal stenosis, and biceps tendon rupture, were not recorded in the DHS. Yet, our findings are directionally consistent with prior observations from population-based cohorts linking the V122I *TTR* to cardiac phenotype differences and a higher risk of incident HF in comparison to non-carriers.^{8, 9} Furthermore, the consistency in the sensitivity analyses, as well as the lack of association between V122I *TTR* carrier status with falsification endpoints (atherosclerotic cardiovascular disease, myocardial infarction, and stroke), support the primary conclusions of our study. Third, we cannot exclude the influence of selection bias for participants with and without follow up testing in DHS-2. While the present analysis leverages a specific genetic variant in persons with African ancestry, these data inform a broader understanding of the natural history of carriers of other far less common *TTR* variants present in other ethnic groups.

CONCLUSION

In individuals with African ancestry in the DHS, the V122I *TTR* carrier status was associated with a larger increase in NT-proBNP over time and a significantly increased risk for incident HF, all-cause mortality, and cardiovascular death. V122I *TTR* was also associated with increased wall thickness in adjusted models. In total, these findings inform the natural history of V122I ATTRv and raise the question of the need of ATTRv screening strategies in V122I *TTR* carriers at younger ages than conventionally considered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DISCLOSURES

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ABBREVIATIONS

ATTR	transthyretin amyloidosis
ATTRv	variant transthyretin amyloidosis
CMR	cardiac magnetic resonance
TTR	transthyretin
HF	heart failure
DHS	Dallas Heart Study
LV	left ventricle
hs-cTnT	high sensitivity cardiac troponin T
NT-proBNP	amino terminal pro-B-type natriuretic peptide
eGFR	estimated glomerular filtration rate

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3 BRIEF BULLET POINTS

- Carriers of the V122I *TTR* mutation may have subtle differences in cardiac structure, greater changes in natriuretic peptides over time, and are at a higher risk of long-term events including heart failure and death.
- Although many of the LV imaging parameters in this study were subtle, contemporary CMR studies with gadolinium, bone scintigraphy, or histologic assessments are needed to better understand whether these observations are evidence of ATTR deposition in V122I *TTR* carriers.
- Carriers of the V122I *TTR* should be made aware that screening for ATTRv may be considered.

PROPOSED TWEET

Data from individuals with African ancestry in Dallas Heart Study suggest that, in comparison with non-carriers, carriers of the V122I *TTR* mutation in mid-life may have subtle differences in cardiac structure, greater changes in NT-proBNP over time, and are at a higher risk of long-term events including heart failure and death.

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HIGHLIGHTS

- The prevalence of the V122I *TTR* variant in individuals with African ancestry is 3.2%
- In mid-life, carriers of the V122I *TTR* have subtle cardiac structural differences in comparison with non-carriers
- Over time, carriers of V122I have a greater increase in NT-proBNP in comparison with non-carriers.
- In comparison with non-carriers, carriers of the V122I *TTR* are at a higher long-term risk of heart failure and death

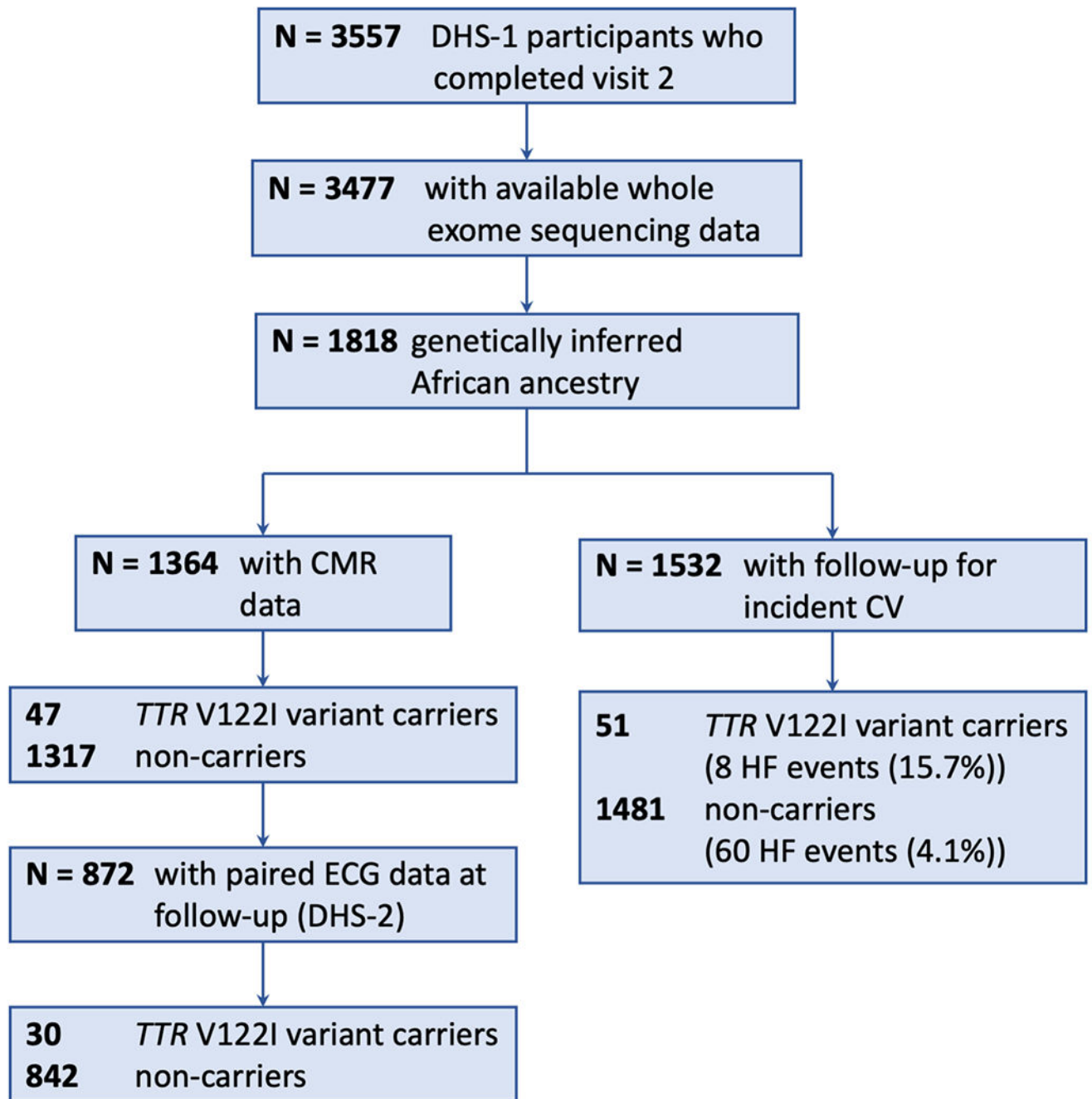


Figure 1.

Consort Diagram. Of the 1,818 DHS participants of African ancestry, n=1,532 had follow-up CV data, n=1,364 had CMR imaging data, and n=1,205 had both. Abbreviations are DHS-1, Dallas Heart Study Phase 1; WES, whole exome sequencing; CMR, cardiac magnetic resonance imaging; ECG, electrocardiogram; and DHS-2, Dallas Heart Study Phase 2.

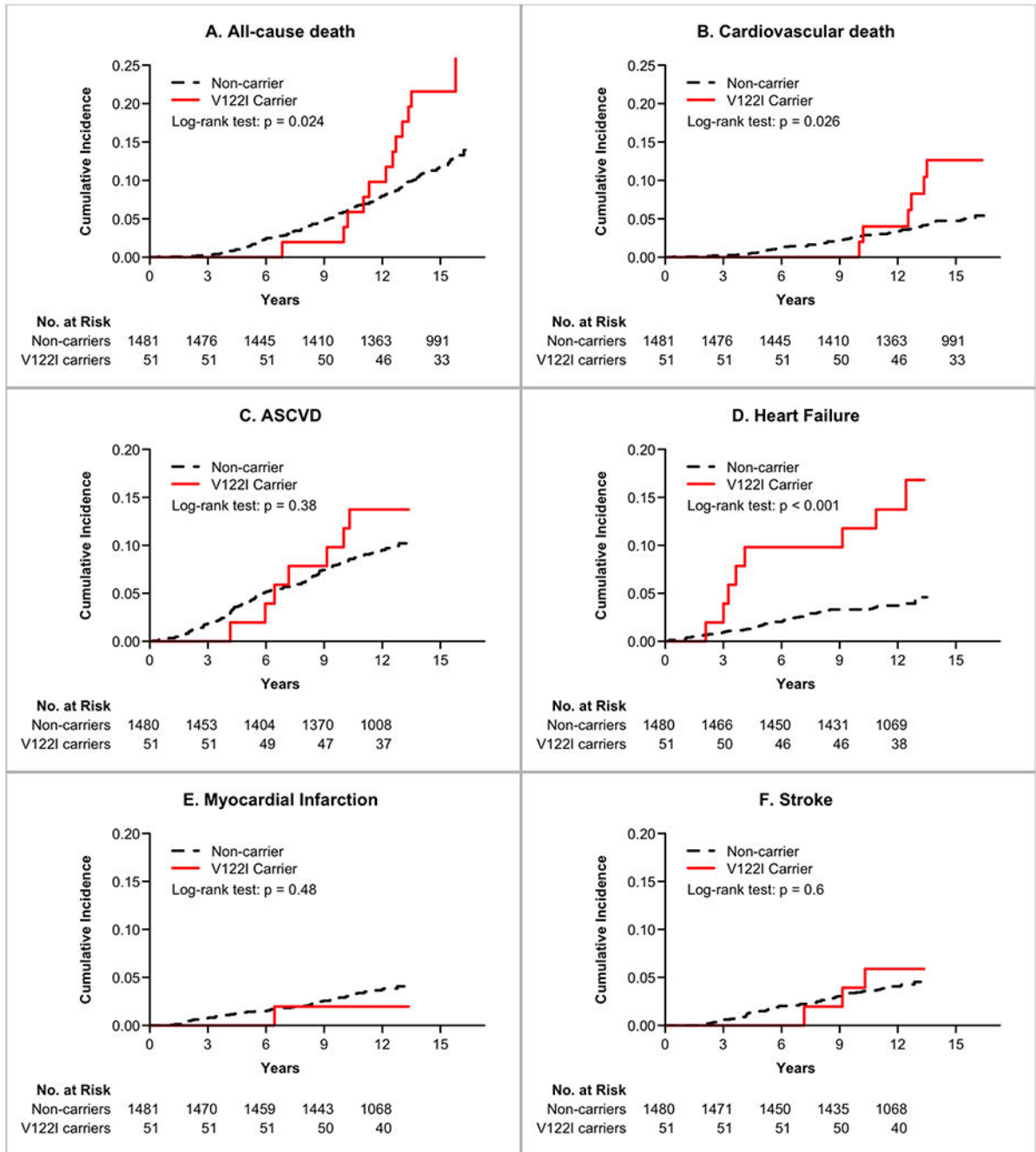


Figure 2. Kaplan-Meier Estimates of Cumulative Incidence of Fatal and Non-fatal Events Stratified by V122I *TTR* Carrier Status. Statistical comparisons are generated by the Log-rank test.

Table 1.Baseline Characteristics of African American DHS-1 Participants Stratified by *TTR* V122I Carrier Status

Characteristic [†]	Participants with baseline CMR data			Participants with event follow-up data		
	<i>TTR</i> V122I Non-carriers	<i>TTR</i> V122I Carriers	<i>P</i> -value	<i>TTR</i> V122I Non-carriers	<i>TTR</i> V122I Carriers	<i>P</i> -value
	(N=1,317)	(N=47)*		(N=1,481)	(N=51)**	
Age [years]	45.1 ± 10	44 ± 9.2	0.43	44.8 ± 10.2	45.2 ± 9.4	0.89
Female	752 (57.1)	28 (59.57)	0.77	898 (60.63)	33 (64.71)	0.66
Hypertension	539 (40.9)	18 (38.3)	0.76	605 (40.9)	21 (41.2)	1
Diabetes Mellitus	179 (13.6)	6 (12.8)	1	203 (13.72)	7 (13.73)	1
Current Smoking	419 (31.9)	17 (36.2)	0.53	447 (30.2)	18 (35.3)	0.44
Weight [kg]	88.8 ± 22.5	86.4 ± 23.6	0.32	90.8 ± 23.7	87.7 ± 23.8	0.30
Income			0.45			0.57
< 16,000	323 (30.3)	10 (25.6)		353 (29.3)	10 (24.4)	
16,000 - 29,999	286 (26.8)	8 (20.5)		317 (26.3)	9 (22)	
30,000 - 49,999	268 (25.1)	15 (38.5)		306 (25.4)	16 (39)	
>= 50,000	189 (17.7)	6 (15.4)		229 (19)	6 (14.6)	
Education			0.71			0.80
< High School	220 (16.7)	5 (10.6)		241 (16.3)	6 (11.8)	
High School	509 (38.6)	22 (46.8)		589 (39.8)	23 (45.1)	
Some college	406 (30.8)	13 (27.7)		442 (29.8)	15 (29.4)	
College grad or higher	182 (13.8)	7 (14.9)		209 (14.1)	7 (13.7)	
Body mass index [kg/m ²]	31.4 ± 7.9	30.8 ± 7.6	0.52	32 ± 8.2	31.5 ± 7.8	0.62
Systolic blood pressure [mm Hg]	129.9 ± 20.1	129.5 ± 26.2	0.25	129.5 ± 19.7	129.9 ± 26.2	0.33
Diastolic blood pressure [mm Hg]	81 ± 10.7	79.8 ± 13.1	0.15	80.9 ± 10.4	79.9 ± 13	0.16
Creatinine [mg/dL]	0.9 (0.8 - 1.0)	0.9 (0.8 - 1.1)	0.24	0.9 (0.8 - 1)	0.9 (0.8 - 1.05)	0.50
eGFR [‡] [mL/min]	102 (88 - 116)	100 (85 - 110)	0.23	102 (88 - 116)	101 (85 - 111)	0.23
NT-proBNP [pg/mL]	26.1 (10.2 - 58.2)	23.6 (8.4 - 54.1)	0.79	24.5 (10.1 - 53.9)	27.8 (12.4 - 59.1)	0.57
Log-NT-proBNP	3.22 ± 1.42	3.2 ± 1.41	0.79	3.15 ± 1.37	3.31 ± 1.38	0.57
hs-cTnT [ng/L]	1.5 (1.5 - 4.2)	1.5 (1.5 - 4.0)	0.56	1.5 (1.5 - 3.94)	1.5 (1.5 - 2.3)	0.51

* 46 individuals are heterozygous and 1 individual is homozygous for the *TTR* V122I allele.

** 50 individuals are heterozygous and 1 individual is homozygous for the *TTR* V122I allele.

[†] Presented as median [interquartile range] or as mean ± standard deviation for continuous variables where appropriate and N (%) for categorical variables. Groups were compared using Wilcoxon rank-sum test (continuous variables), Fisher exact test (categorical variables), or Cochran-Armitage trend test (ordinal categorical variables: income, education).

[‡] eGFR is estimated by the Modification of Diet in Renal Disease equation

Table 2.Cardiac Phenotype of African American DHS-1 Participants Stratified by V122I *TTR* Carrier Status

Characteristic [†]	Non-carriers (N=1,317)		Carriers (N=47)		Unadjusted	Adjusted
	No. of people with data	Value	No. of people with data	Value	P-value	P-value
Cardiac magnetic resonance imaging						
LV ejection fraction [%]	1317	0.67 ± 0.07	47	0.66 ± 0.07	0.34	0.28
LV end diastolic volume [mL]	1317	124.6 ± 34.6	47	123.8 ± 30.1	0.99	0.67
LV end diastolic volume by body surface area [ml/m ²]	1313	62.19 ± 15.33	47	63.43 ± 16.19	0.85	0.76
LV end systolic volume [mL]	1317	42 ± 23.6	47	42.4 ± 15.6	0.41	0.24
LV end systolic volume by body surface area [ml/m ²]	1313	20.93 ± 11.23	47	21.92 ± 9.09	0.55	0.35
Stroke volume [mL]	1317	82.7 ± 17.6	47	81.4 ± 18.4	0.36	0.78
Stroke volume by body surface area [ml/mm ²]	1313	41.25 ± 7.33	47	41.51 ± 8.8	0.74	0.83
Myocardial contraction fraction	1317	0.64 ± 0.16	47	0.62 ± 0.15	0.35	0.19
Left atrial volume [mL]	353	71.4 ± 19	13	75.3 ± 25.2	0.72	0.36
Left atrial volume by body surface area [ml/m ²]	353	36.19 ± 7.88	13	38.37 ± 8.99	0.27	0.33
LV mass [g]	1317	142.3 ± 44.1	47	144.6 ± 45.3	0.80	0.18
LV mass by body surface area [g/m ²]	1313	70.66 ± 18.93	47	73.42 ± 20.15	0.40	0.17
LV wall thickness [mm]	1312	8.21 ± 1.68	44	8.52 ± 1.82	0.51	0.038
LV Concentricity [g/mL]	1317	5.17 ± 0.5	47	5.2 ± 0.51	0.80	0.18
LV hypertrophy by body surface area	1316	230 (17.48)	47	12 (25.53)	0.17	0.14
Electrocardiography						
PR interval [ms]	1304	161 ± 23.8	47	159.8 ± 25.3	0.77	0.81
QRS interval [ms]	1314	85.2 ± 11.7	47	84.8 ± 14.7	0.25	0.49
QT interval [ms]	1312	391.4 ± 32.1	47	392.9 ± 31.7	0.49	0.74
LV hypertrophy	1314	217 (16.51)	47	12 (25.53)	0.11	0.13
Limb lead voltage [mm]	1314	28.1 ± 7.9	47	29.5 ± 7.7	0.066	0.18
Sokolow voltage [mm]	1314	24.5 ± 8.3	47	26.3 ± 7.8	0.094	0.13
Total voltage [mm]	1314	1022 ± 255	47	1075 ± 260	0.13	0.14
Limb lead voltage / LV mass [mm * m ² /g]	1310	0.41 ± 0.13	47	0.42 ± 0.12	0.39	0.81
Sokolow voltage / LV mass [mm * m ² /g]	1310	0.36 ± 0.12	47	0.37 ± 0.12	0.28	0.46
Total voltage / LV mass [mm * m ² /g]	1310	14.9 ± 3.7	47	15.2 ± 3.6	0.35	0.84

* 46 individuals are heterozygous and 1 individual is homozygous for the V122I allele.

[†] Presented as mean ± standard deviation for continuous variables and N (%) for categorical variables. Unadjusted p-values were calculated using Wilcoxon rank-sum test (continuous variables) or Fisher exact test (categorical variables). Adjusted p-values were calculated using linear and logistic regression models (for continuous and categorical variables, respectively), including ancestry, age, sex, systolic BP, height and weight as covariates.

Table 3.

Change in Cardiac Biomarkers, Electrocardiogram, and Cardiac Magnetic Resonance Imaging Parameters from DHS-1 to DHS-2

Characteristic [†]	Non-carriers (N=842)		Carriers (N=30)		Unadjusted P-value	Adjusted P-value
	No. of people with data	Value	No. of people with data	Value		
Cardiac Biomarkers						
NT-proBNP [pg/mL]	834	15.9 (0.0 - 43)	30	28.5 (11.4 - 104.1)	0.034	0.018
hs-cTnT [ng/L]	817	3.24 (0 - 6.31)	29	1.33 (0 - 5.81)	0.19	0.32
Cardiac magnetic resonance imaging						
LV ejection fraction [%]	586	0.01 (-0.03 - 0.05)	22	0.03 (0 - 0.05)	0.058	0.047
LV end diastolic volume [mL]	586	-1.65 (-11.53 - 7.74)	22	-5.57 (-10.95 - 5.37)	0.70	0.60
LV end systolic volume [mL]	586	-1.43 (-7.03 - 4.16)	22	-4.86 (-9.24 - -1.21)	0.044	0.035
Stroke volume [mL]	586	0.13 (-8.4 - 6.43)	22	3.82 (-4.2 - 10.54)	0.18	0.23
Left atrial volume [mL]	349	2.4 (-10.29 - 12.28)	13	6.04 (-16.2 - 17.8)	0.89	0.76
LV mass [g]	586	-2.65 (-12.3 - 9.2)	22	-0.97 (-4.88 - 6.71)	0.57	0.55
LV wall thickness [mm]	581	0 (-0.55 - 0.59)	22	0.11 (-0.26 - 0.39)	0.54	0.21
LV Concentricity [g/mL]	586	0.43 (-0.13 - 1.04)	22	0.58 (0.25 - 1.01)	0.34	0.29
Electrocardiography						
PR interval [ms]	819	4 (-6 - 12)	30	3 (-5.5 - 11)	0.91	0.90
QRS interval [ms]	842	2 (-2 - 6)	30	2 (-2 - 6)	0.66	0.66
QT interval [ms]	841	4 (-16 - 26)	30	6 (-7 - 27.5)	0.40	0.25
Limb lead voltage [mm]	841	3.6 (-0.1 - 7.9)	30	2.6 (0 - 8.6)	0.73	0.32
Sokolow voltage [mm]	841	-2.2 (-5.4 - 0.9)	30	-2.3 (-5 - 0.9)	0.74	0.91
Limb lead voltage / LV mass [mm/g]	585	0.06 (0 - 0.15)	22	0.08 (0.01 - 0.16)	0.42	0.34
Sokolow voltage / LV mass [mm/g]	585	-0.02 (-0.07 - 0.03)	22	-0.03 (-0.07 - 0.02)	0.70	0.89

* 1 individual is homozygous for die V122I allele.

[†] Presented as median [interquartile range] or as mean \pm standard deviation for continuous variables where appropriate and N (%) for categorical variables. Unadjusted p-values were calculated using Wilcoxon rank-sum test. Adjusted p-values were calculated using linear regression models, using the value at DHS-2 as the response variable and adjusted for baseline value, ancestry, age, sex, and systolic blood pressure.

Table 4.Association of the *TTR* V122I Carrier Status with Mortality and Cardiovascular outcomes

Outcomes	N event/N Total, non-carriers	N event/N Total, V122I carriers	Hazard Ratio (95% CI)	Unadjusted P-Value	N event/N Total, non-carriers	N event/N Total, V122I carriers	Adjusted Hazard Ratio (95% CI)	Adjusted P-Value
All-cause mortality	183/1481	12/51	1.93 (1.08 - 3.47)	0.027	182/1478	12/51	1.95 (1.08 - 3.51)	0.026
Cardiovascular death	71/1481	6/51	2.49 (1.08 - 5.74)	0.032	70/1478	6/51	2.65 (1.14 - 6.15)	0.023
Atherosclerotic cardiovascular disease	144/1480	7/51	1.4 (0.66 - 2.99)	0.38	144/1477	7/51	1.1 (0.51 - 2.4)	0.8
Heart failure	59/1480	8/51	4.09 (1.95 - 8.56)	<0.001	58/1477	8/51	3.82 (1.8 - 8.13)	<0.001
Myocardial infarction	57/1481	1/51	0.5 (0.07 - 3.62)	0.49	57/1478	1/51	0.43 (0.06 - 3.15)	0.41
Stroke	63/1480	3/51	1.36 (0.43 - 4.35)	0.6	63/1477	3/51	1.11 (0.34 - 3.59)	0.86

* Hazard ratio for the comparison of carriers of the V122I variant versus non-carriers.

† Multivariable models are adjusted for baseline age, sex, BMI, systolic blood pressure, eGFR, and 2 principal components of ancestry.

‡ All-cause mortality and cardiovascular death were followed till December 31, 2016 and all other outcomes were followed until December 31, 2013.