

# Association of Genome-Wide Variation With the Risk of Incident Heart Failure in Adults of European and African Ancestry

## A Prospective Meta-Analysis From the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium

Nicholas L. Smith, PhD; Janine F. Felix, MD, PhD; Alanna C. Morrison, PhD; Serkalem Demissie, PhD; Nicole L. Glazer, PhD; Laura R. Loehr, MD, PhD; L. Adrienne Cupples, PhD; Abbas Dehghan, MD, PhD; Thomas Lumley, PhD; Wayne D. Rosamond, PhD; Wolfgang Lieb, MD; Fernando Rivadeneira, MD, PhD; Joshua C. Bis, PhD; Aaron R. Folsom, MD; Emelia Benjamin, MD; Yurii S. Aulchenko, PhD; Talin Haritunians, PhD; David Couper, PhD; Joanne Murabito, MD; Ying A. Wang, PhD; Bruno H. Stricker, MMed, PhD; John S. Gottdiener, MD; Patricia P. Chang, MD, MHS; Thomas J. Wang, MD; Kenneth M. Rice, PhD; Albert Hofman, MD, PhD; Susan R. Heckbert, MD, PhD; Ervin R. Fox, MD; Christopher J. O'Donnell, MD; Andre G. Uitterlinden, PhD; Jerome I. Rotter, MD; James T. Willerson, MD; Daniel Levy, MD; Cornelia M. van Duijn, PhD; Bruce M. Psaty, MD, PhD; Jacqueline C.M. Witteman, PhD; Eric Boerwinkle, PhD; Ramachandran S. Vasan, MD

**Background**—Although genetic factors contribute to the onset of heart failure (HF), no large-scale genome-wide investigation of HF risk has been published to date. We have investigated the association of 2 478 304 single-nucleotide polymorphisms with incident HF by meta-analyzing data from 4 community-based prospective cohorts: the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Rotterdam Study.

**Methods and Results**—Eligible participants for these analyses were of European or African ancestry and free of clinical HF at baseline. Each study independently conducted genome-wide scans and imputed data to the  $\approx 2.5$  million single-nucleotide polymorphisms in HapMap. Within each study, Cox proportional hazards regression models provided age- and sex-adjusted estimates of the association between each variant and time to incident HF. Fixed-effect meta-analyses combined results for each single-nucleotide polymorphism from the 4 cohorts to produce an overall association estimate and *P* value. A genome-wide significance *P* value threshold was set a priori at  $5.0 \times 10^{-7}$ . During a mean follow-up of 11.5 years, 2526 incident HF events (12%) occurred in 20 926 European-ancestry participants. The meta-analysis identified a genome-wide significant locus at chromosomal position 15q22 ( $1.4 \times 10^{-8}$ ), which was 58.8 kb from *USP3*. Among 2895 African-ancestry participants, 466 incident HF events (16%) occurred during a mean follow-up of 13.7 years. One genome-wide significant locus was identified at 12q14 ( $6.7 \times 10^{-8}$ ), which was 6.3 kb from *LRIG3*.

**Conclusions**—We identified 2 loci that were associated with incident HF and exceeded genome-wide significance. The findings merit replication in other community-based settings of incident HF. (*Circ Cardiovasc Genet.* 2010;3:256-266.)

**Key Words:** epidemiology ■ genetics ■ heart failure ■ genome-wide variation ■ incidence

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From the Cardiovascular Health Study: Departments of Epidemiology (N.L.S., S.R.H., B.M.P.), Medicine (N.L.G., J.C.B., B.M.P.), Biostatistics (T.L., K.M.R.), and Health Services (B.M.P.) University of Washington, Seattle, Wash.; Seattle Epidemiologic Research and Information Center of the Department of Veterans Affairs Office of Research and Development (N.L.S.), Seattle, Wash.; Group Health Research Institute (S.R.H., B.M.P., N.L.S.) Group Health, Seattle, Wash.; and Department of Cardiology (J.S.G.), University of Maryland, Baltimore, Md; Medical Genetics Institute (T.H., J.I.R.), Cedars-Sinai Medical Center, Los Angeles, Calif; Artherosclerosis Risk in Communities Study: University of Texas at Houston Health Science Center (A.C.M., E.B., J.T.W.), Houston, Tex.; Departments of Medicine (L.R.L., P.P.C.), Biostatistics (D.C.), and Epidemiology (W.D.R., L.R.L.), University of North Carolina at Chapel Hill; National Institute of Environmental Health Sciences (L.R.L.), Research Triangle Park, N.C., University of Minnesota School of Public Health, Division of Epidemiology and Community Health, School of Public Health (A.R.F.), Minneapolis, Minn.; Department of Medicine (E.R.F.), University of Mississippi Medical Center, Jackson, Miss; Texas Heart Institute (J.T.W.), Houston, Tex; Framingham Heart Study, Department of Biostatistics (S.D., L.A.C., Y.A.W.), Boston University School of Public Health, Boston, Mass.; Sections of Preventive Medicine and Cardiology (R.S.V.), Boston University School of Medicine, Boston, Mass.; National Heart Lung and Blood Institute (C.J.O., D.L.), Bethesda, MD; Massachusetts General Hospital (T.J.W., C.J.O.), Harvard Medical School, Boston, Mass.; and Framingham Heart Study (L.A.C., W.L., E.B., J.M., T.J.W., C.J.O., D.L., R.S.V.), Framingham, Mass; Rotterdam Study: Departments of Epidemiology (J.F.F., A.D., Y.S.A., B.H.S., A.H., C.M.v.D., J.C.M.W.) and Internal Medicine (F.R., A.G.U.), Erasmus MC, Rotterdam, the Netherlands; and Member of the Netherlands Consortium on Healthy Aging, Leiden, The Netherlands (J.F.F., J.C.M.W.).

Heart failure (HF) is a serious cardiovascular condition that affects 1 in 5 individuals during their lifetime.<sup>1</sup> Failure arises primarily from the burden of systemic hypertension, coronary ischemia, or cardiac valvular disease.<sup>2,3</sup> Regardless of the clinical mechanism responsible for reduced cardiac output in HF, long-term strain triggers maladaptive cellular function and growth, possibly resulting in irreversible myocardial injury.<sup>4,5</sup>

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Genetic factors contribute to HF onset, and an estimated  $\approx 18\%$  of the risk of HF may be attributable to parental HF.<sup>6</sup> To date, approaches to identify genetic variants associated with HF risk have relied on candidate genes, primarily those of the renin-angiotensin-aldosterone system, adrenergic receptors, sarcomeres, and cytoskeletal proteins.<sup>7,8</sup> No large-scale genome-wide investigation of HF risk has been published.<sup>9</sup> This report presents findings from an investigation of  $\approx 2.5$  million single-nucleotide polymorphisms (SNPs) and their associations with incident HF among 23 821 adults of European or African ancestry.

## Methods

### Setting

The setting for this meta-analysis is the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium and includes data from 4 prospective, population-based cohorts of adults in the USA and the Netherlands: the Atherosclerosis Risk in Communities (ARIC) Study, the Cardiovascular Health Study (CHS), the Framingham Heart Study (FHS), and the Rotterdam Study (RS).<sup>10</sup> The designs of these studies have been described elsewhere.<sup>11–16</sup> In brief, ARIC recruited 15 792 participants 45 to 64 years of age from 1987 to 1989 from 4 US communities. The CHS recruited participants 65 years of age and older from 4 US communities in 2 waves: 5201 participants in 1989–1990 and an additional 687 African Americans in 1992–1993. The FHS recruited 2 generations of participants 28 to 72 years of age in 2 time periods: 5209 Original Cohort participants were recruited in 1948 from Framingham, Massachusetts, and 5124 Offspring Cohort participants were recruited in 1971–1975. The RS recruited 7983 participants 55 years of age and older from 1990 to 1993 from Rotterdam, the Netherlands. The ARIC and CHS studies included African American participants, 27% and 15%, respectively, whereas FHS and RS included almost exclusively participants of European ancestry. Each study independently conducted genome-wide scans of study participants, and within-study analyses were conducted according to a prespecified plan. The results were combined for this prospective meta-analysis.

### Subjects

Eligible participants for these analyses were of European or African ancestry and free of clinical HF. Details about cohort-specific methods for ascertaining prevalent HF at baseline have been published elsewhere.<sup>6,17–20</sup> Race was self-reported. Each study received

an institutional review board approval, and all participants provided written informed consent for the use of their DNA for research.

## Measures

### Baseline

For this analysis, baseline measures of clinical and demographic characteristics were obtained at the time of cohort entry for ARIC, CHS, and RS, and at the time of DNA collection for FHS. Measures were taken using standardized methods as specified by each study and included the following: in-person measures of height, weight, systolic and diastolic blood pressure, and total cholesterol; self-reported history and treatment of hypertension and diabetes; and self-reported history of prevalent coronary artery disease (including a history of myocardial infarction [MI], angina, or coronary revascularization) and cerebrovascular disease (stroke or transient ischemic attack). For CHS, FHS, and RS, prevalent cardiovascular disease was validated by medical record review.

### Follow-Up and HF Diagnosis

Potential incident HF events were identified during follow-up by annual or semiannual self-report, from administrative data, or from periodic in-study examinations. In brief, ARIC relied on *International Classification of Diseases* (ICD)-9 codes collected from hospital discharge summaries and death certificates.<sup>20,21</sup> For CHS, FHS, and RS, HF events identified by self-report or administrative data were validated by physician's review of medical records. CHS and Framingham applied their published criteria,<sup>22,23</sup> and RS applied the European Society of Cardiology criteria.<sup>18,24</sup> Details can be found in supplemental Table I and in previously published reports.

### Genotyping and Imputation

Genotyping used DNA collected from phlebotomy. Genome-wide assays of SNPs were conducted independently in each cohort using various technologies: Affymetrix 6.0 for ARIC, Illumina 370CNV for CHS, Affymetrix 500K and 50K for FHS, and Illumina 550K v3 for RS. Genotype quality control and data cleaning were conducted independently by each study and are summarized in supplemental Table II. Methods were very similar across studies and reflect those used in other published reports on genome-wide associations.<sup>25,26</sup>

We investigated genetic variation in the 22 autosomal chromosomes. For European-ancestry participants, each study independently imputed their genotype data to the  $\approx 2.5$  million SNPs identified in HapMap Centre d'Etude du Polymorphisme Humain from Utah (CEU) samples.<sup>27,28</sup> For African-ancestry participants, CHS imputed to combined CEU and Yoruban (YRI) samples and ARIC did not impute. Supplemental Table II provides details about the imputation methods for each cohort and the quality control criteria applied to the imputed genotypes. Imputation results were summarized as an "allele dosage" defined as the expected number of copies of the minor allele at that SNP (a continuous value between 0 and 2) for each genotype. Each cohort calculated a ratio of observed versus expected variance of the dosage statistic for each SNP. This value, which generally ranges from 0 to 1 (poor to excellent), is a measure of imputation quality. High-signal SNPs that had low observed versus expected variance values in CHS ( $<0.8$ ) were directly measured to improve the quality of the signal. No other cohort conducted additional genotyping.

The online-only Data Supplement is available at <http://circgenetics.ahajournals.org/cgi/content/full/CIRCGENETICS.109.895763/DC1>.

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Drs Smith, Felix, Morrison, Demissie, Psaty, Witteman, Boerwinkle, and Vasan contributed equally to this work.

Correspondence to Nicholas L. Smith, PhD, Cardiovascular Health Research Unit, 1730 Minor Ave, Suite 1360, Seattle WA 98105. E-mail [nlsmith@u.washington.edu](mailto:nlsmith@u.washington.edu)

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**Table 1. Baseline Characteristics of Participants Included in Incident HF Analyses Stratified by Cohort and Race**

Characteristics	ARIC		CHS		FHS	RS
	EA	AA	EA	AA		
Participants, n	7644	2321	3295	574	4268	5719
Mean age, y	54.2	53.3	72.3	72.6	64.6	69.1
Age range, y	45 to 64	45 to 64	65 to 98	65 to 92	30 to 100	55 to 99
Female, %	52.6	61.7	60.9	63.9	55.3	59.1
BMI, kg/m <sup>2</sup>	26.9	29.5	26.3	28.5	27.7	26.3
Systolic blood pressure, mm Hg	118.3	128.7	135.2	142.5	130.7	139.3
Diastolic blood pressure, mm Hg	71.7	80.0	70.4	76.9	74.1	73.8
Total cholesterol, mg/dL	214.8	215.9	212.9	209.4	202.4	255.2
Treated hypertension, %	25.3	54.4	30.2	51.7	35.6	23.2
Treated diabetes, %	8.0*	19.0*	5.4	11.2	6.5	3.8
Prevalent CAD, %	8.8	6.9	0	0	12.1	13.8
Myocardial infarction, %	3.6	3.1	0	0	4.9	10.3
Angina, %	4.4	3.4	0	0	8.6	3.2
Coronary revascularization, %	3.1	1.8	0	0	4.9	2.5
Prevalent CBD, %	2.1	2.9	0	0	5.0	4.1
Current smoker, %	24.9	29.9	11.3	16.6	14.2	22.8
Mean follow-up, y	15.3	14.6	11.8	10.1	5.5	10.8
Incident HF, n (%)	691 (9.0)	331 (14.3)	838 (25.4)	135 (23.5)	249 (5.8)	748 (13.1)

Classification of comorbidities varies by cohort and may not be directly comparable. EA indicates European ancestry; AA, African ancestry; BMI, body mass index; CAD, coronary artery disease (MI, angina, revascularization); and CBD, cerebrovascular disease (stroke, transient ischemic attack, or carotid endarterectomy).

\*Includes diabetics without treatment.

## Statistical Analyses

Investigators from all cohorts jointly developed the prespecified analytic plan. Within each study, Cox proportional hazards regression models were used to test the association between each SNP and time to incident HF, while adjusting for sex and baseline age. Time-to-event models are standard for longitudinal study designs. CHS adjusted for study site and FHS adjusted for generation, and for ancestry using principal components.<sup>29</sup> Time of entry to the analysis was the date of cohort entry (ARIC, CHS, RS) or DNA collection (FHS), and failure time was the time of HF diagnosis. Participants without incident HF were censored at the time of death, last date of contact, or at the end of follow-up, whichever came first. For each SNP, additive genetic models were used to estimate the regression coefficient for the hazard ratio (HR) for allele dosage and its respective standard error. In ARIC and CHS, analyses were conducted separately in 2 ancestry groups, European and African. For each analysis, a genomic control coefficient ( $\lambda$ ) was calculated, which estimated the extent of underlying population structure.

Meta-analyses were performed using MetABEL software (<http://mga.bionet.nsc.ru/~yurii/ABEL/>).<sup>30</sup> For the populations of European ancestry, fixed-effect meta-analyses combined regression  $\beta$  coefficients and  $\lambda$ -adjusted standard errors across the 4 cohorts for each SNP to produce an overall  $\beta$  coefficient, standard error, and  $P$  value. We found that asymptotic assumptions of survival analysis models were not accurate when the number of cases carrying the variant allele was small. Hence, we excluded SNPs for which the postmeta-analysis population-size-weighted minor allele frequency (MAF) was  $<0.015$ .

We created a quantile-quantile (Q-Q) plot of the distribution of the observed and expected  $P$  values associated with the successful SNPs, along with a plot of all the  $P$  values according to location within each of the 22 chromosomes. The a priori threshold of genome-wide significance was set at  $5.0 \times 10^{-7}$ , also used by the Wellcome Trust Case Control Consortium.<sup>31</sup> For 2.5 million tests, this threshold

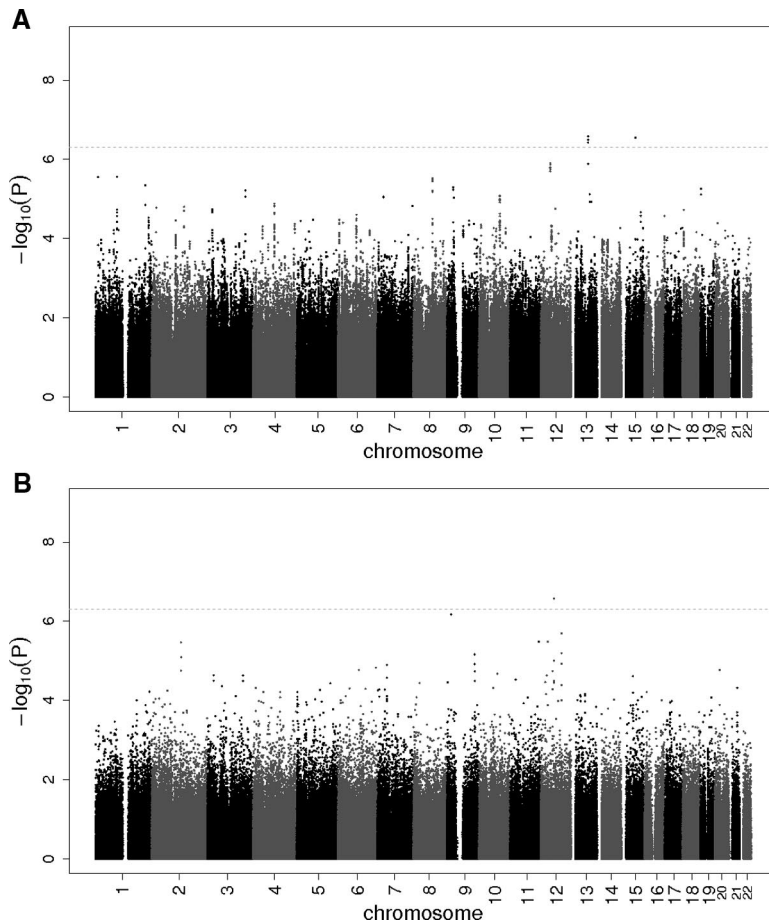
limits the expected number of genome-wide false-positives to approximately 1. We also reported high-signal SNPs that were associated with  $P$  values  $<1.0 \times 10^{-5}$ , the point where observations deviate from those expected on the Q-Q plot. When more than 1 SNP clustered at a locus, we picked the SNP with the smallest  $P$  value as the locus marker. Forest plots that included cohort-specific findings were created for genome-wide significant SNPs.

In sensitivity analyses, we subjected our top associations to time-to-event analyses that excluded participants with a baseline history of clinical MI and censored those with an incident MI during follow-up. This approach allowed us to estimate the association of selected SNPs and HF onset among participants whose HF was less likely to be ischemic in origin.

For participants of African ancestry, a similar analytic approach was undertaken in ARIC and CHS. The ARIC cohort did not have imputed data on those of African ancestry, so testing was restricted to successfully genotyped SNPs on their Affymetrix 6.0 chip that had an average weighted MAF of at least 0.075 in meta-analyses. Genome-wide significance was set at  $5.0 \times 10^{-7}$ . High-signal SNPs from the meta-analysis in the European- and African-ancestry populations were compared.

## Results

A total of 20 926 eligible participants of European ancestry and 2895 participants of African ancestry were free of HF at cohort entry and had genome-wide data that met cohort-specific quality control measures. Characteristics of the 23 821 cohort participants included in these analyses are provided in Table 1. The average age ranged from 53.3 to 72.6 for the 4 cohorts, and the majority of the participants were women (57%). Other demographic and clinical characteristics were similar across the 4 cohorts. A total of 2526



**Figure 1.** The  $-\log_{10}$ , base-10,  $P$  value of the additive genetic model for each SNP with a minor allele prevalence of  $\geq 1.5\%$  for European-ancestry participants (A) and  $7.5\%$  for African-ancestry participants (B) according to its location in the 22 autosomal chromosomes. Horizontal line indicates the  $5 \times 10^{-7}$  threshold of genome-wide significance.

incident HF events (12.1%) were identified over a weighted average of 11.5 years of follow-up among those of European ancestry and 466 events (16.1%) over a weighted average of 13.7 years among those of African ancestry. The average age at the time of HF onset was 73.6 years (range, 44 to 101 years) and 52% of the 2992 events occurred among women. Among European- and African-ancestry participants, 73% and 78%, respectively, of the events were diagnosed in the absence of a prior MI.

### Participants of European Ancestry

A total of 2 478 304 SNPs were investigated across the 4 cohorts. The  $\lambda$  coefficients for analyses of European participants in each of the 4 cohorts were small ( $\leq 1.03$ ) and suggested negligible test statistic inflation. Supplemental Figure 1A presents the Q-Q plot of meta-analyzed  $P$  values derived from the 2 478 304 tests performed plotted against the expected distribution. Overall, evidence for population admixture was small ( $\lambda = 1.02$ ). At the lowest  $P$  values, the observed exceeded the expected.

Figure 1A presents all 2 478 304  $P$  values organized by chromosome and genomic position. Among these SNPs, 5 exceeded the genome-wide significance threshold ( $5.0 \times 10^{-7}$ ) and marked 2 loci, 1 on chromosome 13 (13q22) and another on chromosome 15 (15q22) (see supplemental Table III). Top SNPs at both loci were poorly imputed in CHS, so each was genotyped. After the inclusion of newly measured SNPs in CHS, only the 15q22 locus retained genome-wide significance.

An additional 12 loci on 9 chromosomes were marked by 28 SNPs with  $P$  values  $< 1.0 \times 10^{-5}$  (see supplemental Table III). Top SNPs for 7 loci were poorly imputed in CHS, so these were genotyped. Among the 7 SNPs, 3 retained a  $P$  value  $< 1.0 \times 10^{-5}$  after inclusion of newly measured SNPs. Table 2 lists the top SNP for each of the 10 loci, after genotyping in CHS.

### Genome-Wide Significant Locus in European-Ancestry Participants

Chromosomal position 15q22 was marked by 1 SNP, *rs10519210*, which had a MAF of 0.032 and was associated with a  $P$  value of  $1.4 \times 10^{-8}$ . The HR for this SNP was 1.53 per G allele. This SNP is located between 2 genes: 58.8 kb from *USP3* (ubiquitin-specific peptidase 3) and 63.9 kb from *CA12* (carbonic anhydrase XII). Figure 2A presents detailed information about the linkage disequilibrium (LD) and  $P$  values of regional markers for this locus. This SNP was genotyped in 1 population (ARIC) and imputed in the other 3 populations. The cohort-specific estimates are provided in Table 3 and Figure 3A. Risk estimates were generally consistent across cohorts.

There were 19 195 European-ancestry participants at the risk of HF who did not have a history of clinical MI at baseline. Among these participants, 1854 had an incident HF event without a prior incident MI during follow-up. For locus 15q22 (*rs10519210*), the  $P$  value and the HR were diminished slightly but similar to the findings for all HF events:  $P = 2.8 \times 10^{-6}$  and  $HR = 1.51$  (see supplemental Table IV).



**Table 2. Description and Association of SNP Markers of the Top Loci Associated With Incident HF**

POP	rs No.	CHR	Region	Position	Variant	MAF	P	HR (CI)*	Closest Gene
Genome-wide significant findings ( $<5.0 \times 10^{-7}$ )									
EA	rs10519210	15	15q22	61524978	T→G	0.032	$1.4 \times 10^{-8}$	1.53 (1.05 to 2.24)	58.8 kb from <i>USP3</i>
AA	rs11172782	12	12q14	57545895	A→G	0.290	$6.7 \times 10^{-8}$	1.46 (1.03 to 2.09)	6.3 kb from <i>LRIG3</i>
Other high-signal findings ( $<1.0 \times 10^{-5}$ )									
EA	rs11118620	1	1q41	219095131	T→C	0.292	$7.3 \times 10^{-6}$	1.15 (0.98 to 1.35)	0.0 kb from <i>LOC100129376</i> (intron)
EA	rs1523288	3	3q26.1	166762903	T→C	0.354	$6.1 \times 10^{-6}$	0.87 (0.74 to 1.02)	210.5 kb from <i>BCHE</i>
EA	rs13225783	7	7p15	27296581	C→T	0.048	$6.8 \times 10^{-6}$	1.38 (0.96 to 1.99)	43.9 kb from <i>EVX1</i>
EA	rs6473383	8	8q21.1	83831675	G→A	0.145	$3.1 \times 10^{-6}$	1.19 (0.99 to 1.44)	918.3 kb from <i>SNX16</i>
EA	rs10812610	9	9p21	27523984	A→C	0.496	$5.1 \times 10^{-6}$	1.14 (0.99 to 1.31)	4.1 kb from <i>MOBK2B</i>
EA	rs11203032	10	10q23	90954594	T→G	0.103	$8.3 \times 10^{-6}$	1.22 (0.97 to 1.53)	1.1 kb from <i>CH25H</i>
EA	rs1520832	12	12q12	41539682	G→T	0.036	$1.2 \times 10^{-6}$	1.39 (0.99 to 1.95)	269.9 kb from <i>PRICKLE1</i>
EA	rs548097	13	13q22	74674269	T→G	0.018	$6.4 \times 10^{-7}$	1.62 (1.00 to 2.63)	82.5 kb from <i>TBC1D4</i>
EA	rs11880198	19	19p13.3	3110769	A→G	0.134	$5.6 \times 10^{-6}$	1.23 (0.98 to 1.54)	0.0 kb from <i>GNA15</i> (intron)
AA	rs13418717	2	2q20	127379367	T→A	0.202	$3.4 \times 10^{-6}$	1.46 (0.97 to 2.20)	4.8 kb from <i>LOC339760</i>
AA	rs2210327	9	9p22	18099235	T→A	0.185	$6.8 \times 10^{-7}$	3.14 (0.99 to 10.0)	282.0 kb from <i>SH3GL2</i>
AA	rs563519	11	11q24	125533927	C→T	0.344	$3.3 \times 10^{-6}$	0.69 (0.46 to 1.03)	43.6 kb from <i>RPUSD4</i>
AA	rs2046383	12	12p11.2	29995409	T→G	0.302	$3.3 \times 10^{-6}$	1.39 (0.97 to 1.97)	166.5 kb from <i>TMT1</i>
AA	rs17019682	12	12q22	90829401	A→T	0.165	$2.0 \times 10^{-6}$	1.47 (0.98 to 2.22)	231.6 kb from <i>BTG1</i>

POP indicates population; CHR, chromosome; MAF, minor allele frequency; HR, hazard ratio from additive genetic model; EA, European ancestry; and AA, African ancestry.

\*Represents CIs based on a 2-sided  $\alpha=0.000005$ .

### Loci With a P Value $<1.0 \times 10^{-5}$ in European-Ancestry Participants

Nine additional loci on chromosomes 1, 3, 7, 8, 9, 10, 12, 13, and 19 were associated with SNPs that had  $P$  values  $<1.0 \times 10^{-5}$  (Table 2 and supplemental Table III). Among the 9 loci, 2 were intronic: rs11118620 (MAF=0.292) in *LOC100129376* and rs11880198 (MAF=0.134) in *GNA15* (guanine nucleotide binding protein [G protein], alpha 15 [Gq class]). Four loci were close to known genes: rs13225783 (MAF=0.048) within 43.9 kb of *EVX1* (even-skipped homeobox 1); rs10812610 (MAF=0.496) within 4.1 kb of *MOBK2B* (MOB1, Mps One Binder kinase activator-like 2B) and 4.1 kb of *IFNK* (interferon, kappa); rs11203032 (MAF=0.103) within 1.1 kb of *CH25H* (cholesterol 25-hydroxylase) and 8.7 kb of *LIPA* (lipase A, lysosomal acid, cholesterol esterase); and rs548097 (MAF=0.018) within 82.5 kb of *TBC1D4* (TBC1 domain family, member 4). The remaining 3 loci were not within 100 kb of known genes, and the nearest genes were *BCHE* (butyrylcholinesterase) for rs1523288, *SNX16* (sorting nexin 16) for rs6473383, and *PRICKLE1* (prickle homolog 1) for rs1520832. Supplemental Figure IIA through III present detailed information about the LD and  $P$  values of regional markers for these 9 loci. Cohort-specific details on these loci are presented in Table 3, and sensitivity analyses restricted to those without a prior MI are presented in supplemental Table IV.

### Participants of African Ancestry

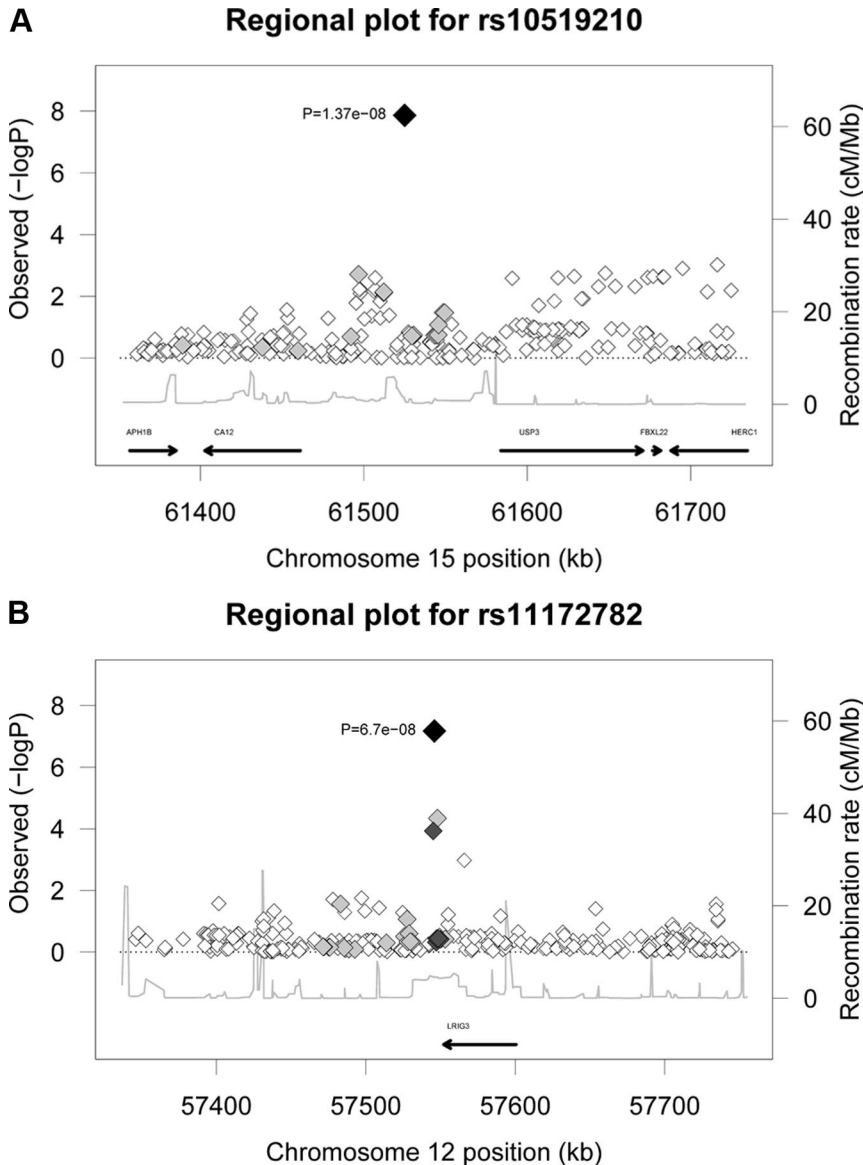
A total of 692 330 SNPs were investigated across the 2 cohorts that included participants of African ancestry. The  $\lambda$

coefficients were small ( $<1.02$ ) and suggested negligible population structure. Supplemental Figure IB presents the Q-Q plot from the 692 330 tests performed and Figure 1B presents all  $P$  values organized by chromosome and position. No SNP had a  $P$  value that exceeded the genome-wide significance threshold ( $5.0 \times 10^{-7}$ ). Seven loci on 4 chromosomes were marked by 10 SNPs with  $P$  values  $<1.0 \times 10^{-5}$  (see supplemental Table III). Five loci were marked by poorly imputed SNPs in CHS and 2 of these were genotyped. Among the 2 loci, 1 reached genome-wide significance (12q14) and the other did not retain a  $P$  value  $<1.0 \times 10^{-5}$ . Table 2 lists the top SNP for the 6 loci with a  $P$  value  $<1.0 \times 10^{-5}$ .

### Genome-Wide Significant Locus in African-Ancestry Participants

Chromosomal position 12q14 was marked by 1 SNP, rs11172782, which had a MAF of 0.29 and was associated with a  $P$  value of  $6.7 \times 10^{-8}$ . The HR for this SNP was 1.46 per G allele. This SNP is located 6.3 kb from *LRIG3* (leucine-rich repeats and immunoglobulin-like domains 3). Figure 2B presents detailed information about the LD and  $P$  values of regional markers for this locus. The cohort-specific estimates are provided in Table 3 and Figure 3B. Risk estimates were consistent across both cohorts.

There were 2631 participants of African ancestry at the risk of HF who did not have a history of clinical MI at baseline. Among these participants, 372 had an incident HF event without a prior incident MI during follow-up. For locus 12q14 (rs11172782), the  $P$  value and the HR were similar to



**Figure 2.** Regional plots for genome-wide significant marker loci: *rs10519210* in European-ancestry participants (A); *rs11172782* in African-ancestry participants (B). The SNP with the smallest *P* value is presented in black font and neighboring variants are presented in different black saturations based on linkage disequilibrium: dark gray:  $r^2 \geq 0.5$  and  $< 0.8$ ; light gray:  $r^2 \geq 0.2$  and  $< 0.5$ ; white:  $r^2 < 0.2$ . There were no SNPs in the region with a linkage disequilibrium  $r^2 \geq 0.8$  with the top hit.

the findings for all HF events: *P* value =  $6.5 \times 10^{-8}$  and HR = 1.53 (see supplemental Table IV).

#### **Loci With a *P* Value $< 1.0 \times 10^{-5}$ in African-Ancestry Participants**

Five loci on chromosomes 2, 9, 11, and 12 were associated with SNPs that had *P* values  $< 1.0 \times 10^{-5}$  (Table 2). Two loci were close to hypothesized or known genes: *rs13418717* (MAF = 0.202) within 4.8 kb of LOC339760 and *rs563519* (MAF = 0.344) within 43.6 kb of *RPUSD4* (RNA pseudouridylation synthase domain containing 4). The remaining 3 loci were not within 100 kb of known genes and the nearest genes were *SH3GL2* (SH3-domain GRB2 [growth factor receptor-bound protein 2]-like 2) for *rs2210327*, *TMTCL1* (transmembrane and tetratricopeptide repeat containing 1) for *rs2046383*, and *BTGL1* (B-cell translocation gene 1, antiproliferative) for *rs17019682*. Supplemental Figure IIIJ through IIIN presents detailed information about the LD and *P* values of regional markers for the 5 loci. Cohort-specific details on these loci are presented in Table 3, and sensitivity analyses restricted to those without a prior MI are presented in supplemental Table IV.

#### **Across-Ancestry Comparisons**

The genome-wide significant SNP in European-ancestry participants, *rs10519210*, was not associated with HF in those of African ancestry:  $7.6 \times 10^{-1}$  (HR = 1.06; MAF = 0.179). The genome-wide significant SNP in African-ancestry participants, *rs11172782*, was uncommon and not associated with HF in those of European ancestry:  $5.6 \times 10^{-1}$  (HR = 0.91; MAF = 0.018).

From among the 9 high-signal SNPs identified in European-ancestry participants, none was genotyped in ARIC; therefore, none was included in African-ancestry genome-wide analyses. When these 9 SNPs were examined in CHS participants of African ancestry, 1 of the 9 SNPs was associated with a *P* value  $< 0.05$ : *rs10812610* had a *P* value of  $9.1 \times 10^{-3}$  (HR = 1.52; MAF = 0.766 [C allele was not a minor allele in these participants]). When meta-analyzed with the European-ancestry findings, the resulting combined-ancestry *P* value for this locus was  $7.5 \times 10^{-7}$ . From among the 5 high-signal SNPs in African-ancestry participants, none was associated with HF risk in European-ancestry participants (all *P* values  $> 5.0 \times 10^{-2}$ ).

**Table 3. Cohort Specific Results for the Top Loci Associated With Incident HF**

POP	rs No.	CHR	ARIC			CHS			FHS			RS		
			HR	P	OEV*	HR	P	OEV	HR	P	OEV	HR	P	OEV
Genome-wide significant findings (<5.0×10 <sup>-7</sup> )														
EA	rs10519210	15	1.40	1.6×10 <sup>-2</sup>	0.998	1.18	1.9×10 <sup>-2</sup>	Remx	2.35	2.4×10 <sup>-7</sup>	0.977	1.32	1.2×10 <sup>-1</sup>	0.897
AA	rs11172782	12	1.54	3.5×10 <sup>-7</sup>	NA	1.32	2.9×10 <sup>-2</sup>	Remx	NAA	NAA	NAA	NAA	NAA	NAA
Other high-signal findings (<1.0×10 <sup>-5</sup> )														
EA	rs11118620	1	1.16	1.2×10 <sup>-2</sup>	0.994	1.08	1.7×10 <sup>-1</sup>	Remx	1.17	9.8×10 <sup>-2</sup>	1.024	1.22	7.3×10 <sup>-4</sup>	0.863
EA	rs1523288	3	0.88	2.6×10 <sup>-2</sup>	0.934	0.85	2.9×10 <sup>-3</sup>	0.966	0.85	2.4×10 <sup>-1</sup>	0.364	0.87	1.3×10 <sup>-2</sup>	0.962
EA	rs13225783	7	1.40	1.9×10 <sup>-2</sup>	0.649	1.19	2.3×10 <sup>-1</sup>	Remx	1.60	3.5×10 <sup>-2</sup>	0.531	1.47	1.9×10 <sup>-3</sup>	0.816
EA	rs6473383	8	1.04	6.4×10 <sup>-1</sup>	0.996	1.30	2.3×10 <sup>-5</sup>	1.002	1.30	2.0×10 <sup>-2</sup>	1.004	1.16	3.1×10 <sup>-2</sup>	0.995
EA	rs10812610	9	1.11	5.8×10 <sup>-2</sup>	0.986	1.19	3.5×10 <sup>-4</sup>	0.992	1.25	1.3×10 <sup>-2</sup>	1.002	1.08	1.6×10 <sup>-1</sup>	0.999
EA	rs11203032	10	1.23	1.3×10 <sup>-2</sup>	0.999	1.13	1.1×10 <sup>-1</sup>	0.958	1.49	4.3×10 <sup>-3</sup>	0.924	1.23	1.7×10 <sup>-2</sup>	0.985
EA	rs1520832	12	1.37	1.6×10 <sup>-2</sup>	0.986	1.29	5.0×10 <sup>-2</sup>	Remx	0.81	4.4×10 <sup>-1</sup>	0.972	1.61	3.6×10 <sup>-5</sup>	0.991
EA	rs548097	13	1.89	4.5×10 <sup>-4</sup>	0.998	1.12	6.0×10 <sup>-1</sup>	Remx	1.38	3.1×10 <sup>-1</sup>	0.992	1.81	6.4×10 <sup>-4</sup>	0.966
EA	rs11880198	19	1.34	4.6×10 <sup>-2</sup>	0.313	1.23	7.1×10 <sup>-4</sup>	1.021	2.05	3.0×10 <sup>-2</sup>	0.168	1.17	4.9×10 <sup>-2</sup>	0.867
AA	rs13418717	2	1.41	5.6×10 <sup>-5</sup>	NA	1.91	8.0×10 <sup>-3</sup>	0.348	NAA	NAA	NAA	NAA	NAA	NAA
AA	rs2210327	9	3.51	7.1×10 <sup>-7</sup>	NA	1.95	2.1×10 <sup>-1</sup>	0.189	NAA	NAA	NAA	NAA	NAA	NAA
AA	rs563519	11	0.69	1.5×10 <sup>-5</sup>	NA	0.72	6.7×10 <sup>-2</sup>	0.512	NAA	NAA	NAA	NAA	NAA	NAA
AA	rs2046383	12	1.30	1.5×10 <sup>-3</sup>	NA	1.62	2.0×10 <sup>-4</sup>	0.972	NAA	NAA	NAA	NAA	NAA	NAA
AA	rs17019682	12	1.53	4.4×10 <sup>-6</sup>	NA	1.31	1.1×10 <sup>-1</sup>	0.817	NAA	NAA	NAA	NAA	NAA	NAA

EA indicated European ancestry; AA, African ancestry; POP, population; CHR, chromosome; HR, hazard ratio from additive genetic model; OEV, observed-expected variance; Remx, remeasured variants; NA, OEV results not available since all SNPs were measured; and NAA, no African-ancestry participants.

\*Estimated using  $r^2$ .

## Discussion

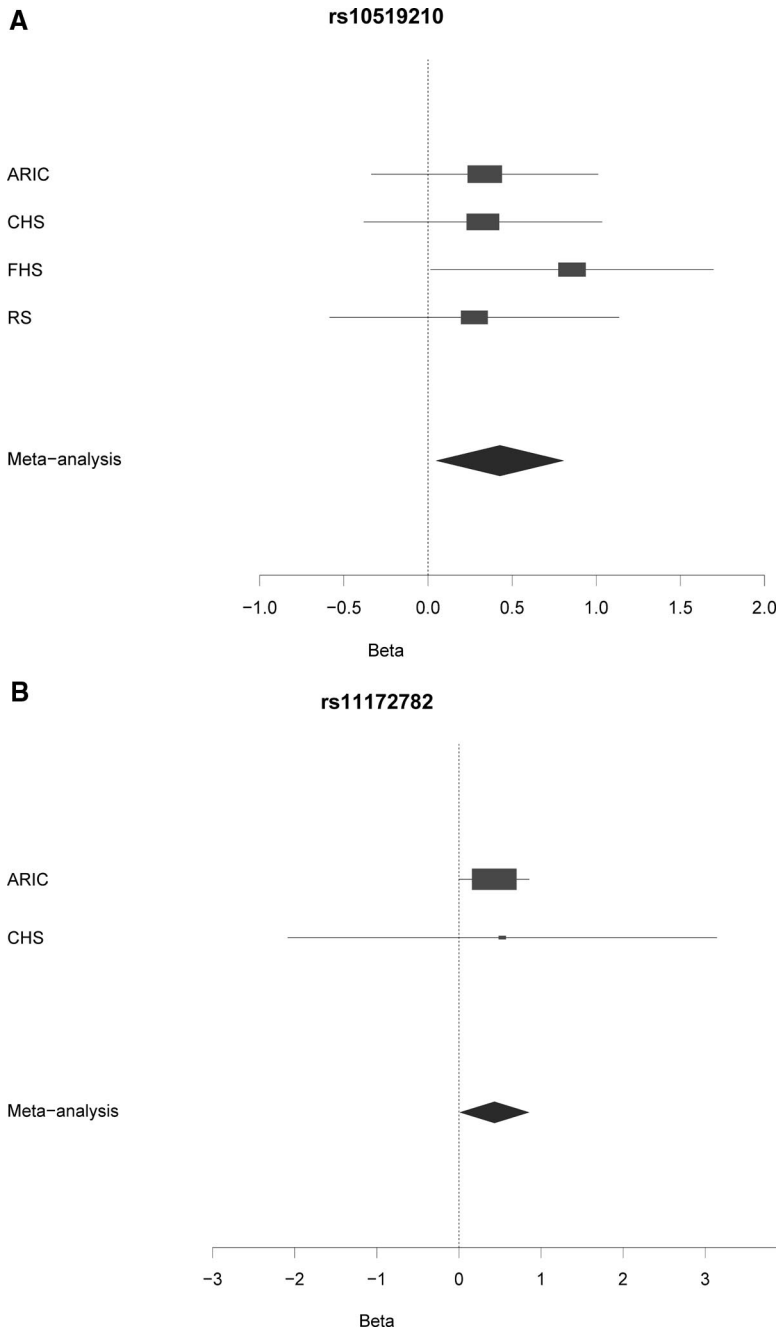
Among 20 926 European-ancestry participants with 2526 incident HF events, we identified 1 locus with a SNP whose  $P$  value ( $1.4 \times 10^{-8}$ ) exceeded the genome-wide statistical significance threshold of  $5 \times 10^{-7}$  and was associated with a 53% increase in HF risk. Among 2895 African-ancestry participants who had 466 incident events, we identified 1 locus with a SNP whose  $P$  value ( $6.7 \times 10^{-8}$ ) exceeded the genome-wide statistical significance and was associated with a 46% increase in HF risk. For both loci, risk estimates were similar for those subjects who HF was not attributable to MI. We identified an additional 14 loci in European-ancestry and African-ancestry participants that were marked by high-signal SNPs,  $P$  value  $<1.0 \times 10^{-5}$ , but that did not reach genome-wide significance. For most loci, risk estimates were modest and did not seem to differ in a subset of participants without an MI preceding HF onset.

### Genes Associated With Genome-Wide Significant and High-Signal Findings

Our results suggest that there may be several genomic regions associated with new-onset HF in older adults, and support the hypothesis that common genetic variation, regardless of the clinical mechanism responsible for reduced cardiac output in HF, contributes to risk. The 2 loci with marker SNPs that reached genome-wide significance were within 60 kb of known genes: *USP3* and *CAI2* in European-ancestry participants and *LRIG3* in African-ancestry participants. The product of the *USP3* gene is an ubiquitin-specific protease (USP).

Ubiquitin is a highly conserved 76-amino acid protein involved in the regulation of intracellular protein breakdown, cell-cycle regulation, and stress response. It is released from degraded proteins by the disassembly of polyubiquitin chains, which is mediated by USPs.<sup>32</sup> The *USP3* gene may also have a role in genome stability.<sup>33</sup> Several papers have recently been published that describe the role of ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies and ubiquitin myocardial expression in pathogenic cardiac hypertrophy.<sup>34,35</sup> The gene product from *CAI2* is a membrane protein highly expressed in many tissue beds and may have a role in cancer prognosis.<sup>36</sup> The *LRIG3* gene is a member of the LRIG family of genes that is widely expressed and impacts tissue development and tumor progression.<sup>37–39</sup>

Among the 14 high-signal SNPs, 2 were intronic, 1 of which was located in a known gene, *GNAI5*, and the other in a hypothetical gene *LOC100129376*. The *GNAI5* gene, marked by rs11880198, is a guanine nucleotide binding protein of the Gq class, found in some G-protein-coupled receptors. This family of receptors include adrenergic, endothelin, and angiotensin II receptors, which regulate cardiac output, arterial pressure, and blood volume.<sup>40,41</sup> Three important amino acid substitutions (1 nonsense and 2 missense) and 1 frameshift variant have been identified in the *GNAI5* gene. None of these variants were included in the imputed SNP panel, and LD with the HF SNP in our data are not known. Two additional high-signal SNPs were within 10 kb of known genes, which included *MOBK2B* and *IFNK*, and *CH25H*



**Figure 3.** Meta-analysis and study-specific  $\beta$  coefficients and confidence intervals ( $\alpha=0.0000005$ ) for genome-wide significant findings for genome-wide significant SNPs: rs10519210 in European-ancestry participants (A); rs11172782 in African-ancestry participants (B).

and *LIPA*. None seems to have known functional qualities related to cardiac processes.<sup>42,43</sup> Three additional high-signal SNPs were within 100 kb of known genes including *EVXI*, *TBC1D4*, and *RPUSD4*. Several papers have been published on the protein product of the *TBC1D4* gene, known as AS160, and its role in handling insulin in skeletal muscles.<sup>44–46</sup> The remaining 6 SNPs were not within 100 kb of genes.

Although we have described genes nearest to high-signal markers as potential culprits in the observed associations, it is possible that the true casual variant, if one exists, lies in neighboring genes or within the non-protein-coding transcripts in intergenic regions.<sup>45</sup> In this article, we have identified the genes nearest to the high-signal marker as good

candidates, but identifying the true causal genes will require further scientific investigation.

The consistency of findings across the European-ancestry and African-ancestry populations was modest. For the 2 genome-wide significant findings, the MAFs differed greatly between African-ancestry and European-ancestry participants. These high-signal markers are probably not themselves causal and are likely to be in linkage disequilibrium with the causal variants. In view of the large differences in MAFs and the different patterns of LD between the populations, it is perhaps not surprising that the associations are not consistent across ancestral groups. Indeed, the optimal replication sample for the findings in those of European ancestry would be other similar populations of European ancestry.



**Strengths and Limitations**

To our knowledge, this is the first large-scale attempt to discover genetic risk variants for incident HF, and included more than 23 000 individuals and  $\approx 2.5$  million markers spread throughout the genome. Data came from 4 large, population-based cohort studies that included cardiovascular outcomes as primary study endpoints. Over 2500 incident HF events among European-ancestry participants were included in our analyses, which allowed 80% power (2-sided  $\alpha = 5 \times 10^{-7}$ ) to detect HRs as small as 1.6, 1.4, and 1.3 for variants with a MAF of 0.05, 0.1, and 0.2, respectively. For African-ancestry participants, minimal detectable HRs were larger: 2.6, 2.2, and 1.9, respectively. Statistical power for African-ancestry analyses was limited. We found 1 SNP of genome-wide significance for each ancestry population, on par with what one would expect by chance alone. Replication of associations between these SNPs and incident HF will clarify what role, if any, these SNPs have in the genesis of clinical HF. Each cohort relied on slightly different criteria to classify HF events, and it was not feasible to reclassify all 2992 events using a standard protocol. The between-study variation in HF classification likely introduced some heterogeneity that may have diminished our power to detect associations. Furthermore, we did not have comparable and complete data across cohorts on the type of failure. Previously published data from CHS and FHS indicated that half of the patients with new-onset HF had cardiac systolic dysfunction at the time of clinical onset.<sup>48,49</sup> Not all 2 478 304 variants tested in patients of European ancestry were directly genotyped, and the imputation quality varied across SNPs. For poorly imputed SNPs, there was reduced statistical power to detect an association. We excluded SNPs with low MAF from meta-analyses because asymptotic assumptions of survival analysis models may not be accurate if the number of cases with the variant allele is low. For European-ancestry participants, the MAF threshold of 0.015 translates to  $\approx 75$  participants with incident HF who are carriers of 1 or more variant alleles. This relatively large number of events is not expected to violate asymptotic assumptions, and *P* values are likely accurate. Most of the across-ancestry comparisons were limited to data from CHS, where imputation quality for African-ancestry participants was generally poor, because the Illumina 370CNV chip was not designed to optimize coverage in this ancestry.

**Summary**

Using data from 4 prospective cohort studies that included 23 821 participants and prospectively identified 2992 incident HF events in older adults, we identified 2 SNPs that reached genome-wide significance, 1 in European-ancestry and 1 in African-ancestry participants. An additional 14 subthreshold but high-signal loci were identified and included intronic markers in attractive candidate genes. Our findings merit replication in other community-based settings of incident HF.

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**Disclosures**

None.

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### CLINICAL PERSPECTIVE

Genetic factors contribute to heart failure (HF) onset and, to date, most approaches to identify genetic variants associated with HF risk have relied on candidate genes. No large-scale genome-wide investigation of HF risk has been published. We investigated the association of  $\approx 2.5$  million single-nucleotide polymorphisms with incident HF by meta-analyzing data from 4 community-based prospective cohorts: the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Rotterdam Study. Among 20 926 European-ancestry participants with 2526 incident HF events, we identified 1 locus with a single-nucleotide polymorphism, whose  $P$  value ( $1.4 \times 10^{-8}$ ) exceeded the genome-wide statistical significance threshold of  $5 \times 10^{-7}$  and was associated with a 53% increase in HF risk. Among 2895 African-ancestry participants who had 466 incident events, we identified 1 locus with a single-nucleotide polymorphism, whose  $P$  value ( $6.7 \times 10^{-8}$ ) exceeded the genome-wide statistical significance and was associated with a 46% increase in HF risk. We identified an additional 14 loci in European-ancestry and African-ancestry participants that were marked by high-signal single-nucleotide polymorphisms with a  $P$  value  $< 1.0 \times 10^{-5}$ . For most loci, risk estimates were modest and did not seem to differ in subjects without a myocardial infarction preceding HF onset. Our results suggest that there may be several genomic regions associated with new-onset HF in older adults, and support the hypothesis that common genetic variation, regardless of the clinical mechanism responsible for reduced cardiac output in HF, contributes to the risk. These findings merit replication in other community-based settings of incident HF.