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ORIGINAL ARTICLE

Identification of Functional Genetic Determinants of Cardiac Troponin T and I in a Multiethnic Population and Causal Associations With Atrial Fibrillation

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BACKGROUND: Elevated cardiac troponin levels in blood are associated with increased risk of cardiovascular diseases and mortality. Cardiac troponin levels are heritable, but their genetic architecture remains elusive.

METHODS: We conducted a transethnic genome-wide association analysis on high-sensitivity cTnT (cardiac troponin T; hs-cTnT) and high-sensitivity cTnI (cardiac troponin I; hs-cTnI) levels in 24 617 and 14 336 participants free of coronary heart disease and heart failure from 6 population-based cohorts, followed by a series of bioinformatic analyses to decipher the genetic architecture of hs-cTnT and hs-cTnI.

RESULTS: We identified 4 genome-wide significant loci for hs-cTnT including a novel locus rs3737882 in *PPFIA4* and 3 previously reported loci at *NCOA2*, *TRAM1*, and *BCL2*. One known locus at *VCL* was replicated for hs-cTnI. One copy of C allele for rs3737882 was associated with a 6% increase in hs-cTnT levels (minor allele frequency, 0.18; $P=2.80\times10^{-9}$). We observed pleiotropic loci located at *BAG3* and *ANO5*. The proportions of variances explained by single-nucleotide polymorphisms were 10.15% and 7.74% for hs-cTnT and hs-cTnI, respectively. Single-nucleotide polymorphisms were colocalized with *BCL2* expression in heart tissues and hs-cTnT and with *ANO5* expression in artery, heart tissues, and whole blood and both troponins. Mendelian randomization analyses showed that genetically increased hs-cTnT and hs-cTnI levels were associated with higher odds of atrial fibrillation (odds ratio, 1.38 [95% CI, 1.25–1.54] for hs-cTnT and 1.21 [95% CI, 1.06–1.37] for hs-cTnI).

CONCLUSIONS: We identified a novel genetic locus associated with hs-cTnT in a multiethnic population and found that genetically regulated troponin levels were associated with atrial fibrillation.

Key Words: alleles ■ cardiovascular diseases ■ heart failure ■ Mendelian randomization analysis ■ troponin T

Cardiac troponin is a biomarker of cardiomyocyte necrosis,[1] consisting of 3 units, T, I, and C, collocated with tropomyosin on the actin filament. The troponin complex is essential for calcium-mediated regulation of cardiac muscle contraction.[2] cTnT (cardiac troponin T) and cTnI (cardiac troponin I) are established biomarkers for myocardial infarction diagnosis and prognosis[1] and have been shown to be associated with increased risk for cardiovascular disease (CVD) and mortality in the general population.[3–6]

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Nonstandard Abbreviations and Acronyms

AF	atrial fibrillation
CADD	combined annotation dependent depletion score
cTnI	cardiac troponin I
cTnT	cardiac troponin T
CVD	cardiovascular disease
eQTL	expression quantitative trait locus
GWAS	genome-wide association study
hs-cTnI	high-sensitivity cardiac troponin I
hs-cTnT	high-sensitivity cardiac troponin T
MR	mendelian randomization
MTAG	multi-trait analysis of genome-wide association study
PPARγ	peroxisome proliferator-activated receptor gamma
SNP	single-nucleotide polymorphism

Circulating cardiac troponin levels are heritable; the estimated heritability is 35% for cTnT and 25% for cTnI.[6] A genome-wide association study (GWAS) of serum levels of high-sensitivity cTnT (hs-cTnT) identified 2 loci—an intergenic region at 8q13 and *TNNT2* (1q32)—in 11 544 Europeans and African Americans.[7] Recently, a GWAS in 19 130 Scottish subjects has identified multiple loci for high-sensitivity CTnI (hs-cTnI; *KLKB1* [4q35.2], *VCL* [10q22.2], *ANO5* [11p14.3], *CEP95* [17q23.3], and *CPLX4* [18q21.32]) and added 4 novel loci at *C1orf112* (1q24.2), *TRABD2A* (2p11.2), *SORBS2* (4q35.1), and *PTPRD* (9p24.1) for hs-cTnT.[6,7] Yet, the impact of genetic variation on the levels of hs-cTnT and hs-cTnI in ethnically diverse populations has not been described. Using the most updated high-sensitivity assays,[8] we aimed to identify novel genetic variants associated with circulating cTnT and cTnI levels in a large multiethnic population consisting of African, Asian, European, and Hispanic ancestries and furthermore, to investigate causal associations with CVDs.

METHODS

Availability of Data and Materials

Full summary GWAS statistics generated in this study are available upon reasonable request made to the corresponding authors. The Genotype-Tissue Expression, version 8, expression quantitative trait loci (eQTL) data used in this study are available from eQTL catalogues (ftp://ftp.ebi.ac.uk/pub/databases/spot/eQTL). The authors declare that all other supporting data are available within the article and Materials in the Supplemental Material.

Ethical Declarations and Methods

All studies were approved by appropriate institutional review committees, and all subjects provided written informed consent. Full details of data and methods used in this study are presented in the Supplemental Material and Methods.

RESULTS

Multiethnic GWAS Identifies a Novel Locus Associated With hs-cTnT

We conducted multiethnic GWAS for hs-cTnT levels in 24 617 participants, including 18 590 from European, 3806 from African, 775 from Asian, and 1446 from Hispanic ancestries. The hs-cTnI analyses included 14 336 participants, consisting of 12 730 European and 1606 African ancestry subjects. The studies had mean ages ranged from 47.13 (SD, 16.05) to 76.21 (SD, 5.23), with proportions of women ranging from 50.8% to 65.1%. Baseline characteristics were comparable among studies. Detailed demographic information is presented in Data S5.

We identified 67 variants at 4 independent loci that were associated with hs-cTnT at genome-wide significance ($P<5\times10^{-8}$; Figure 1A; Table). One locus, mapping to the intron of *PPFIA4* (liprin-alpha-4), has not been reported previously. One copy of a C allele (minor allele frequency, 0.18) for the lead single-nucleotide polymorphism (SNP) rs3737882 in *PPFIA4* was associated with 6% increased hs-cTnT level ($P=2.80\times10^{-19}$). The minor allele frequency of rs3737882 was similar, and the direction of effect was consistent across ethnic groups (Table S1). We also replicated 3 previously reported loci near *NCOA2* and *TRAM1* and at *BCL2* (apoptosis regulator). We did not observe any genome-wide significant association for hs-cTnI (Figure 1B); however, one previously reported locus at *VCL* showed suggestive association with hs-cTnI ($P=5.51\times10^{-8}$). No genomic inflation was observed for both troponin analyses (Figure S1).

The European-specific analysis resulted similar findings comparing to the transethnic analysis (Table S2). The proportions of phenotypic variance explained by common variants were estimated at 10.15% (SE, 0.025) for hs-cTnT and 7.74 % (SE, 0.038) for hs-cTnI in the European ancestry. In the African ancestry–specific analysis, we identified a genome-wide significant locus at LOC105378816;LOC107985037 (rs150095447; $P=4.63\times10^{-9}$) for hs-cTnT and one at CD2BP2 (rs116215614; $P=1.11\times10^{-8}$) for hs-cTnI (Table S3). We did not observe genome-wide significant association in Asian or Hispanic ancestries (Tables S4 and S5). We presented ancestry-specific allele frequencies and association statistics for transethnic significant associations in Table S1.

Variant Effects on Protein Coding Sequence

We investigated the predicted deleterious effects of troponin-associated loci using the Combined Annotation

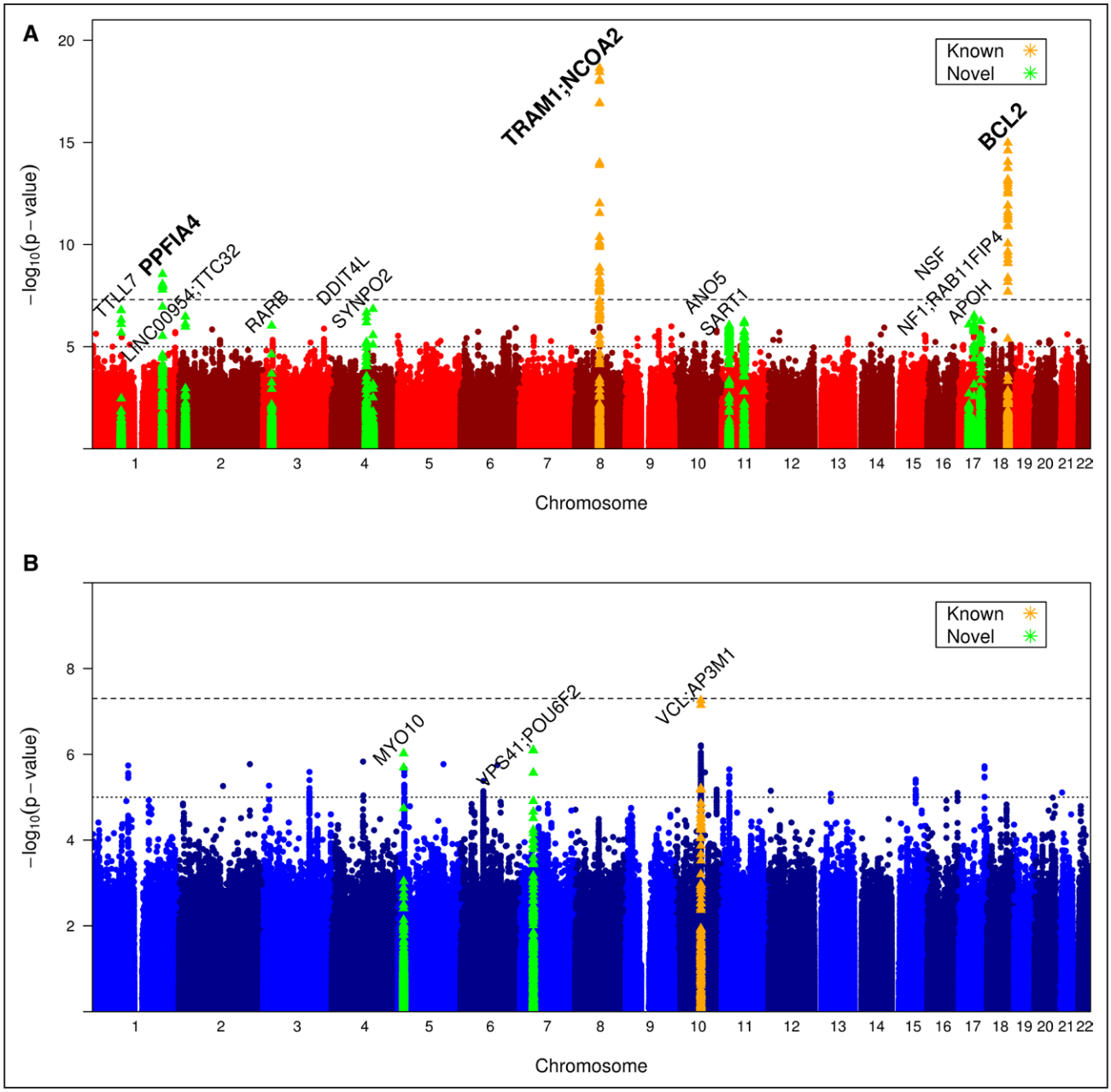

Figure 1. xxx.

Manhattan plots of genome-wide associations for high-sensitivity cardiac troponin T (cTnT; **A**) and high-sensitivity cardiac troponin I (cTnI; **B**). Single-nucleotide polymorphisms (SNPs) are positioned along the *x* axis according to chromosomal position with −log10(*P*) along the *y* axis. Genome-wide significance threshold ($P=5\times10^{-8}$) is presented as a dashed black horizontal line, and suggestive significance threshold ($P=1\times10^{-5}$) is presented as a dotted black horizontal line. Sentinel SNPs (\$\pm \$50 kb) with $P<1\times10^{-6}$ are labeled with the nearest genes. Novel findings are colored in green, while the previously reported loci are highlighted in yellow.

Dependent Depletion (CADD) scores. Sentinel SNPs and their proxies with CADD scores >12 are shown in Table and Table S6. Among the genome-wide significant loci associated with hs-cTnT, the CADD score was only significantly high (12.64) for the sentinel SNP at *PPFIA4* (rs3737882). SNPs associated with hs-cTnI in the *VCL* and *ADK* region showed significant CADD score. Additionally, a proxy for the sentinel variant in the pleiotropic *BAG3* region, rs2234962, was predicted to be deleterious (CADD score, 21.50).

Pleiotropic Locus for Troponin T and I

We identified 3 candidate pleiotropic loci, *BCL2*, *ANO5*, and *BAG3*, associated with both hs-cTnT and hs-cTnI at genome-wide significance (Figure S2; Table S7). The sentinel SNP at *BCL2*, rs12457700, was identified by multitrait analysis of GWAS (MTAG) with *P* of 3.93×10^{-12} and 4.84×10^{-12} for hs-cTnT and hs-cTnI, respectively. Two loci, *BAG3* and *ANO5*, were previously identified with suggestive evidence in both

Table. Lead Variants ($P<1\times10^{-6}$) Associated With hs-cTnT and hs-cTnI

rsID	Chr	Position (hg19)	Locus	Nearest gene(s)*	Relation to gene	A1/A2	AF	β (SE)	*P* value	CADD
hs-cTnT (n=24 617)										
rs10091864	8	71359103	8q13.3	*NCOA2;TRAM1*	Intergenic	c/g	0.56	−0.07 (0.008)	2.28×10^{-19}	0.22
rs9944895	18	60859974	18q21.33	*BCL2*	Intronic	c/g	0.69	0.07 (0.008)	1.05×10^{-15}	2.32
rs3737882	1	203034955	1q32.1	*PPFIA4*	Intronic	c/g	0.82	0.06 (0.010)	2.80×10^{-9}	12.64
rs28581409	8	71407059	8q13.3	*TRAM1*	Intergenic	a/g	0.34	−0.05 (0.008)	6.63×10^{-9}	0.62
rs75244633	4	119879588	4q26	*SYNPO2*	Intronic	t/c	0.02	0.14 (0.027)	1.44×10^{-7}	4.14
rs146737477	1	83763281	1p31.1	*TTLL7*	Intergenic	a/g	0.03	−0.25 (0.047)	1.65×10^{-7}	1.81
rs12506869	4	101000987	4q23	*DDIT4L*	ncRNA_intronic	a/g	0.26	−0.05 (0.009)	2.13×10^{-7}	0.02
rs199460	17	44764775	17q21.31	*NSF*	Intronic	a/c	0.74	−0.05 (0.010)	3.07×10^{-7}	4.26
rs17618762	2	19846104	2p24.1	*LINC00954;TTC32*	Intergenic	a/g	0.93	−0.09 (0.017)	3.37×10^{-7}	0.58
rs13341435	17	64250605	17q24.2	*APOH*	Intronic	a/g	0.06	0.08 (0.016)	5.61×10^{-7}	3.86
rs1192168	11	65730945	11q13.1	*SART1*	Intronic	t/g	0.50	0.04 (0.007)	7.27×10^{-7}	0.18
rs9899998	17	29711014	17q11.2	*NF1;RAB11FIP4*	Intergenic	a/g	0.06	−0.19 (0.039)	8.22×10^{-7}	1.22
rs4922982	11	22237365	11p14.3	*ANO5*	Intronic	t/c	0.31	−0.04 (0.009)	9.21×10^{-7}	0.63
rs116819086	3	25449004	3p24.2	*RARB*	Intronic	c/g	0.04	−0.24 (0.048)	9.23×10^{-7}	0.10
hs-cTnI (n=14 336)										
rs7915720	10	75774139	10q22.2	*VCL;AP3M1*	ncRNA_intronic	a/g	0.32	0.07 (0.012)	5.51×10^{-8}	0.63
rs2915700	7	38984277	7p14.1	*VPS41;POU6F2*	Intergenic	a/g	0.17	0.09 (0.019)	7.97×10^{-7}	1.53
rs26742	5	16664769	5p15.1	*MYO10*	Downstream	a/g	0.57	−0.06 (0.012)	9.45×10^{-7}	0.71

This table presents the top 14 and 3 independent variants associated with hs-cTnT and hs-cTnI, respectively, at the significance level of $P<1\times10^{-6}$. Six studies were meta-analyzed using an inverse variance–based fixed-effect approach. The statistics are based on A1. A1 indicates allele 1; A2, allele 2; AF, atrial fibrillation; CADD, combined annotation dependent depletion score; Chr, chromosome; hs-cTnI, high-sensitivity cardiac troponin I; and hs-cTnT, high-sensitivity cardiac troponin T.

*Nearest gene with a functional protein or RNA (eg, anti-sense RNA) product that either overlaps with the sentinel variant or for intergenic variants, the nearest genes up- and downstream, respectively.

hs-cTnT and hs-cTnI GWAS analyses and had improved significance in MTAG analyses. The sentinel pleiotropic variants at *BAG3* and *ANO5* were rs7938061 (MTAG *P* for hs-cTnT, 1.38×10^{-9} and MTAG *P* for hs-cTnI, 1.36×10^{-9}, respectively) and rs72842207 (MTAG *P* for hs-cTnT, 1.17×10^{-8} and MTAG *P* for hs-cTnI, 1.11×10^{-8}, respectively).

Gene-Based Association Test and Gene-Set Enrichment

The Multi-Marker Analysis of GenoMic Annotation gene-based association analysis identified 7 and 3 loci associated with hs-cTnT and hs-cTnI ($P<2.58\times10^{-6}$), respectively (Table S8). The significant associations for hs-cTnT included the GWAS loci at *BCL2* and *PPFIA4*, with 5 other novel genes, *NSF*, *MANBA*, *NPC1*, *TMEM127*, and *C18orf8*. For hs-cTnI, *VCL*, *ADK*, and *AP3M1* were identified as significant. Genes mapped to GWAS associations with $P<1\times10^{-5}$ were further investigated for gene-set enrichment (Table S9). Two genome-wide significant loci for hs-cTnT, *BCL2* and *PPFIA4*, were enriched in the hypoxia hallmark gene set composed of genes upregulated in response to low oxygen levels (adjusted $P=9.60\times10^{-3}$). Genes mapped to hs-cTnI SNPs were enriched among the gene ontologies associated with mitochondrion targeting (adjusted $P=6.38\times10^{-6}$) and protein localization to mitochondrion (adjusted $P=1.19\times10^{-5}$).

Tissue-Specific Colocalization and Transcriptome-Wide Association Analyses

We performed colocalization analysis for the 19 loci identified in the GWAS and MTAG analysis with gene expression using Genotype-Tissue Expression v8 eQTL data (Table S10). We identified SNPs associated with *ANO5* expression and either hs-cTnT or hs-cTnI in aortic artery, coronary artery, heart atrial appendage, and whole blood (Figure 2). The eQTL associations for *ANO5* were remarkably high in 2 artery tissues. We also identified SNPs at *BCL2* in left ventricular and atrial appendage tissues (Figure S3) and SNPs at *NSF* in aorta artery tissue, which colocalized with either hs-cTnT or hs-cTnI levels. Using predicted expression levels, we performed a transcriptome-wide association analysis in aorta artery, coronary artery, atrial appendage, left ventricle, and whole blood (Table S11). At the transcriptome-wide significance level ($P<1.59\times10^{-6}$), we found that *ANO5* in whole blood ($P=1.51\times10^{-6}$) and in atrial appendage ($P=6.94\times10^{-7}$) and *BCL2* in left ventricle ($P=4.41\times10^{-11}$) and in atrial appendage ($P=3.49\times10^{-8}$) were associated with hs-cTnT. For hs-cTnI, we identified a novel locus at *PLAU* in the left ventricle ($P=8.05\times10^{-7}$).

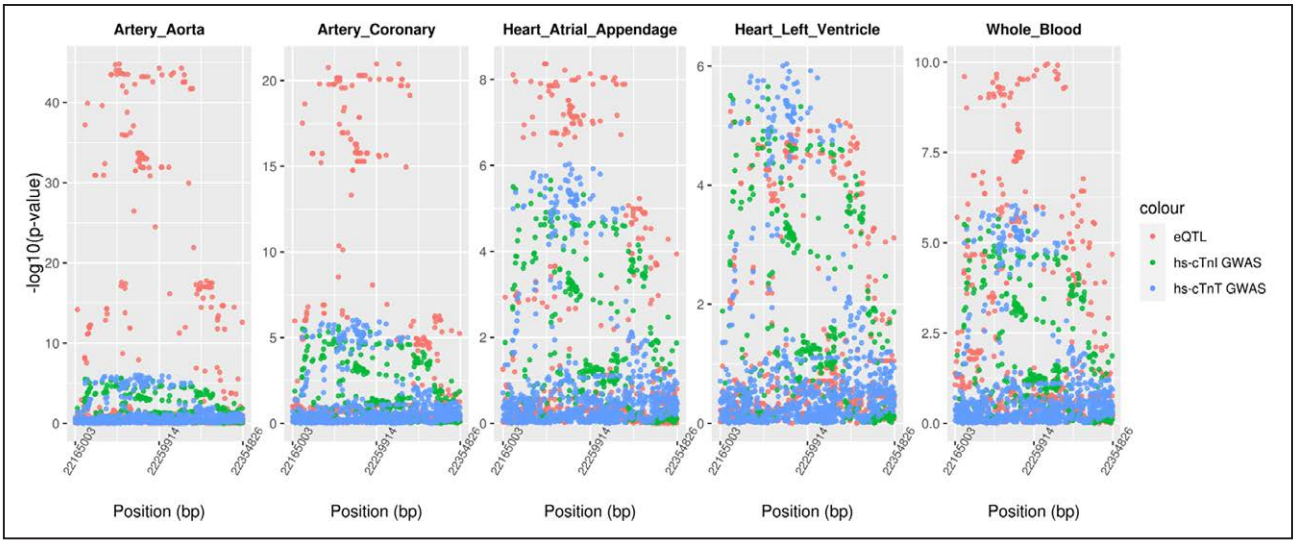

Figure 2. Scatter plot of genome-wide association studies (GWAS) and expression quantitative trait locus (eQTL) associations at *ANO5*.

SNPs located $\pm $50 kb of *ANO5* are plotted with $-\log_{10}(P)$ along the *y* axis against their genomic positions on the *x* axis. Associations for gene expression, high-sensitivity cTnT (cardiac troponin T; hs-cTnT), and high-sensitivity cTnI (cardiac troponin I; hs-cTnI) are shown in red, green, and blue points, respectively.

Phenotypic Effects of Troponin-Associated Loci

The genetic correlation between hs-cTnT and hs-cTnI was estimated to be 0.99 (P=2.00×10^{-3}). Genetic correlations with CVDs and related traits are provided in Table S12. Atrial fibrillation (AF; r=0.27; P=1.00×10^{-4}), body mass index (r=0.18; P=2.00×10^{-4}), and estimated glomerular filtration rate (r=−0.30; P=1.17×10^{-5}) were significantly genetically correlated with hs-cTnT, and heart failure was correlated with hs-cTnI (r=0.53; P=2.00×10^{-3}).

Causal Pathway From Troponins to CVDs

To establish a causal pathway from troponins to CVDs, we performed a Mendelian randomization (MR) analysis for coronary artery disease, heart failure, AF, and stroke. As shown in Figure 3, both troponins were causally associated with the risk of AF. A 1-SD higher level of inverse-normalized hs-cTnT and hs-cTnI was associated with a 32% (odds ratio, 1.32 [95% CI, 1.17–1.50]; P=1.14×10^{-5}) or a 21% (odds ratio, 1.21 [95% CI, 1.06–1.37]; P=4.72×10^{-3}) greater risk of AF, respectively. We did not observe any association with coronary artery disease, heart failure, or stroke. The MR associations of hs-cTnT for AF showed the presence of heterogeneity and horizontal pleiotropy (Table S13), so we conducted sensitivity analyses by excluding outliers identified by MR-Pleiotropy Residual Sum and Outlier. The adjusted odds ratio (95% CI) for an association between hs-cTnT and AF association was 1.38 (1.25–1.54; P=1.04×10^{-9}), consistent with the primary analysis. Scatter plots of causal estimates for different MR test methods are presented in Figure S4.

DISCUSSION

We are the first study to examine genetic determinants of hs-cTnT and hs-cTnI in a multiethnic population, and we identified novel genetic determinants and validated previous findings to improve our understanding of troponin genetic susceptibility. Beyond mapping to the nearest genes, we also showed the biological impacts of our findings using in silico functional analyses and increasingly abundant publicly available data. Our results demonstrated that multiple genetic loci were coupled with gene expression information, which imply biologically relevant pathways. Furthermore, we observed a putative causal association between troponins and AF using MR approaches. Our study provides insights into the genetic etiology of circulating troponin levels and its potential impact on CVD.

We identified a novel hs-cTnT locus at 1q32.1 mapped to *PPFIA4*. *PPFIA4* encodes liprin-alpha-1, which may regulate cell interaction with the extracellular environment. Our sentinel variant, rs3737882, is a *PPFIA4* intron variant with a deleterious effect (CADD score, 12.64). A previous multiethnic GWAS[7] has reported another gene at 1q32.1, *TNNT2*, associated with a 99th percentile dichotomized hs-cTnT trait. *PPFIA4* is a novel finding, as it lies 1.6 Mb away from *TNNT2* and the sentinel SNPs at the two genes are independent (r^2<0.01). Our study, for the first time, identified *BCL2* with genome-wide significance in hs-cTnT. *BCL2* showed only suggestive evidence in Yu et al[7] (P=6.57×10^{-6}). The sentinel variant mapped to *BCL2* was rs9944895, and we functionally confirmed its colocalization with *BCL2* expression in heart atrial appendage and left ventricular tissues (Figure S3).

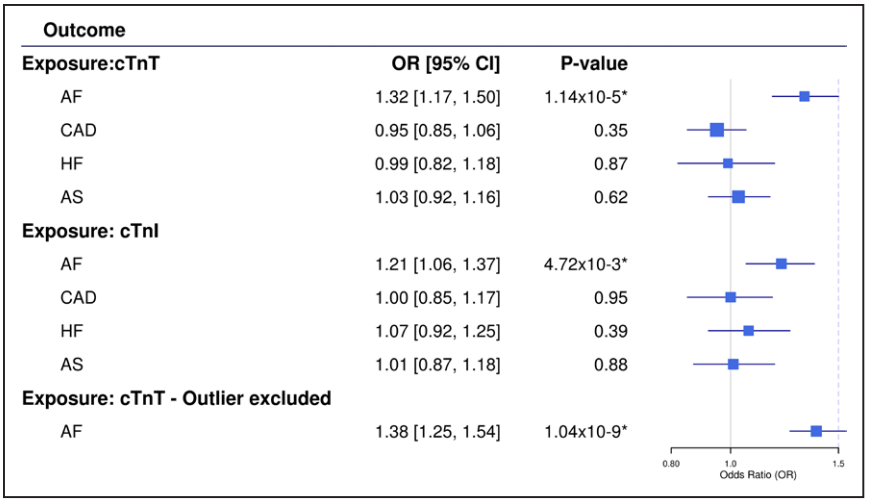

Figure 3. Forest plots for Mendelian randomization (MR) associations between troponins and cardiovascular diseases.
AF indicates atrial fibrillation; AS, all stroke; CAD, coronary artery disease; cTnI, cardiac troponin I; cTnT, cardiac troponin T; HF, heart failure; and OR, odds ratio. *Indicates significant MR association after Bonferroni adjustment for multiple testing burden.

Troponin-associated loci are involved in cardiac cell responses to oxidative stress,[9,10] which can potentially influence the devilment of AF. hs-cTnT–associated loci, *PPFIA4* and *BCL2*, are enriched in the hypoxia-mediated mechanism (Table S9). When cells are stressed by hypoxia, *PPFIA4* (liprin-alpha-4) is upregulated by a hypoxia-inducible factor HIF-1a[9] and dissociates cell contacts.[11] *PPFIA4* is specifically expressed in the brain and skeletal and cardiac muscle tissues (The Human Protein Atlas, https://www.proteinatlas.org/).[12] According to Genotype-Tissue Expression, version 8, data, *PPFIA4* is significantly overexpressed in the cerebellum and cerebellar hemispheres. The cerebellum is believed to have a unique modular structure made, including the control tower of the cardiovascular system, especially blood vessels.[13] Overexpression of *PPFIA4* in the cerebellum may potentially implicate a role for the brain in controlling the cardiovascular system under hypoxic conditions.

BCL2 encodes an integral outer mitochondrial membrane protein that inhibits the apoptotic death of cells such as lymphocytes. *BCL2* proteins have highly redundant structures indicating the evolutionary importance of apoptosis, generally implicated in the pathogenesis of many conditions including cardiac failure.[14] Apoptosis of cardiomyocytes is the major pathological change in cardiomyopathy, leading to excessive intercellular space. In vivo, decreased expression of Bcl-2 is associated with the production of reactive oxygen species,[10] which can cause arrhythmic conditions.[15] Cardiomyocytes with overexpressed PPARγ (peroxisome proliferator-activated receptor gamma) were reportedly resistant to oxidative stress–induced apoptosis, and a knockdown study suggested that *Bcl-2* upregulation mediated the protective effect of PPARγ by regulating cell's sensitivity to oxidative stress.[16] At the apex and both ventricles of the heart, 3 transplanted individuals with dilated cardiomyopathy exhibited increased *Bcl-2* expression possibly as a compensatory mechanism to the increased level of apoptosis.[17] In the colocalization analysis, we observed that the sentinel SNP, rs9944895, was associated with decreased hs-cTnT levels and an increased *BCL2* expression in heart tissues (for an additional G allele, β=–0.07 in GWAS; β=0.25 [left ventricle] and 0.38 [atrial appendage] in Genotype-Tissue Expression, version 8, eQTL data), supporting the antiapoptotic protective effect of *BCL2*. *BCL2* expression associated with hs-cTnT variants is specific to heart tissues (Figure S3), highlighting the importance of hs-cTnT as a detectable biomarker in the blood.

Two loci, *ANO5* and *BAG3*, were identified as valid pleiotropic loci for both hs-TnT and hs-cTnI, and included as genetic instruments in the MR analysis. *BAG3* encodes an antiapoptotic cochaperone protein, and variants in *BAG3* have been established as causes of dilated cardiomyopathy and myofibrillar myopathy.[18] *BAG3* interacts with the best characterized inhibitor of apoptosis, *BCL2*, in preventing cell death.[19] In hypoxia-injured cardiomyocytes, *BAG3* overexpression activated autophagy and NF-κB promoting cell proliferation and inhibiting apoptosis.[20]

ANO5 encodes a member of the anoctamin family, a transmembrane protein, and a putative calcium activated chloride channel.[21] In our study, both troponin associations are significantly colocalized with *ANO5* expression in all tissues of interest, but more significant expression is found in artery tissues taken from the left and right coronary arteries and the ascending aorta (rising from the left ventricle of the heart; Figure 2; Table S10). Few is known about the function of *ANO5* in artery, but anoctaminopathies may apply to arteries since the tunica media or a middle layer of the arterial wall contains muscular tissue.[22] The recent GWAS in a Scottish family identified *ANO5* for hs-cTnI only and stated its relevance to adult-onset cardiomyopathy.[6] An increased risk of ventricular arrhythmia has been observed in *ANO5* mutation carriers.[23]

The association between hs-cTnT, hs-cTnI, and the risk of AF has been observed repeatedly, where increased troponin levels were observed in patients with AF.[24,25] Evidence has also shown that troponin is associated with the risk of CVDs and mortality in patients with AF.[26] The underlying mechanism between troponin and AF is not

clear; however, we observed that genetically regulated high hs-cTnT and hs-cTnI levels related to increased risk of AF. The genetic instruments of troponin we used to test the potential causality with AF included *PPFIA4*, *BCL2*, *BAG3*, and *ANO5*. The novel hs-cTnT locus, *PPFIA4*, has shown genome-wide significance in the association with AF in large genome-wide studies,[27,28] suggesting shared genetic architecture between troponin and AF. The link between *PPFIA4* and AF is understudied; we suspect that hypoxia-induced cell disassociation can lead to structural remodeling of the atria, which increases the risk of AF.[29] In a canine model of congestive heart failure, the increased apoptosis (ie, the increased ratio of proapoptotic [Bax] to antiapoptotic *BCL2* expression) developed within 24 hours after the onset of tachypacing, which leads to increased cell death and leukocyte infiltration, and progressively increased AF till 5 weeks after the onset.[30] The association between *BAG3*, *ANO5*, and AF is largely unknown. Mutations in *BAG3* and *ANO5* could induce dilated cardiomyopathy,[18,23,31] and myocardial interstitial fibrosis was reported in *ANO5* knockout rabbits.[32] Cardiac troponin is a sensitive biomarker of cardiomyopathy, and elevated hs-cTnT and hs-cTnI levels are associated with myocardial fibrosis.[33,34] AF has shared pathology with cardiomyopathy[35] and myocardial fibrosis,[36] suggesting that troponin may mediate the effect of those candidate genes to AF. The potential causal relation between troponin and AF deserves further investigation.

Our study is the largest multiethnic GWAS analysis of cardiac troponins; however, it also has limitations. Due to modest sample sizes of African, Asian, and Hispanic subjects, the statistical power to detect ancestry-specific associations in these ancestries was limited. In addition, we lacked replication studies to reproduce our novel findings. Of note, we reproduced 3 previously reported loci: an intergenic region near *NCOA2* for hs-cTnT and *VCL* and *ADK* for hs-cTnI.[6,7] For our novel finding in *PPFIA4*, we were not able to get an independent study to replicate the locus. Nevertheless, for the sentinel SNP in *PPFIA4*, rs3737882, we observed homogeneous positive effect across all ancestries and statistical significance in African, Asian, and European ancestries. We anticipate our novel findings can be generalized to other populations. Lastly, AF polygenic risk score has provided prognostic information into clinical factors in risk stratification algorithms.[37] Our work can be extended by constructing a troponin polygenic risk score and integrating AF loci. Future studies are warranted to exam the added value of troponin-AF polygenic risk score in the clinical risk management.

CONCLUSIONS

In summary, we identified a novel genome-wide significant locus for hs-cTnT, rs3737882 in *PPFIA4* in a large multiethnic population. Previously reported loci were also confirmed for hs-cTnT, *BCL2* at 8q13.3 and an intergenic region near *NCOA2* at 18q21.33, and for hs-cTnI, *VCL* at 10q22.2. Pleiotropic loci for both hs-cTnT and hs-cTnI were identified at *ANO5* and *BAG3*, supported by colocalization evidence of gene expression in heart and artery tissues. MR analysis showed that hs-cTnT and hs-cTnI were causally associated with 38% and 21% higher risk of AF, respectively. Our findings provide new sights into CVD etiology and demonstrate potential clinical utility of troponin as a preventive target of AF.

ARTICLE INFORMATION

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Supplemental Material

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