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Myocardial Fibrosis and Cardiomyopathy Risk: A Genetic Link in the Multi-Ethnic Study of Atherosclerosis

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Abstract

BACKGROUND: Common genetic variants are associated with risk for hypertrophic (HCM) and dilated cardiomyopathy (DCM), and with left ventricular (LV) traits. Whether these variants are associated with myocardial fibrosis (MF), an important pathophysiological mediator of cardiomyopathy, is unknown.

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Disclosures

Authors of this work has nothing to disclose. All authors declare that the submitted work is original and has not been published and is not under consideration for publication elsewhere. The views expressed in this manuscript are those of the authors and do not necessarily represent the views of the National Heart, Lung, and Blood Institute; the National Institutes of Health; or the U.S. Department of Health and Human Services.

Supplemental Materials

Table S1-S5; Figure S1-S3

METHODS: Multi-Ethnic Study of Atherosclerosis participants with T1-mapping cardiac MRI (CMR) in-whom extracellular volume (ECV) was assessed, and genotyping information was available were included (n=1,255). Log ECV (%) was regressed on 50 candidate SNPs (previously identified to be associated with HCM, DCM and LV traits) adjusting for age, sex, diabetes, blood pressure and principal components of ancestry. Ancestry-specific results were pooled by fixed-effect meta-analyses. Gene knockdown (KD) experiments were performed in human cardiac fibroblasts (HCF).

RESULTS: The *SMARCB1* rs2186370 intronic variant (minor allele frequency: 0.18 in Whites, and 0.50 African Americans), previously identified as a risk variant for DCM and HCM, was significantly associated with increased ECV (p=0.0002) after adjusting for confounding clinical variables. The *SMARCB1* rs2070458 locus previously associated with increased LV wall thickness and mass, was similarly significantly associated with increased ECV (p=0.0002). The direction of effect was similar in all four ancestry groups, but the effect was strongest in African Americans. The variants are strong expression quantitative loci (eQTLs) in human LV tissue and associated with genotype-dependent decreased expression of *SMARCB1* ($p=7.3\times10^{-22}$). *SMARCB1* KD in HCFs resulted in increased TGF-β1 mediated α-smooth muscle actin and collagen expression.

CONCLUSION: Common genetic variation in *SMARCB1* previously associated with risk for CM and increased LV wall thickness is associated with increased CMR-based MF and increased TGF-β1 mediated MF in-vitro. Whether these findings suggest a pathophysiologic link between MF and CM risk remains to be proven.

Keywords

Fibrosis; genetic; cardiomyopathy; polymorphism; SMARCB1

INTRODUCTION

Cardiomyopathies (CM) are the most common Mendelian inherited cardiovascular diseases (CVD), with a prevalence of 1 in 200 to 500 individuals in the general population.[1,2] Although rare pathogenic or likely pathogenic variants in structural sarcomere or sarcomere associated genes could account for 20–50% of cardiomyopathy cases, it has been well established that common genetic variants with smaller effect sizes also contribute to the risk and pathogenesis of cardiomyopathy.[3,4] Genome-wide association studies have identified such common genetic variants associated with both hypertrophic (HCM) and dilated cardiomyopathy (DCM).[5,6] Further cross-trait analyses of these candidate variants with left ventricular (LV) traits have broadened our understanding of the disease.[6] However, the functional significance of these common genetic variants that predispose patients to the development of CM remains unknown.

Myocardial fibrosis (MF) is a prominent finding in cardiomyopathic hearts and plays an important role in myocardial dysfunction that characterizes CM. MF can appear long before the process of anatomical cardiac remodeling and sometimes represent its only initial manifestation.[7] Indeed, such a profibrotic state characterized by dysregulation of fibroblast physiology or other injurious factors that precipitate the fibrotic process maybe

one of the initiating steps in the process of CM development as demonstrated by cardiac MRI (CMR).[8] Whether genetic variation mediates this fibrosis process resulting in the variable disease penetrance observed in CM is unknown. Although endomyocardial biopsy is the gold-standard for detection of MF, determination of extracellular volume (ECV) using contrast-enhanced CMR is a reproducible and validated non-invasive technique to quantify MF.[9] T1 times measured by this T1 mapping method are inversely and strongly correlated with histologic fibrosis detected through endomyocardial biopsy.[10] Tissue characterization by T1 mapping serves as a sensitive, therapeutic and prognostic marker at early stages of many cardiac pathologies enabling effective patient care.[11,12] ECV, unlike Native T1, is a more accurate index of extracellular expansion that is less affected by non-fibrotic processes, and has been shown to be a reliable surrogate marker for earlier stages of cardiac remodeling[12–15] that is affected by fibrosis including in cardiomyopathy.[16]

Determining the functional significance of common genetic variation that predisposes individuals to CM, may help provide pathophysiological insights that may explain the inter-individual variability in risk of developing CM. To explore the association of these risk variants with CM relevant biological traits such as MF, we conducted a cross-trait analysis on the shared genetic pathways between CM and LV traits with interstitial MF detected by contrast enhanced CMR in the Multi-Ethnic Study of Atherosclerosis (MESA) using ECV as the phenotype.

METHODS

Study Population and Participant Selection

The data other than that from MESA that support the findings of this study are available from the corresponding author upon reasonable request and release of data related to MESA will be governed by that study's rules and regulations. MESA was approved by the institutional review boards of each of the participating field sites in the United States (Wake Forest University, Winston-Salem, NC; Columbia University, New York City, NY; Johns Hopkins University, Baltimore, MD; University of Minnesota, Minneapolis, MN; Northwestern University, Evanston, IL; and University of California, Los Angeles, CA), and all participants provided written informed consent. All sites were compliant with the Health Insurance Portability and Accountability Act. The MESA began in the year 2000 recruiting 6,814 individuals aged 45–84 years with no history of CVD. The MESA cohort included White, African American, Hispanic, and Chinese American participants from six US field centers.[17] In the fifth MESA exam (2010–11), 2,124 participants had T1 mapping cardiac MRI studies, 1,345 of whom accomplished contrast-enhanced phase. Participants with a history of myocardial infarction (MI) or heart failure (HF) at the time of cardiac MRI were excluded to filter out participants with potential replacement fibrosis. Among these, 1,255 participants had genotyping information available with ECV phenotype measures. There were 1,954 subjects with Native T1 analysis and genotyping results available.

Myocardial Fibrosis Measurement

The cardiac MRI protocol for the quantification of MF including T1 mapping in MESA was explained elsewhere.[18] ECV fraction derived from T1-mapping CMR were used as direct

surrogates of interstitial MF. In T1 mapping method, the T1 times at pre-contrast (native) phase and 12- and 25-minute post gadolinium contrast injection are estimated through a single breath-hold ECG-synchronized Modified Look-Locker Inversion recovery approach. ECV fraction is calculated using pre- and post-contrast T1 times and partition coefficient. Native T1 time a more commonly used but less specific marker of fibrosis as compared to ECV was also measured. Further information on CMR analyses is available in the literature.[11,18]

Genotyping

Genotyping array data and imputed genotypes for 6,364 individuals that passed quality control was obtained from the National Heart, Lung, and Blood Institute SNP Health Association Resource (SHARe) project.[19] Briefly, participants were genotyped on the Affymetrix 6.0 array, with 934969 SNPs available. Genotypes were called using the Birdseed algorithm and monomorphic SNPs, those failing Hardy-Weinberg with a p-value<0.001, and those with per SNP call rates<0.97 or call rates per sample <0.97 were removed for QC purposes. Genotype imputation was performed using the Michigan Imputation Server with Minimac4 and TOPMED r2 (Version r2 2020) reference haplotype panel. Pre-imputation QC was conducted using the “HRC/1KG Imputation Preparation and Checking Tool” available through the Michigan Imputation Server. The imputation for each MESA ethnic group (European, Chinese, African American and Hispanic) was performed separately.

Relatedness

Kinship coefficients between pairs of participants within each racial group were estimated using KING v2.2.6 software from imputed SNPs with MAF>5% where regions of local influence (2q21/LCT, HLA, 8p23, and 17q21.31) were removed and linkage disequilibrium (LD) thinned.[20] Pairs of individuals with a kinship coefficient >1/16 were considered related and within the same family (maximum family size was 3). One participant was chosen among each cluster of related participants at random resulting in the exclusion of 20 participants due to relatedness.

Population Stratification

To address possible population stratification, principal components (PCs) were computed by ancestral background based on the same LD thinned SNPs with MAF>5% used for kinship coefficient calculations using PC Analysis (PCA) implemented in the SNPRelate R package.[21,22] Except for the PC1 being associated with ECV in Chinese Americans (p=0.008), there was no significant population stratification within the ancestries in the first 10 PCs.

Candidate SNPs

To determine whether cardiac fibrosis plays a role in the risk for developing CM we sought to determine the association of cardiomyopathy risk loci and LV trait variants with ECV determined by MRI. SNPs found to be previously associated with risk for HCM (n=16), DCM (n=13) and LV morphologic traits (n=24) were selected from recent literature[6]

(Table S1). The LV trait SNPs were associated with LV end-diastolic volume (LVEDV), end-systolic volume (LVESV), ejection fraction (LVEF), mass (LVM), concentricity (LVM/LVEDV), mean wall thickness, and global peak radial (GRS), longitudinal (GLS) and circumferential (GCS) strain. Two SNPs were common for HCM and DCM and one SNP overlapped with DCM and LV traits, resulting in a total of 50 SNPs in our candidate list. (Table S1).

Functional Genomics

Expression quantitative trait loci (eQTL)—To determine whether genetic variants were eQTL, data from the Genotype-Tissue Expression (GTEx) Portal was queried in June 2023 (https://gtexportal.org/). The GTEx project, is a resource database funded by the National institutes of Health and has an associated tissue bank for the scientific community to study the relationship between genetic variation and gene expression in various human tissues.

Cell culture, siRNA transfection and treatment—Cryopreserved adult human cardiac fibroblasts (HCF) (Catalog#: 306V-05a) were purchased from Cell Applications, Inc. (San Diego, CA). Cell culture and subculture were performed following a standard protocol as follows. In brief, primary cells were cultured with HCF Growth Medium (Catalog#: 316–500, Cell Application) in a 37°C, 5% CO2 humidified incubator. Culture medium were changed every 48 hrs. Cells were subcultured when HCFs were 80% confluent using the Subculture Reagent Kit (Catalog#: 090K, Cell Application). HCFs at passage 2 to passage 4 were used for in vitro experiments.

RNA sequencing.—Total RNA was extracted from HFCs with TRIzol® Reagent (ThermoFisher) and the miRNAeasy kit (QIAGEN) per the manufacturer's instructions. RNA quality control was performed before RNA-seq, which showed that the RNA integrity number (RIN) was ≥9 for all samples. RNA-seq libraries were generated using the Illumina TruSeq RNA Library Prep Kit v2 (Illumina, San Diego, CA). Paired-end sequencing 2×100bp was performed on an Illumina HiSeq 4000 with approximately 50 million fragment reads per sample. Each sample was sequenced in duplicate. RNA sequencing quality was determined with FastQC (Babraham Institute, Cambridge, UK), and the reads were aligned to hg38 using STAR.7 Raw counts were generated with featureCounts.

SMARCB1 and MMP11 knock-down (KD) with TGF-β1 stimulation—KD in HCF was carried out via transient transfection of ON-TARGETplus human SMARCB1 siRNA (Catalog#: L-010536, Dharmacon, Lafayette, CO), ON-TARGETplus human MMP11 siRNA (Catalog#: L-005953, Dharmacon, Lafayette, CO) or ON-TARGETplus Human CTRL siRNA (Catalog#: L-005834) using the Lipofectamine™ RNAiMAX Transfection Reagent (ThermoFisher, 13778075). Recombinant Human TGF- β1 Protein (R&D system, 7754-BH) was reconstituted at 100 μg/mL in sterile reconstitution buffer 4 (BSA/HCL, R&D system, RB04). In brief, when the HCFs reached ~80% confluent, HCFs Growth Medium was replaced by Fibroblast Medium-2, basal (FM-2, Catalog#: 2331-b, ScienCell Research Laboratory, Carlsbad, CA) for 24 hrs serum-free starvation, meanwhile siRNA was transfected into HCFs to KD *SMARCB1, MMP11* and control. After 24 hrs starvation

and transfection, 10ng/mL of TGF-β1 or vehicle (reconstitution buffer) in FM-2 (1% FBS) were applied. After another 48 hrs, stimulated HCFs were lysed in a cell lysate buffer for RNA/protein extraction.

Real-time polymerase chain reaction—HCFs were lysed in a cell lysate buffer. Total RNA was extracted using Quick-RNA Microprep Kit (Zymo Research, R1051). Reverse transcript-PCR and real-time PCR were completed in one step using Power SYBR™ Green RNA-to-CT™ 1-Step Kit (Applied Biosystems™, 50–591-795) on a StepOneTM system (Applied Biosciences). Ct values were standardized to 'housekeeping' gene, GAPDH, and relative quantification calculated using ΔΔCt methodology.

Western blot—Quantification of protein by Western blot was carried out using standard techniques and the following primary and secondary antibodies: anti-αSMA (A5228, Sigma-Aldrich, dilution: 1:1,000), anti-Collagen I (72026, Cell Signaling, dilution:1:1,000), anti-SMARCB1 (91735, Cell Signaling, dilution:1:1,000), anti-GAPDH (Cell Signaling, dilution: 1:2,000) and species-specific HRP conjugated secondary antibodies (GE Healthcare). ECL Advanced Western blot detection reagent (34075, ThermoFisher Scientific) was applied to the membrane and signal intensity measured on a Gel Doc Imager (BioRad). Data were normalized to GAPDH expression.

Statistical Analysis

Ancestry stratified linear regression models were used to model the effect of SNPs (as additive count of allele) on log (ECV) and Native T1 time in unrelated individuals adjusting for: age, sex and the first 5 PCs. The additive genetic SNP coefficients were then combined across the four racial groups, pooling effects using an inverse variance weighted fixed effects meta-analysis implemented in METASOFT (v2.0.1).[23,24] The significance level for this study was set at p value cutoff of 0.001 based on Bonferroni correction for 50 SNPs. An additional analysis adjusting for: age, sex, 10 PCs, SBP, DBP, diabetes and current smoking was conducted for SNPs found statistically significant at the Bonferroni threshold. As well, model sensitivity analyses were conducted without adjustments of age and or PCs; with additional adjustment of 10 or 15 PCs; none of these analyses made any meaningful change to the results. A sensitivity analysis of the meta-analysis method was conducted using the random-effects meta-analysis method of Han and Eskin's but was not found to contribute more to the analysis.[23] For analyzing the functional genomics experimental results statistical analyses were performed using GraphPad Prism 9 (GraphPad). Two-tailed T-test was used for single comparisons.

RESULTS

Population Characteristics

A total of 1,255 participants (51.6% male), including 663 (52.8%) Whites, 275 (21.9%) African Americans, 142 (11.3%) Chinese American, and 175 (13.9%) Hispanics were included in this study. The prevalence of diabetes mellitus was 24.4% in African Americans, 11% in Whites, 15.5% in Chinese American and 20.6% in Hispanics. Median ECV fraction was 27.1% (IQR: 3.6) in African Americans, 26.9% (3.7) in Whites, 26.3% (2.8) in Chinese,

and 26.5% (3.6) in Hispanics. Population characteristics and cardiac MRI data are shown in Table 1.

Cardiomyopathy and LV Trait Alleles and ECV

Cardiomyopathy related SNPs and their association with ECV in the study cohort are shown in Tables 2–4. One shared SNP among the DCM and HCM related variants, rs2186370, an intronic variant in *SMARCB1*, was significantly associated with ECV (p=4.09e-04) (Tables 2–3). The frequency of the A risk allele was 0.18 in Whites, and 0.50 in African Americans of this study cohort). The A allele was associated with 1.6% increase in ECV (95% CI: 0.7–2.5) accounting for sex and first 5 PCs (Table 2). The association of the A allele with ECV was strongest in the African American sub-population (1.9% increase in ECV), although the associations were in the same direction of effect for all four ancestries after adjustment for 10 PCs and for confounding variables including age, sex, diabetes mellitus, blood pressure and tobacco use (Table 5).

Another *SMARCB1* intronic LV trait associated variant, rs2070458, was also associated with ECV (p=0.0004) (Table 4). According to the 1000 Genomes Project, this variant is in near complete linkage disequilibrium (R^2 >0.998 in all races) with rs2186370 described above. With an equivalent effect size to rs2186370, the A allele was associated with a 1.6% increase in ECV (95% CI:0.7–2.5). This A allele was previously reported in the UK Biobank among other LV traits to be significantly associated with increased LV concentricity and wall thickness. The population frequency of this allele was similar to that of rs2186370 in this cohort. Similarly, its association with ECV was strongest in African Americans, and the effect was in the same direction for all four ancestries after adjustment for 10 PCs and for confounding variables including age, sex, diabetes mellitus, blood pressure and tobacco use (Table 5).

The association of CM risk and LV trait related genetic variants with Native T1 time was not significant as outlined in Tables S2–4.

eQTL in LV Tissue

Based on data from GTEx, the rs2186370 variant is a strong eQTL for the *SMARCB1* gene in human myocardial tissue, including the left ventricle and atrial appendage. The variant allele “A” was significantly associated with decreased mRNA levels for *SMARCB1* (p=7.3e-22) in LV myocardial tissue (Figure S1). In addition, the rs2186370 variant was also an eQTL for *MMP11* and *VPREB3* in left ventricular tissue. The variant allele “A” was significantly associated with decreased and increased expression for *MMP11* (p=2.5e-24) and *VPREB3* (p=1.9e-8) respectively (Figure S1). However, *VPREB3* is not expressed in the left ventricle as quantified by RNA-seq in GTEx (mean RNA level was <0.5 TPM versus 19.24 for *SMARCB1* and 3.35 for *MMP11*). Moreover, based on single cell RNA-sequencing data acquired from left ventricular tissue[25] and HCF (Figure S2), *VPREB3* is not expressed in HCF. Therefore, further functional genomic studies of *SMARCB1* and *MMP11*, and not *VPREB3*, were pursued in the HCF cell model.

SMARCB1 Mediates TGF-β1 Induced MF

To evaluate the functional significance of rs2186370 and its resultant effect of decreased expression of its associated genes *SMARCB1* and *MMP11* in fibrosis, we knocked down (KD) their expression in HCFs using siRNA. Our results indicate that HCFs treated with TGF-β1 (10 ng/ml) for 48 hrs, result in significantly increased expression of α-smooth muscle actin (α-SMA, ACTA2) and collagen I (COL1A1) (Figure 1, Table S5). The expression of *SMARCB1* itself is also downregulated with TGF-β1 treatment. In addition, *SMARCB1* KD in HCFs resulted in increased TGF-β1 mediated fibrosis indicated by increased expression of α-smooth muscle actin and collagen. However, *MMP11* KD did not signifcantly attenuate TGF-β1 mediated induction of α-SMA and collagen I (Figure S3).

DISCUSSION

Our study demonstrates the association of cardiomyopathy risk loci with increased MF as determined by MRI determined ECV and in vitro fibrosis (Figure 2). We also demonstrate the novel association of SMARCB1 with MF, decreased expression of which associated with increased MF. We therefore demonstrate the functional significance of common genetic variation identified by prior GWAS that predisposes patients to CM and relevance of its effect by ancestral background. *SMARCB1* variants previously associated with CM risk, increased LV mass and wall thickness, were associated with increased ECV especially in African Americans. Furthermore, we identified that this variant is eQTL for or decreased expression of *SMARCB1* and decreasing the expression of *SMARCB1* enhanced TGF- β1 mediated MF in-vitro.

There are multiple reports of the role of MF preceding the development of HCM, implicating MF as an important pathophysiological mediator of HCM.[7,8] MF can be a subclinical finding in HCM or sometimes it is the only pathophysiological manifestation as reflected by increased serum C-terminal pro-peptide of type I procollagen levels in HCM mutation carriers who do not yet have imaging evidence of hypertrophy.[7,8] We have also previously demonstrated that carrier status of rare pathogenic variants in CM-related genes in the MESA cohort who have not yet developed CM is more prevalent in individuals with CMR-detectable MF as compared to individuals with lower MF.[26] Although the prognostic role of MF has been well established in DCM,[27] its role in the risk for DCM has not been defined.

The alternate alleles of the rs2186370 intronic variant identified in our study, A and G, are risk alleles for HCM and DCM, respectively.[6,28] According to GnomAD, the overall frequency of the A allele is 0.31, with the highest AF in African-ancestry (53%) and lowest in European-ancestry populations (20%). We found that the A allele was associated with increased interstitial MF with a more pronounced effect in African Americans. This finding suggests the importance of MF, more so in African Americans given the risk variant's higher prevalence and effect size in that population. Consistent with this finding, clinically African Americans with HCM are younger, have more severe disease and a higher prevalence of MF than European-ancestry patients.[29,30] Conversely, the association of the alternate allele G that is predictive of DCM risk, was associated with decreased ECV, suggesting that

increased MF mediated by this genetic mechanism may not play a primary role in MF associated with DCM.

The other intronic variant in *SMARCB1*, rs2070458, [6] has previously been identified as a proxy SNP (r^2=0.85) to the UK biobank sentinel variant, rs6003909, a regulatory region variant to *SLC2A11* and *DERL3* in the same cytogenetic location. The latter is a unique sentinel variant for LV mass to volume ratio and is also associated with *MMP11* expression.[31] We however demonstrate that decreased *MMP11* expression in HCF did not attenuate TGF-β1 mediated fibrosis.

The rs2186370 “A” allele was also associated with decreased *SMARCB1* expression in human LV tissue and we demonstrate that *SMARCB1* KD is profibrotic, consistent with our finding that the A allele is associated with increased ECV. SMARCB1 plays a key role in cell-cycle control and causes cell cycle arrest in G0/G1.[32,33] It is a core component of the BAF (hSWI/SNF) chromatin-remodeling complex which plays an important role in cell proliferation and differentiation.[34,35] In fetal lung fibroblast transcriptome studies, SMARCB1 has been shown to be associated with inflammatory pathways and binds to the interleukin-6 (IL6) promoter repressing it expression.[36] IL6 levels have been associated with the development of new-onset HF and MF.[37,38] The potential direct role of SMARCB1 in modulating TGF-β mediated MF was not known until this study.

Our study had several limitations. Our results demonstrate a functional association of cardiomyopathy risk loci with MF but the role of MF in the development of CM remains to be proven. The smaller number of participants of certain ancestries such as Chinese and Hispanics in this study population confines our significant findings to those with European or African ancestry. However, the direction and magnitude of the effect measures are similar in all ancestral/ethnic groups. The prevalence of pathogenic/likely pathogenic CM related genetic variants in MESA was small (0.5%) and therefore given their extremely low prevalence our overall results are not likely to be affected by these Mendelian variants. Although African Americans in our study population had a higher prevalence of risk factors of MF such as diabetes mellitus, hypertension, and smoking, these potentially confounding variables were adjusted for our analysis thus demonstrating an independent association of these cardiomyopathy risk alleles with MF. Finally, the effect size of the variants on ECV is relatively small. However, many common but functional variants in genes that encode targets of approved drugs have modest effect on the phenotype.[39] A specific example is the *HMGCR* locus which encodes HMG-CoA reductase, the target of statin therapy. Functional variants in *HMGCR* have a small effect on LDL levels, yet statins substantially reduce LDL with significant clinical impact. Unlike their association with ECV, none of the genetic variants examined were associated with Native T1 time which is another MRI surrogate measure of fibrosis. However Native T1, although easier to measure and does not require the use of contrast, is not as accurate as ECV in determining interstitial fibrosis since it is affected by intracellular, vascular and other processes[40] that do not involve fibrosis such as inflammation and volume status.[41] In addition, the finding of a significant association of an increased LV mass and LV wall thickness genetic variant with increased ECV that was not observed with Native T1 also validates that ECV indeed more accurately reflects the interstitium.

In summary, our study demonstrates a functional association between common *SMARCB1* cardiomyopathy risk variants and MF. The possible pathophysiologic significance of MF in the development of CM and its role as a potential mechanistic target in understanding the variable penetrance of CM needs to be further explored especially in African Americans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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NONSTANDARD ABBREVIATIONS AND ACRONYMS

CM	cardiomyopathies
HCM	hypertrophic
DCM	dilated cardiomyopathy
LV	left ventricle
ECV	extracellular volume
MF	myocardial fibrosis
MESA	Multi-Ethnic Study of Atherosclerosis
MI	myocardial infarction
HF	heart failure
AF	allele frequency
LD	linkage disequilibrium
PCs	principal components
LVEDV	LV end-diastolic volume
LVESV	end-systolic volume

LVEF	ejection fraction
GRS	global peak radial
eQTL	expression quantitative trait loci
GTEx	Genotype-Tissue Expression
HCF	human cardiac fibroblasts

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CLINICAL PERSPECTIVE

What is new?

- Common genetic variants in two linked *SMARCB1* loci that were previously associated with cardiomyopathy risk and increased left ventricular (LV) wall thickness, were found to be significantly associated with CMR-based interstitial myocardial fibrosis (MF) in the Multi-Ethnic Study of Atherosclerosis cohort, especially in African Americans.
- These genetic variants are associated with significantly decreased expression of *SMARCB1* in human LV tissue.
- Decreased expression or knockdown of *SMARCB1* in turn results in increased TGF-β1 mediated α-smooth muscle actin and collagen expression or in-vitro fibrosis as demonstrated by functional genomic studies using human cardiac fibroblasts.

What are the clinical implications?

- Known common genetic variation associated with cardiomyopathy risk and increased LV wall thickness in *SMARCB1* is associated with MF.
- Whether MF predisposes patients to the development of cardiomyopathy and targeting *SMARCB1* is a potential therapeutic strategy to attenuate MF and the risk for developing cardiomyopathy remains to be proven.

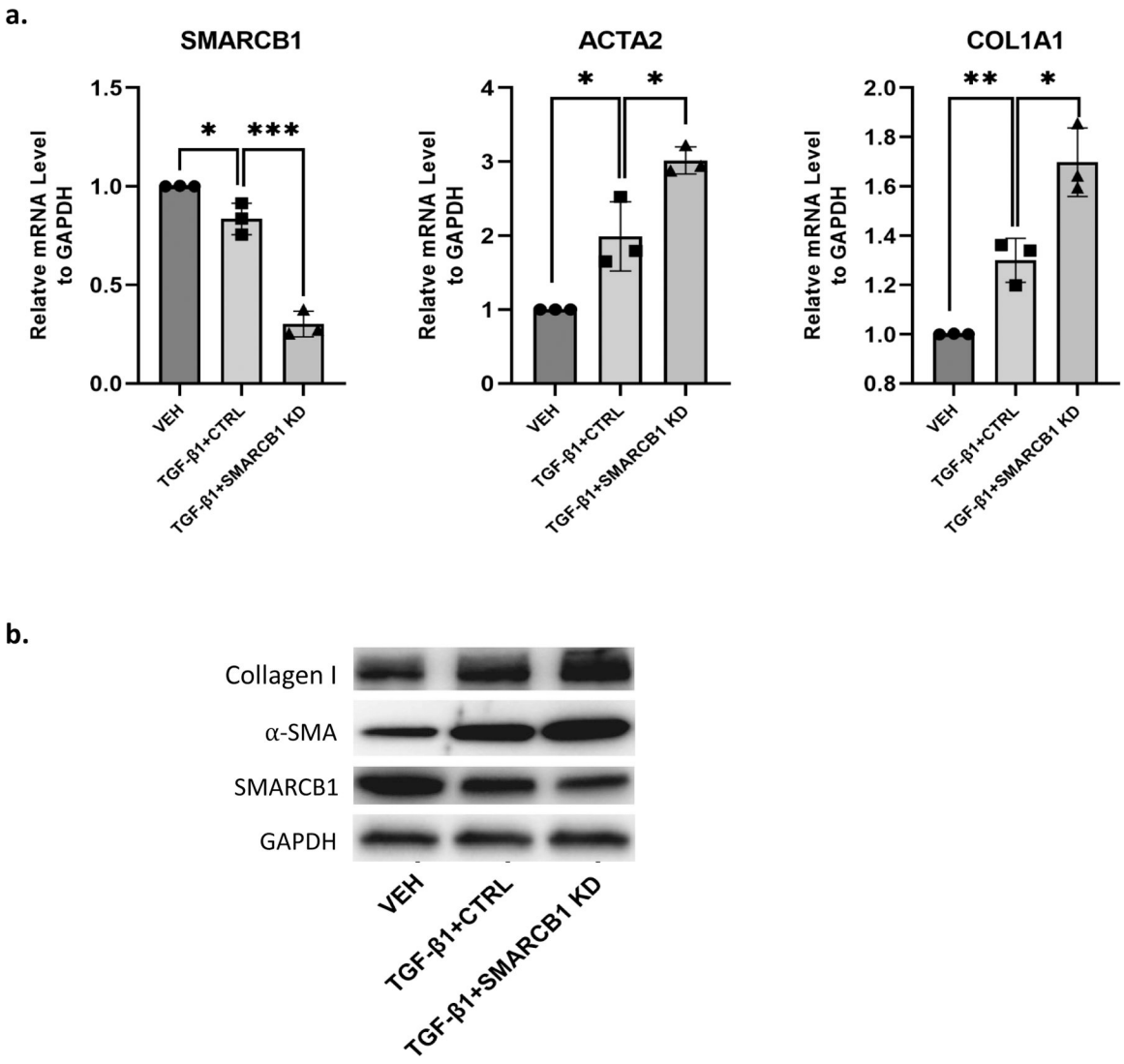

Figure 1.

SMARCB1 KD increases expression of profibrotic genes induced by TGF-β1 treatment. a) Quantitative RT-PCR analysis of *SMARCB1, ACTA2* (α-SMA) and *COL1A1* (collagen I) after being treated by vehicle/TGF-β1 (*SMARCB1* KD/CTRL); b) Western blot (performed in triplicate) demonstrating similar changes in protein expression of SMARCB1, ACTA2 and COL1A1. For statistical analysis, a paired two-tailed *t*-test was used. $*p<0.05$, $**p<0.01$, $***p<0.001$.

VEH, Vehicle, CTRL, Control, KD, Knock-down, α-SMA, α-smooth muscle actin

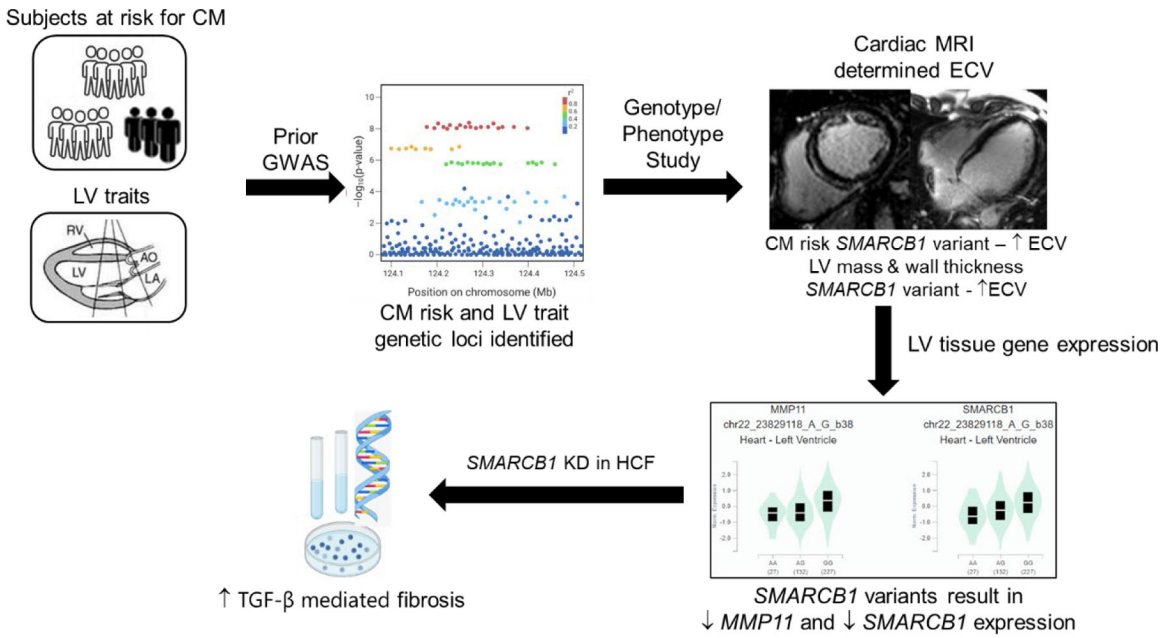

Figure 2.

Study schema with results describing the link between cardiac fibrosis and development of cardiomyopathy. A genotype-phenotype association study using previously identified and validated 50 genetic loci determining cardiomyopathy risk and LV traits was performed with cardiac MRI determined ECV as a phenotype. *SMARCB1* variants that are known to be associated with increased LV mass, LV wall thickening and cardiomyopathy risk were significantly associated with increased ECV. These variants result in decreased *SMARCB1* and *MMP11* expression in LV tissue and knockdown of *SMARCB1* results in increased in-vitro cardiac fibrosis.

RV, right ventricle, AO, aorta, LA, left atrium, LV, left ventricle, CM, cardiomyopathy, ECV, extracellular volume, GWAS, genome wide association study, MRI, magnetic resonance imaging, HCF, human cardiac fibroblasts, KD, transforming growth factor

Table 1.

Study population characteristics stratified by ancestry.

	African American (n=275)	White (n=663)	Chinese (n=142)	Hispanic (n=175)
Male gender	138 (50.2%)	335 (50.5%)	74 (52.1%)	101 (57.7%)
Age (years)	67 (60,74)	67 (61,75)	63 (59,72)	65 (59,73)
BMI (kg/m^2)	28.9 (26,33)	27.1 (24.1,30.1)	23.9 (22,26.5)	28.9 (26.5,31.3)
DBP (mmHg)	69.5 (63.5,76)	67.5 (60.8,74)	69.5 (62.1,74.9)	68.5 (62,74.2)
SBP (mmHg)	122.5 (113.5,140)	116.5 (106.5,132)	113.8 (104.6,127.9)	118 (107.5,132)
Diabetes	66 (24.4%)	73 (11%)	22 (15.5%)	36 (20.6%)
Current smoker	35 (12.8%)	48 (7.3%)	7 (4.9%)	9 (5.2%)
Estimated GFR (mL/min)	74.6 (65.8,84.4)	66.7 (59.1,73.9)	71.9 (65.8,80)	72.8 (65,77.5)
Total Cholesterol (mg/dl)	177 (158,201)	181 (157,206)	190.5 (161.2,217.5)	176 (158.5,200.5)
ECV fraction (%)	27.1 (25.5,29.1)	26.9 (25.2,28.9)	26.3 (25.3,28.1)	26.5 (24.7,28.3)
LV end-diastolic mass (g)	129.7 (110.4,157.7)	119.5 (97.2,146.2)	106 (92.2,127.2)	123.5 (105.7,144.7)
LV end-diastolic volume (mL)	126.7 (105.2,151.2)	120.3 (100.8,145.6)	110.7 (95.2,123.4)	118.2 (102.6,141.3)
LV ejection fraction (%)	60.3 (55.2,66)	61.7 (57.3,66.2)	65 (61,68.9)	63.7 (59,67.2)
LV end-systolic volume (mL)	47.9 (38.1,63.1)	45.2 (35.8,58.3)	37.9 (31.4,45.9)	42.8 (34.4,54.3)
Global Circumferential Strain	−16.7 (−19,−15.2)	−18.1 (−19.8,−16)	−19.5 (−21.4,−17.8)	−17.9 (−19.8,−16.2)

BMI, body mass index, SBP, systolic blood pressure, DBP, diastolic blood pressure, GFR, glomerular filtration rate, LV, left ventricular. values are median (25th, 75th %tile) or N (%).

Table 2.

MESA ECV association results for SNPs predictive of HCM.

				Allele Frequency				Meta-analysis	
RSID	**Chr**	**Position (GRCh38)**	**HCM Risk Allele**	**African American**	**Caucasian**	**Chinese**	**Hispanic**	**Difference in log(ECV) per HCM Risk Allele (95%CI)**	**p-value**
rs10927886	1	16012818	G	0.28	0.41	0.19	0.42	0.0031 (−0.0053,0.0115)	0.4688
rs11196078	10	112728053	A	0.36	0.25	0.47	0.33	0.0037 (−0.0048,0.0121)	0.3966
rs17099139	10	119659975	G	0.29	0.29	0.07	0.18	−0.0009 (−0.01,0.0083)	0.8557
rs3740293	10	73646383	C	0.35	0.14	0.22	0.14	−0.0041 (−0.0143,0.006)	0.4239
rs11073729	15	84806850	C	0.27	0.49	0.23	0.42	−0.0031 (−0.0114,0.0053)	0.4719
rs9928278	16	2102650	C	0.47	0.18	-	0.15	0 (−0.0107,0.0106)	0.997
rs1378358	17	46709946	T	-	0.19	-	0.16	−0.0133 (−0.0256,−0.0009)	0.036
rs9892651	17	66307675	T	-	-	-	-	-	-
rs503274	18	36673782	C	0.23	0.3	0.2	0.43	0.0009 (−0.0078,0.0095)	0.8425
rs2186370	**22**	**23829118**	**A**	**0.5**	**0.18**	**0.43**	**0.24**	**0.0157 (0.0069,0.0246)**	**0.0005**
rs9647379	3	172067378	C	0.13	0.38	-	0.44	0.0046 (−0.0049,0.0142)	0.3428
rs4385202	5	139407567	A	0.31	0.33	0.32	0.36	−0.0011 (−0.0096,0.0074)	0.7975
rs2191445	5	57715642	T	0.07	0.19	0.07	0.09	−0.0076 (−0.0193,0.004)	0.2
rs12212795	6	118333145	C	-	-	-	-	-	-
rs66761782	6	36668303	C	0.28	0.25	0.18	0.19	−0.0026 (−0.0119,0.0067)	0.5853
rs60871386	7	128790383	T	0.08	0.1	0.19	0.06	0.0015(−0.0114,0.0145)	0.8182

HCM, hypertrophic cardiomyopathy, Chr, chromosome, ECV, extracellular volume. SNPs with low imputation quality in each ancestral group of this study were not analyzed (blank cells).

Note: Missing values indicate certain SNPs that were not included in the analysis because they were not present in the TOPMED imputation reference panel, they had poor imputation quality and were rare in the population with phenotype information available.

Table 3.

MESA ECV association results for SNPs predictive of DCM.

				Allele Frequency				Meta-analysis	
RSID	**Chr**	**Position (GRCh38)**	**DCM Risk Allele**	**African American**	**Caucasian**	**Chinese**	**Hispanic**	**Difference in log(ECV) per DCM Risk Allele (95%CI)**	**p-value**
rs10927875	1	15972817	C	0.2	0.34	-	0.3	0.0026 (−0.0068,0.012)	0.5854
rs2234962	10	119670121	T	-	0.21	-	0.09	−0.0029 (−0.0152,0.0094)	0.6448
rs2182400	10	29418622	G	0.24	0.24	0.44	0.4	−0.0009 (−0.0096,0.0079)	0.8479
rs1051168	15	84657289	T	0.05	0.27	0.15	0.17	0.002 (−0.008,0.012)	0.6984
rs9892651	17	66307675	C	-	-	-	-	-	-
rs2303510	18	36744128	G	0.41	0.31	0.24	0.37	−0.0014 (−0.0097,0.0069)	0.7455
rs2042995	2	178693639	T	0.45	0.25	0.43	0.36	0.0034 (−0.0051,0.0118)	0.4374
rs2186370	**22**	**23829118**	**G**	**0.5**	**0.18**	**0.43**	**0.24**	**-0.0157 (−0.0246,−0.0069)**	**0.0005**
rs6807275	3	14232951	G	0.13	0.32	0.31	0.22	0.0015 (−0.0072,0.0103)	0.7317
rs4713999	6	36665292	G	0.37	0.36	0.3	0.27	0.001 (−0.0075,0.0096)	0.8172
rs2291569	7	128848680	G	-	0.1	0.11	-	−0.0157 (−0.031,−0.0003)	0.0453
rs13265989	8	11922131	G	0.12	0.26	-	0.19	−0.0001 (−0.0103,0.0101)	0.9788
rs12541595	8	124845117	G	0.1	0.28	0.46	0.3	0.0008 (−0.0082,0.0097)	0.8695

DCM, dilated cardiomyopathy, Chr, chromosome, ECV, extracellular volume. SNPs with low imputation quality in each ancestral group of this study were not analyzed.

Note: Missing values indicate certain SNPs that were not included in the analysis because they were not present in the TOPMED imputation reference panel, they had poor imputation quality and were rare in the population with phenotype information available.

Table 4.

MESA ECV association results for SNPs predictive of 9 LV traits.

				Allele Frequency				Meta-analysis	
RSID	**Chr**	**Position (GRCh38)**	**Effect Allele**	**African American**	**Caucasian**	**Chinese**	**Hispanic**	**Difference in log(ECV) per Effect Allele (95%CI)**	**p-value**
rs9428221	1	115757516	A	0.16	0.49	0.3	0.32	0.0024 (−0.0062,0.0109)	0.5904
rs945425	1	16021917	T	0.25	0.34	0.06	0.42	−0.004 (−0.013,0.005)	0.3881
rs185178768	1	50952706	A	-	-	-	-	-	-
rs6755784	2	178525015	C	-	-	-	-	-	-
rs11675573	2	178976191	A	0.17	0.34	0.34	0.4	0.0026 (−0.0061,0.0112)	0.5658
rs62371001	5	65030114	G	0.07	0.17	0.09	0.09	0.0002 (−0.0116,0.0121)	0.9727
rs1743241	6	118358811	A	0.38	0.47	0.27	0.37	0.0054 (−0.0029,0.0137)	0.199
rs79567239	6	2908668	T	0.16	0.11	0.24	0.12	−0.0001 (−0.0115,0.0112)	0.9848
rs9462210	6	36661176	A	0.28	0.25	0.18	0.18	−0.0019 (−0.0112,0.0074)	0.6833
rs144567740	7	139415067	T	-	-	-	-	-	-
rs13278982	8	11731524	A	0.1	0.35	0.16	0.3	0.0039 (−0.0053,0.0131)	0.403
rs12541595	8	124845117	T	0.1	0.28	0.46	0.3	−0.0008 (−0.0097,0.0082)	0.8695
rs11784619	8	143939607	A	-	-	-	-	-	-
rs72840788	10	119656173	A	-	0.21	-	0.09	0.0041 (−0.0083,0.0165)	0.5156
rs3184504	12	111446804	T	0.12	0.49	-	0.28	0.0032 (−0.006,0.0123)	0.5
rs116904997	12	120230731	G	-	-	-	-	-	-
rs2801617	13	55896707	C	-	0.07	-	-	-	-
rs8039472	15	84818413	G	0.22	0.46	0.2	0.4	−0.004 (−0.0125,0.0046)	0.3622
rs12906223	15	98712731	T	0.31	0.41	0.49	0.47	0.0008 (−0.0072,0.0088)	0.844
rs4790351	17	1392798	G	-	0.1	0.15	0.31	0.0096 (−0.0029,0.0221)	0.1341
rs2668674	17	45580074	C	-	-	-	-	-	-
rs74174203	19	45661508	G	-	-	-	-	-	-
rs2070458	22	23817120	A	0.5	0.19	0.44	0.25	0.0158 (0.0069,0.0247)	0.0005
rs4820654	22	25766935	G	0.34	0.37	0.5	0.48	−0.0041 (−0.0122,0.004)	0.3216

Chr, chromosome, ECV, extracellular volume. SNPs with low imputation quality in each ancestral group of this study were not analyzed.

Note: Missing values indicate certain SNPs that were not included in the analysis because they were not present in the TOPMED imputation reference panel, they had poor imputation quality and were rare in the population with phenotype information available.

Table 5.

Effect of SMARCB1 genetic variation (rs2070458 and rs2186370) by ancestry adjusting for: age, sex, 10 PCs, SBP, DBP, diabetes and current smoking.

RSID	Chr	Position (GRCh38)	Risk Allele	Group	Risk Allele Frequency	Difference in log(ECV) per Risk Allele (95%CI)	p-value
rs2070458	22	23817120	A	African American	0.5	0.0228(0.0042,0.0414)	0.0168
				Caucasian	0.19	0.0143(0.0034,0.0283)	0.0451
				Chinese	0.44	0.0195(−0.0008,0.0399)	0.0623
				Hispanic	0.25	0.0116(−0.0112,0.0344)	0.3193
				Meta-analysis		0.0169(0.0079,0.0259)	0.0002
rs2186370	22	23829118	A	African American	0.5	0.0231(0.0044,0.0418)	0.016
				Caucasian	0.18	0.0145(0.0004,0.0285)	0.0439
				Chinese	0.43	0.0198(−0.0006,0.0403)	0.0596
				Hispanic	0.24	0.0114(−0.0116,0.0345)	0.3331
				Meta-analysis		0.0171(0.0080,0.0261)	0.0002

Chr, chromosome, ECV, extracellular fraction.