Approximately 100 years ago, primary or idiopathic left ventricular (LV) hypertrophy developing in the absence of a defined volume or pressure load called hypertrophic cardiomyopathy (HCM) was first described.1 HCM commonly presents during the second decade of life, yet the presentation may be delayed into adulthood,2 with a phenotype that includes thickened or enlarged ventricular walls and obstruction of blood flow at the LV outflow tract. Excessive LV wall thickening and marked obstruction of the LV outflow tract in the setting of HCM are associated with an increased risk of death.3–6 HCM is a leading cause of sudden death in young athletes.7

Background—Incomplete penetrance and variable expression of hypertrophic cardiomyopathy (HCM) is well appreciated. Common genetic polymorphisms variants that may affect HCM penetrance and expression have been predicted but are not well established.

Methods and Results—We performed a case-control genomewide association study to identify common HCM-associated genetic polymorphisms and then asked whether such common variants were more represented in HCM or could explain the heterogeneity of HCM phenotypes. We identified an intronic FHOD3 variant (rs516514) associated with HCM (odds ratio, 2.45; 95% confidence interval, 1.76–3.41; \( P=1.25 \times 10^{-7} \)) and validated this finding in an independent cohort. Next, we tested FHOD3-V1151I (rs2303510), a nonsynonymous variant in partial linkage disequilibrium with rs516514, and we detected an even stronger association with HCM (\( P=1.76 \times 10^{-9} \)). Although HCM patients were more likely to carry these, FHOD3 allele subjects homozygous for FHOD3-1151I had similar HCM phenotypes as carriers of the V1151 allele. FHOD3 expression is increased in the setting of HCM, and both alleles of FHOD3-V1151I were detected in HCM myectomy tissue. Previously, FHOD3 was found to be required for formation of the sarcomere, and here we demonstrate that its fly homolog fhos is required for normal adult heart systolic contraction.

Conclusions—Here we demonstrate the association of a common nonsynonymous FHOD3 genetic variant with HCM. This discovery further strengthens the potential role of gene mutations and polymorphisms that alter the amino acid sequence of sarcomere proteins and HCM. (Circ Cardiovasc Genet. 2013;6:10-18.)

Key Words: contractility ■ genomewide analysis ■ hypertrophic cardiomyopathy

A

Received September 7, 2012; accepted November 26, 2012.
From the Molecular Cardiology Research Institute Center for Translational Genomics (E.C.W., S.R.G., I.D., J.E.C., N.K.K., G.S.H.), Department of Medicine, Cardiology Division (N.O., N.K.K., M.S.M., G.S.G.), Tufts Medical Center, Boston, MA; Department of Medicine, Division of Cardiovascular Diseases (V.B.H., I.J.K., S.R.O., M.J.A.), Department of Molecular Pharmacology and Experimental Therapeutics (J.M.B., M.J.A.), Department of Pediatric and Adolescent Medicine/Division of Pediatric Cardiology (M.J.A.), Mayo Clinic, Rochester, MN; and Department of Medicine, Duke University Medical Center, Durham, NC (M.J.W.).

*These authors contributed equally and are co-equal first authors.
**These authors are co-equal senior authors.

Christopher Semsarian, MB, BS, PhD, FRACP, was the Guest Editor for this article.

The online-only Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.111.965277/-/DC1.

Correspondence to Gordon S. Huggins, MD, MCRI Center for Translational Genomics, Molecular Cardiology Research Institute, Tufts Medical Center and University School of Medicine, 800 Washington St, Box 7703, Boston, MA 02111. E-mail ghuggins@tuftsmedicalcenter.org.

© 2013 American Heart Association, Inc.
people with a normal heart structure can carry a contractile gene mutation that causes HCM in related family members. Factors that protect mutation carriers from manifesting LV hypertrophy and outflow tract obstruction are poorly understood. In addition to incomplete penetrance, variable LV wall thickening and outflow tract obstruction between patients carrying the same mutation are well described. Variability in HCM disease penetrance and expression can only be partially explained by currently known HCM-causing cardiac sarcomere gene mutations because consistent and mutation-specific effects have not been identified.

Several lines of evidence support a role for genetic factors in HCM beyond contractile gene mutations ultimately responsible for triggering HCM itself. First, chromosomal loci associated with differences in LV mass have been found within a large HCM family carrying a single contractile gene mutation. Second, mice engineered with a human HCM-causing gene mutation exhibit strain-specific HCM phenotypes. Third, extreme HCM phenotypes, including the development of advanced heart failure and the need for heart transplantation, are associated with the presence of >1 contractile gene mutation. Finally, in the absence of HCM, LV mass is associated with >1 contractile gene mutation because consistent and mutation-specific effects have not been identified.

We estimated that we had 80% power to detect SNPs associated with HCM at an odds ratio (OR) of >2.0 with 153 individuals assuming that the associated SNP has a minor allele frequency (MAF) of 0.4. Population structure in the Tufts cohort was analyzed using the software package Structure (version 2.3.2.1), which found no evidence of significant allele frequency divergence at any value of K within the control and experimental groups when considered singly or as a whole. The PCA-population–based covariates had no effect on the reported results. GWA study analyses were performed using PLINK v1.07. Total genotyping call rate across all individuals (174 cases, 823 controls) was 0.98, and 311399 SNPs were available for analysis. Logistic analysis was performed for the binary disease trait (eg, HCM/no-HCM) with covariate adjustments for sex, presence of known HCM mutations, and age. Raw probability values were adjusted relative to number of tests performed and data-derived genomic inflation values (λ=1.01 in the initial association analysis). Imputation of variants throughout the associated region was accomplished using the HapMap CEU (release 23a) and PLINK as described.

The Mayo Clinic institutional review board approved all studies and study procedures after participant informed consent, described to and signed by voluntary participants in accordance with the principles expressed in the declaration of Helsinki. Subjects were recruited from the Mayo Clinic Hypertrophic Cardiomyopathy Clinic from 1998 to 2009, and all individuals were unrelated. The diagnosis of HCM was made via physical examination, ECG, and echocardiography. DNA was purified from whole blood collected from each HCM subject using the AutoGen FlexStar automated DNA extraction system (AutoGen, Holliston, MA). Genomic DNA was genotyped with the ABI 7900HT real-time polymerase chain reaction (PCR) system (Applied Biosystems, Carlsbad, CA). Duplicate internal controls and a blank control were included. The call rate was 97.0% for rs516514 and 98.3% for rs2303510.

The Mayo control cohort for this study consisted of patients with normal ECG identified who had either peripheral arterial disease (n=1648 cases) or no evidence of peripheral artery disease (n=1675), had European ancestry, and were recruited between October 2006 and May 2009. All participants gave their written consent for participation in the studies and the use of their data for future research. In the Mayo control cohort, rs516514 was genotyped in Illumina Human660W-quadv1-A genotyping platform, and rs2303510 genotypes were imputed by MACH based on HapMap II CEU database (release 21).

Clinical and echocardiographic data were extracted from the electronic medical record for each patient in the Mayo Clinic HCM cohort. Each patient had been analyzed previously for mutations in the 9 most common HCM-susceptibility genes (MYH7, MYBPC3, TNNT2, TNNI3, TNNC1, TPM1, ACTC, MYL3, MYL2). T tests were performed to assess the relationship between the predictor variable genotype (CC+CT versus TT) and continuous outcome variables (age at diagnosis, mean septal thickness, mean resting gradient). χ² tests were performed to assess the relationship between the predictor variable genotype (CC+CT versus TT) and categorical variables (obstruction, family history of HCM and sudden cardiac death, myectomy, implantable cardioverter-defibrillator). These analyses were conducted using JMP 8 (SAS Institute Inc, Cary, NC).

The gene expression Omnibus data sets derived from multiple human organs were searched through the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/geo/). Quantitative real-time PCR was performed with RNA extracted from 17 control donor LV tissues and 19 HCM myectomy

**Tufts HCM Cohort, Genomewide Array Analysis**

The Tufts Medical Center/Tufts University institutional review board approved all studies and study procedures after participant informed consent, described to and signed by voluntary participants in accordance with the principles expressed in the declaration of Helsinki. All subjects were unrelated and were identified through their diagnosis of HCM, which was confirmed by echocardiography. DNA was collected and purified by manufacturer protocols (PaxGene); DNA concentration was measured using the PicoGreen assay (Invitrogen, Carlsbad, CA). Genomic DNA was genotyped on the Illumina 370CNV array by deCode (Reykjavik, Iceland). The Tufts HCM replication cohort was genotyped using TaqMan assays for rs516514 and rs2303510 (Applied Biosystems Assays on Demand) using a 7900HT. Genotypes for 823 control individuals (all of CEU ancestry, average age 43, range 30–88) were obtained from Illumina Genotype Control Database (tControlDB, www.illumina.com). These individuals were genotyped on the same platform as our experimental study, with the exception of copy number probes present on the 370CNV array variant that were not included in the control population study; all nonoverlapping probes were set to missing for the purposes of this study.
interindividual heterogeneity of maximal LV wall thickness as well as resting and inductive outflow tract obstruction (Table 1). Analysis of 8 contractile genes by denaturing high-performance chromatography identified a contractile gene mutation with a high probability of being disease-causing in 27.4% of cases, which is similar to other cohorts. The Tufts HCM cohort was genotyped on the Illumina Hap370CNV array. We obtained a genetic data set including all available white control individuals (n=823) from the Illumina iControlDB database, genotyped on the Illumina Hap300 array, which is an identical genotyping platform as the 370CNV but lacking copy number variation probes. The control cohort had a similar age distribution as the Tufts HCM cohort. After applying standard quality control techniques, 31,139 SNPs were available for analysis across the combined case and control data sets. Structure analysis of ancestry informative markers present on the arrays proved the cases and controls to be well matched for the European ancestry; no significant subpopulations were found between (or within) case or control groups. After applying an additive logistic regression test to the case and control groups followed by a correction for multiple testing, 2 separate chromosomal loci were found to be associated with HCM defined by having a Bonferroni-corrected probability value <0.05 (Table 2). We were unable to confirm the association of rs12341266 with HCM in the Mayo Clinic cohort (data not shown). Here, we report the association of rs516514, which is located within an intron of the FHOD3 gene, with HCM (OR, 2.45; 95% confidence interval [CI], 1.76–3.41; P=1.25x10^-6) (Figure 1A). The MAF for rs516514 reported by HapMap was =0.48 in all tested racial and ethnic groups, which is considerably higher than the prevalence of HCM itself. The observed MAF in HCM-affected individuals was 0.61 (control MAF 0.44). We then genotyped 136 additional cases whose DNA had been collected after the GWA samples. Analysis of this second set of cases against the same control cohort again demonstrated an association of rs516514 with HCM (OR, 2.04; 95% CI, 1.52–2.73; P=1.85x10^-6). Next, we searched the gene expression Omnibus to determine whether any gene near rs516514 is expressed in the heart. Review of several Omnibus gene expression data sets, a representative example is GDS1096, demonstrated that FHOD3 is strongly expressed in the heart compared with other organs. We confirmed strong preferential expression of FHOD3 in the heart compared with other organs by
quantitative real-time PCR analysis of a panel of mouse organ total RNA (Figure 1B). Further support for FHOD3 as opposed to other genes near rs516514 was derived from the observation that FHOD3 belongs to a family of proteins that include an FH2 domain, whose function is to promote actin filament formation. Finally, FHOD3 was reported to be required for assembly of the neonatal cardiomyocyte cardiac contractile apparatus.34

Independent Replication of rs516514 Association With HCM
We sought to confirm the association of rs516514 with HCM by testing the Mayo Clinic HCM (Table 1) and control cohorts. The Mayo Clinic HCM cohort includes 1012 FHOD3 genotyped cases. The Mayo Clinic control cohort included 1326 subjects available from the electronic medical records and genomics network not known to have HCM and who all

Table 2. Significant Loci

<table>
<thead>
<tr>
<th>dbSNP rsID</th>
<th>Gene</th>
<th>Chr</th>
<th>BP (hg18)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>SNP Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs516514</td>
<td>FHOD3</td>
<td>18</td>
<td>32515046</td>
<td>1.25×10⁻⁷</td>
<td>0.04</td>
<td>2.45 (1.76–3.41)</td>
</tr>
<tr>
<td>rs12341266</td>
<td>RGS3</td>
<td>9</td>
<td>115396337</td>
<td>1.32×10⁻⁷</td>
<td>0.04</td>
<td>4.06 (2.40–6.80)</td>
</tr>
</tbody>
</table>

Logistic regression analyses were performed with correction for the following covariates: sex, presence of known hypertrophic cardiomyopathy mutations, and age. Probability values before and after adjustment for multiple hypothesis testing (Bonferroni protocol) are shown. BP indicates chromosomal base pair; Chr, chromosome; CI, confidence interval; OR, odds ratio; and SNP, single-nucleotide polymorphism.

Figure 1. Chromosome 18 locus that includes FHOD3 is associated with hypertrophic cardiomyopathy (HCM). A, Graph shows the –log(probability value) and the location of each single-nucleotide polymorphism (SNP) contained on the 370CNV array that is within the chromosome 18 locus identified by the HCM genomewide association study. The SNP with the strongest association with HCM is shown in blue. SNPs in linkage disequilibrium (LD) with rs516514 are shown from red (strong LD) to yellow (weak LD). Relative sites of recombination are shown in blue lines. B, Quantitative real-time polymerase chain reaction demonstrating expression of FHOD3 RNA in mouse tissues. The strongest expression was found in the heart. C, Alignment of FHOD3 peptide showing conservation of FHOD3-V1151 (red box) in mammals. Amino acids conserved across all species shown are marked by asterisk.
have a normal ECG. Our a priori threshold for significance in this replication study was P<0.01. Applying an additive model to rs516514 genotypes in the Mayo Clinic case and control samples revealed a significant replication of the Tufts GWA HCM association (OR, 1.26; 95% CI, 1.12–1.41; P=0.0001). This finding confirmed the association of rs516514 with HCM identified by our GWA study and suggested that a genetic variant within FHOD3 increased the risk of clinically apparent HCM.

FHOD3-V1151I Shows a Stronger Association With HCM Compared With rs516514

Approximately 50% of all clinically diagnosed HCM and 80% of reverse curvature HCM are caused by mutations involving genes that encode key sarcomeric contractile proteins (myofilaments). Consequently, we speculated whether the associated FHOD3 intronic polymorphism might be in LD with a FHOD3 peptide variant that has the potential to biologically impact the physiology of the cardiac sarcomere. We queried the exome variant server (http://evs.gs.washington.edu/ EVS/) to ask whether an FHOD3 nonsynonymous FHOD3 SNP, which changed the peptide sequence, was in LD with rs516514. This survey identified 114 FHOD3 nonsynonymous variants, and only FHOD3-V1151I was in LD with rs516514. This survey identified 114 FHOD3 nonsynonymous variants, and only FHOD3-V1151I was found to be in partial LD with rs516514 (rs2303510; D′=0.903, r2=0.287). FHOD3-V1151I is highly conserved among mammals and other species; nearby amino acids are completely conserved (Figure 1C). Using an additive model, we found that FHOD3-V1151I was more strongly associated with HCM (OR, 1.36; 95% CI, 1.20–1.54; P<0.0001) in the Mayo Clinic cohort compared with the intronic rs516154 variant. Similarly, the probability value for association with HCM of the FHOD3-V1151I polymorphism was lower in the Tufts HCM cohort (additive model OR, 2.01; 95% CI, 1.64–2.64; P=1.76×10−9) consistent with a stronger association of rs2303510 compared with rs516154. When rs514516 was added as a covariate in our logistic regression model, the association of rs2303510 with HCM became nonsignificant, indicating that the association of both SNPs with HCM is related to their being in partial LD and less likely to be caused by their representing >1 HCM association signal. Finally, we found no significant statistical interaction between FHOD3 rs2303510 genotype status and contractile gene mutation. The discovery of a common variant that changes the amino acid sequence of FHOD3 associated with HCM suggests that the association of FHOD3 with HCM is based on an effect mediated within the cardiac sarcomere as opposed to differences in expression level.

FHOD3 Variants Are Not Associated With Differences in LV Phenotypes

Next, we asked whether carriers of the FHOD3 polymorphisms demonstrated differences in LV phenotypes. We tested this question in the Mayo Clinic HCM cohort because its large number of subjects offered greater power to detect a significant difference for quantitative traits. Patients homozygous for both the rs516514 and the FHOD3-V1151I minor alleles exhibited similar HCM phenotypes when compared with the reference genotypes (Table 3). Although we find evidence that FHOD3-V1151I is associated with HCM, these studies demonstrate that FHOD3-V1151I is not associated with a particular LV phenotype, including LV wall thickness or outflow tract obstruction.

Both Alleles of FHOD3-V1151I Are Expressed in HCM Heart Tissue

Next, we analyzed FHOD3 expression in heart tissue taken from patients with HCM at the time of septal myectomy, an operation in which a portion of the LV septum is surgically removed to alleviate outflow tract obstruction. These studies confirmed expression of FHOD3 transcripts in the HCM heart. FHOD3 transcript abundance was increased in the HCM heart 1.67-fold (P=0.012) by quantitative PCR (Figure 2A). Western blotting revealed 2.05-fold (P=0.03) greater FHOD3 protein in HCM heart tissue versus control hearts (Figure 2B). Finally, we sought to determine whether transcripts encoding both alleles of the FHOD3-V1151I variant were expressed in the HCM tissue. Sanger sequencing of cDNA produced from HCM myectomy heart RNA identified the expression of both FHOD3-V1151I alleles in 3 cases heterozygous for the variant (Figure 2C). Increased expression of FHOD3 transcript and protein in HCM heart tissue, as well as expression of both alleles of FHOD3-V1151I, suggests that FHOD3 may have an important role in heart function.

FHOD3 Is Required for Normal Heart Contractile Function

We next sought to further define whether FHOD3 is plausibly related to myocardial phenotypes by defining whether it is required for normal adult heart function. Animal models provide well-recognized approaches to analyze genes whose human disease mutations are associated with disease. Drosophila has emerged as a tractable genetic model to determine whether genes associated with human disease may have a plausible role in heart function. Therefore, to determine whether FHOD3 is required for contractile function in the fully formed adult heart, we analyzed flies genetically engineered for RNA interference-mediated knockdown
(RNAi) of fhos, the fly homolog of the mammalian FHOD genes. Overexpression of fhos-specific RNAi (achieved using the Act5C-Gal4 driver line) produced a significant reduction in the fhos transcript when compared with control (online-only Data Supplement Figure). Heart-specific expression of fhos RNAi (achieved using the TinC-Gal4 driver line) produced a significant decrease in fly heart fractional shortening with increases in the end-systolic and end-diastolic dimensions (Figure 3A–3D), indicative of significantly impaired contractile function. These results in flies and published work in mammalian cardiomyocytes are consistent with the requirement of FHOD3/fhos for normal heart contractile function and support a plausible role for FHOD3 variants being associated with HCM. This demonstration in Drosophila support future studies that will define the role of FHOD3 amino acid variants on the function of the mammalian heart.

**Discussion**

To our knowledge this is the first GWA study to identify a common gene variant associated with HCM, a disease caused by rare single gene mutations. In this GWA study, we identified an intronic SNP in FHOD3, a gene required for formation of the contractile apparatus, to be associated with HCM in 2 independent case-control cohorts of unrelated patients with HCM. This finding, together with the modestly increased FHOD3 expression at both the transcript and protein level in human HCM myectomy tissue, is
consistent with an important role for FHOD3 in the HCM. However, these FHOD3 polymorphisms cannot and should not be considered as HCM-causative variants because their MAFs are higher than the prevalence of HCM itself. The over-representation of FHOD3 alleles in the setting of HCM may instead reflect a greater risk of overt HCM disease expression. Our finding of a common genetic variant affecting a gene with a plausible role in heart phenotypes in HCM should encourage the application of GWA testing to other monogenic disorders to identify common gene variants associated with trait.

Our findings are consistent with carriers of the FHOD3-V1151I having an altered risk of manifesting clinically apparent HCM that would lead them to seek care in a dedicated HCM treatment center. Considering that several laboratories have demonstrated that patients carrying >1 contractile gene mutation have more severe HCM phenotypes, we tested whether FHOD3-V1151I was also associated with differences in HCM phenotypes. We found no evidence that FHOD3-V1151I is associated with differences in LV wall thickness, LV outflow tract obstruction, or other HCM clinical endpoints. We also speculated that by affecting sarcomere formation, FHOD3-V1151I may alter remodeling of the cardiomyocyte in response to a contractile gene mutation; however, we found no evidence to suggest that FHOD3-V1151I had a selective effect in patients found to carry a contractile gene mutation. Although we found no evidence that FHOD3-V1151I affects HCM disease expression, we acknowledge that our analyses may have lacked sufficient power to detect small phenotypic effects. Our results complement recent studies that have identified common polymorphisms associated with dilated nonischemic cardiomyopathy, further supporting a role for common genetic variants in cardiomyopathies otherwise caused by rare variants. We acknowledge that because the Tufts and Mayo Clinic HCM cohorts do not have sufficient numbers of family members that include HCM-causing mutation positive subjects who do not have HCM, we are unable to formally test whether FHOD3-V1151I affects penetrance of HCM. Our results should encourage future studies, perhaps based on testing families that include members carrying an HCM mutation but lack phenotypic expression of HCM, to determine whether FHOD3 genotype status is associated with penetrance of HCM.

We find it compelling that a FHOD3 variant is associated with HCM because 2 reports have demonstrated that FHOD3 has a vital role in actin filament formation and maintenance. Mutations of genes encoding components of the thin filament, including α-cardiac actin, have been previously implicated as HCM disease-associated genes. We found increased FHOD3 protein levels in patients with HCM, whereas patients with a dilated cardiomyopathy have reduced FHOD3 protein levels. Our demonstration that reducing flos fly heart transcript levels produces cardiac contractile dysfunction is consistent with an important role for FHOD3 in cardiomyopathy. Future studies will define the role of FHOD3 overexpression and peptide-variant expression on cardiomyocyte and heart phenotypes.

The FHOD3 protein includes a formin homology 2 (FH2) domain and an FH1 domain. FH2 domains promote extension of actin filaments by blocking capping proteins from binding the actin filament end, which would terminate filament extension. The FH2 crystal structure indicates 2 states of the FH2 domain: one that binds to actin and the other that releases actin, both while protecting the filament from capping proteins. Alanine mutants of 2 FHOD3 FH2 domain residues conserved with critical residues of the yeast protein Bni1p FH2 domain are unable to promote actin-nucleating activity and sarcomere formation in rat neonatal cardiomyocytes. Intriguingly, we identified the FHOD3-V1151I peptide variant, which is conserved in the FH2 domain, to be associated with HCM. We speculate that the V1151I variant may alter the kinetics of FHOD3 actin binding, resulting in either a permissive or restrictive effect on actin filament formation.

We acknowledge several limitations to our study. First, our discovery cohort is smaller than most GWA study cohorts. Despite this limitation, the association of rs516514 found in the Tufts HCM cohort was independently replicated in the larger Mayo Clinic HCM cohort. Second, because our study has been performed in subjects of European background, our results may not be generalizable to HCM patients from different racial or ethnic backgrounds. Despite this limitation, the MAF of FHOD3 rs516514 is similar in whites, blacks, and Asians, which should motivate testing the association of FHOD3-V1151I with HCM in non-Europeans. The effects of population stratification were controlled by the inclusion of ancestry informative marker-derived covariates in our GWA study analysis. A significant proportion of the patients included in the Tufts and Mayo Clinic HCM cohorts were treated with septal reduction therapy (myectomy or alcohol septal ablation), suggesting that our results may not be generalizable to HCM patients with purely nonobstructive disease. Finally, our unbiased GWA study did not identify gene variants in the renin–angiotensinogen–aldosterone pathway that modify HCM, nor did we identify polymorphisms previously associated with LV mass in the absence of HCM or incident heart failure. Failure to find these associations in our GWA study may reflect associations that could not overcome the strict correction for multiple hypothesis testing that is necessary as part of our GWA study discovery strategy or, perhaps, that the array platform did not adequately profile those alleles.

Our results support a model by which a common FHOD3 genetic variant alters the HCM disease phenotype in such a manner that increases the risk of clinically apparent disease. The requirement of flos for fly heart contractility suggests that effects of FHOD3 on mammalian heart contractility may be responsible for its association with HCM. Future investigations will explore the molecular mechanisms supporting the association of FHOD3 variants with HCM.

Acknowledgments
We thank Randy Kring, Westley Spiro, Michelle Arya, and Alessandra Alicea for their contribution to this article.
Sources of Funding
This project was supported by a grant from the John T. Babbitt Foundation (Dr Huggins), the National Institutes of Health grant HL077378, the American Heart Association (Dr Wooten), the Windland Smith Rice Comprehensive Sudden Cardiac Death Program, and the National Institutes of Health grant P01HL 94291 (Dr Ackerman). The project described was supported by the National Center for Research Resources grant UL1RR025752 and the National Center for Advancing Translational Sciences, National Institutes of Health, grant UL1TR000073.

Disclosures
None.

References
Hypertrophic cardiomyopathy (HCM) is characterized by excessive thickening and enlargement of the heart muscle, causing heart failure, syncope, and death. HCM is caused by rare mutations that change the peptide sequence of proteins in the cardiac sarcomere. We asked whether genetic polymorphisms common in the general population might also be found more frequently in HCM as has been recently shown for dilated cardiomyopathy. To address this question, we performed a case-control genomewide association study in the Tufts HCM cohort, and we validated our findings in the Mayo Clinic HCM cohort. These studies identified a peptide-altering variant in the FHOD3 gene associated with HCM. Although HCM patients were more likely to carry the FHOD3 polymorphism, carriers had similar HCM phenotypes as noncarriers. We found that the FHOD3 protein is expressed in the heart, and its expression was increased in the setting of HCM. To answer whether this family of genes is important in muscle function, as might be expected of a gene associated with HCM, we studied the fruit fly whose homolog of FHOD3 is called fhos. Genetic knockdown of the fhos transcript specifically in the fly heart caused a decrease in heart contractile function. In summary, these studies have identified a new gene associated with HCM that is common in the general population and likely required for normal heart contractile function. Future studies will define the role of FHOD3 in cardiac function and cardiomyopathy.
**Formin Homology 2 Domain Containing 3 Variants Associated With Hypertrophic Cardiomyopathy**


*Circ Cardiovasc Genet*. 2013;6:10-18; originally published online December 19, 2012; doi: 10.1161/CIRCGENETICS.112.965277

*Circulation: Cardiovascular Genetics* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 2012 American Heart Association, Inc. All rights reserved.

Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circgenetics.ahajournals.org/content/6/1/10

Data Supplement (unedited) at:

http://circgenetics.ahajournals.org/content/suppl/2012/12/19/CIRCGENETICS.112.965277.DC1

**Permissions**: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation: Cardiovascular Genetics* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

**Reprints**: Information about reprints can be found online at:

http://www.lww.com/reprints

**Subscriptions**: Information about subscribing to *Circulation: Cardiovascular Genetics* is online at:

http://circgenetics.ahajournals.org/subscriptions/
Supplementary Figure 2. Analysis of *fhos* transcript abundance relative to Actin 5C transcript abundance by reverse transcriptase PCR. Analysis performed by A. Agarose chromatography and B. Quantitative PCR. Total RNA was isolated from (i) wild type flies, (ii) W1118;Act5C-Gal4 control flies (i.e. driver line backcrossed in the genetic background) and (iii) Act5C-Gal4/UAS-*Fhos* RNAi flies. In the double transgenics expression of the Fhos interference construct (VDRC construct ID 108388) results in reduced levels of the *fhos* transcript compared with levels found in either the wild type or corresponding control flies. PCR control housekeeping gene: Actin5C.