Title

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Authors

Jiang, Xuan
Shao, Yanqiu
Araj, Faris G
et al.

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BRIEF COMMUNICATION

Heterozygous Cystic Fibrosis Transmembrane Regulator Gene Missense Variants Are Associated With Worse Cardiac Function in Patients With Duchenne Muscular Dystrophy

Xuan Jiang, PhD; Yanqiu Shao, MS; Faris G. Araj, MD; Alpesh A. Amin, MD; Benjamin M. Greenberg, MD, MHS; Mark H. Drazner, MD, MSc; Chao Xing, PhD; Pradeep P. A. Mammen, MD

BACKGROUND: Duchenne muscular dystrophy (DMD) is a neuromuscular disorder caused by mutations within the dystrophin gene. DMD is characterized by progressive skeletal muscle degeneration and atrophy and progressive cardiomyopathy. It has been observed the severity of cardiomyopathy varies in patients with DMD.

METHODS AND RESULTS: A cohort of male patients with DMD and female DMD carriers underwent whole exome sequencing. Potential risk factor variants were identified according to their functional annotations and frequencies. Cardiac function of 15 male patients with DMD was assessed by cardiac magnetic resonance imaging, and various cardiac magnetic resonance imaging parameters and circulating biomarkers were compared between genotype groups. Five subjects carrying potential risk factor variants in the cystic fibrosis transmembrane regulator gene demonstrated lower left ventricular ejection fraction, larger left ventricular end-diastolic volume, and higher NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels compared with 10 subjects who did not carry the potential risk factor variants (P=0.023, 0.019 and 0.028, respectively).

CONCLUSIONS: This study revealed heterozygous cystic fibrosis transmembrane regulator gene missense variants were associated with worse cardiac function in patients with DMD. The cystic fibrosis transmembrane regulator gene may serve as a genetic modifier that accounts for more severe cardiomyopathy in patients with DMD, who would require more aggressive management of the cardiomyopathy.

Key Words: Duchene muscular dystrophy–associated cardiomyopathy ■ genetic modifier ■ whole exome sequencing

Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder resulting from mutations within the dystrophin gene. DMD affects 1 in 3500 to 5000 boys, resulting in muscle degeneration, dilated cardiomyopathy, and premature death. The majority of patients with DMD die before age 40 because of complications of cardiomyopathy. Pathological changes in DMD-associated cardiomyopathy are associated with an increase in myocardial fibrosis leading to worsening cardiac function and eventual death.

The initial medical treatment for young patients with DMD, who have preserved left ventricular (LV) systolic function, involves coadministration of an angiotensin-converting enzyme inhibitor or angiotensin-II receptor blocker and a mineralocorticoid receptor

Correspondence to: Chao Xing, PhD, and Pradeep P. A. Mammen, MD, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390. E-mail: chao.xing@utsouthwestern.edu; pradeep.mammen@utsouthwestern.edu

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antagonist. As patients with DMD develop progressive cardiomyopathy, combination therapy with a β-blocker, angiotensin-converting enzyme inhibitor/angiotensin-II receptor blocker, and mineralocorticoid receptor antagonist are recommended.[1] Despite aggressive guideline-directed medical therapy, the LV ejection fraction (LVEF) among adult patients with DMD reveals a skewed distribution. Some patients with DMD develop worsening cardiomyopathy at a faster rate. Patients with DMD who develop LV systolic dysfunction before the age of 18 years succumb to death at an earlier age compared with patients with DMD who have preserved cardiac function during their youth.

We hypothesize that worsening cardiomyopathy in patients with DMD is associated with a genetic modifier aside from the primary mutation within the dystrophin gene; the 2 genetic factors work in synergy, leading to an accelerated deterioration in cardiac function. In this study, we sought to identify the genetic modifiers by undertaking whole exome sequencing (WES) on adult patients with DMD. We showed that patients with DMD carrying potential risk factor variants in the cystic fibrosis transmembrane regulator (*CFTR*) gene had worse cardiac function.

METHODS

Because of the sensitive nature of the data collected for this study, requests to access the data set from qualified researchers trained in human subject confidentiality protocols may be sent to Dr. Pradeep P.A. Mammen at pradeep.mammen@utsouthwestern.edu.

Study Population

The study was approved by the University of Texas Southwestern Medical Center's Institutional Review Board. Patients with DMD and carriers, who had been verified with a pathogenic mutation within the dystrophin gene and had established care in the UT Southwestern Adult Neuromuscular Cardiomyopathy Clinic, were approached for enrollment into the study. Written informed consent was obtained from all patients. There were 22 male patients with DMD and 12 female DMD carriers from 24 independent families enrolled, including 11 DMD singletons, 7 mother-son pairs, 1 affected sibling pair, 1 affected cousin pair plus the proband's carrier mother, and 4 female carriers without their affected sons enrolled (Table S1). Cardiac function was assessed by cardiac magnetic resonance imaging (cMRI) in the majority of the subjects (n=22). For individuals who were unable to undergo cMRI because of technical issues, the cardiac structure and function were obtained by the relatively less precise imaging modalities of either echocardiography (n=9) or cardiac computed tomography scan (n=1). There were 2 individuals without cardiac function recorded. Since cMRI provides a more accurate cardiac assessment compared with echocardiography or cardiac computed tomography scan and the measurements by different imaging modalities are not directly comparable, we focused on the 15 male patients with DMD assessed by cMRI to evaluate the association between cardiac function and genetic variants (Table). Particular attention was focused on the LV end-diastolic volume and LVEF measurements obtained by cMRI, as both of these variables are associated with poor prognosis in cardiomyopathy. Standard clinical blood work was measured with a particular focus on circulating cardiac biomarker levels, including NT-proBNP (N-terminal pro-B-type natriuretic peptide), total serum creatinine kinase, and cardiac troponin T.

Self-reported race was confirmed by ancestry inference based on the WES data. There were 18 families of European ancestry, 4 of Hispanic ancestry, 1 of East Asian ancestry, and 1 of South Asian ancestry.

DNA Sequencing and Analysis

Genomic DNA was isolated from whole blood using the KAPA Hyper Prep Kit (Kapa Biosystems). WES was performed at the UT Southwestern McDermott Center Sequencing Core, using xGen Exome Research Panel v1.0 (Integrated DNA Technologies) on an Illumina platform to generate paired-end 150-bp reads. Sequences were aligned to the human reference genome b37. Variants were called using the Genome Analysis Toolkit and annotated using SnpEff.

As we aimed to identify genetic modifiers that exacerbate DMD-associated cardiomyopathy, the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines,[2] which serve to evaluate pathogenicity of Mendelian disorders, cannot be directly followed to determine pathogenicity in this study. Rather, we filtered variants using the following criteria (Figure S1): (1) minor allele frequency <0.05 in each subpopulation of the 1000 Genomes Project and genome aggregation database (gnomAD v2.1.1); (2) nonsense, missense, canonical splicing, or frameshift variants; (3) Genomic Evolutionary Rate Profiling score >2.0; (4) PolyPhen-2 score equal to or >0.9 for a missense variant; and (5) variants submitted as "pathogenic" to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) on at least 1 occasion—clinical significance annotated with at least one "5" in the ASN.1 file from the National Center for Biotechnology Information. We termed those variants passing the filtering criteria *potential risk factors* in line with the

Table. Clinical Characteristics in Male Patients With DMD With Cardiac Function Measured by cMRI

Family	Subject	cMRI-Related Information					Circulating Biomarkers		
		LVEF (%)	RVEF (%)	LVEDV (mL)	LV Mass (g)	Fibrosis	TnT (ng/mL)	Total CK (U/L)	NT-proBNP (pg/mL)
F1	1499	71	63	64	62	N	<0.01	307	59
F2	1564*	70	58	77	68	Y	<0.01	344	25
F3	1567*	52	47	109	70	Y	<0.01	384	46
	1806	42	55	145	84	Y	0.03	770	135
F4	1620†	25	43	225	127	Y	0.02	602	183
	1621†	45	44	134	97	Y	<0.01	463	116
F5	1680	60	52	87	87	N	0.02	3059	16
F7	1947	69	63	106	78	Y	0.06	6790	57
F10	1398	15	26	411	151	Y	<0.01	329	1424
F11	1407*,†	37	76	163	119	Y	0.01	781	399
F12	1457	53	47	107	103	Y	0.02	701	17
F13	1500†	33	45	170	91	Y	0.03	958	172
F14	1622	57	51	104	110	Y	0.05	1072	20
F15	1887*,†	41	64	151	65	Y	0.03	227	98
F16	1895	60	56	71	…	Y	<0.01	614	21

CK indicates creatine kinase; cMRI, cardiac magnetic resonance imaging; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RVEF, right ventricular ejection fraction; TnT, troponin T.

*Carriers of *MC1R* potential risk factor variants.

†Carriers of *CFTR* potential risk factor variants.

ClinVar clinical significance value options. Note that the criterion (5) was arbitrarily used to reduce the number of variants to be considered given the small sample size, filtering for biologically important variants regardless of the phenotypes associated with the variant deposition.

The targeting regions of the *CFTR* gene were amplified by polymerase chain reaction with gene-specific primers as listed in Table S2. The polymerase chain reaction product was purified to remove primers and deoxyribonucleotide triphosphates by using the QIAquick PCR Purification Kit (Qiagen), and Sanger sequencing was performed at the UT Southwestern Sanger Sequencing Core using an ABI Prism 3100 machine (Applied Biosystems).

Structural Analysis

Exonic *CFTR* variants identified in patients with DMD were mapped onto a crystal structure using the cryo-electron microscopy-derived structure of CFTR at 3.9 Å resolution in ATP-free state (PDB ID: 5UAK) as a template.[3] PyMOL (v2.2.3) was used to visualize protein structure and render graphic images. Protein crystal structure was schematized, with the variant sites shown as spheres.

Statistical Analysis

Comparisons of clinical measurements between groups were performed by the Mann–Whitney–Wilcoxon test because of the small sample size. Enrichment of *CFTR* risk factor variants was tested by Fisher's exact test. R software was used for statistical analysis. $P<0.05$ was considered statistically significant.

RESULTS

The DMD mutations previously tested by clinical assays were confirmed using the WES data (Table S1). To search for a second genetic variant predisposing to cardiomyopathy susceptibility, we started with the 24 independent exomes (20 DMD probands and 4 female carriers), one from each family. There were 48 variants in 45 genes passing the variant filtering criteria (Table S3), though none of them were pathogenic for cardiomyopathy by the ACMG/AMP criteria. Of note, there were 2 genes, *CFTR* and *MC1R*, harboring >1 qualified variant, and in both genes there existed a variant appearing in >1 family (Figure 1A and Table S4). All potential risk factors in *CFTR* and *MC1R* were confirmed by Sanger sequencing with the 3 *CFTR* variant chromatographs shown in Figure 1B. There were 2 individuals (1407 and 1887) carrying 1 potential risk factor in both genes. Because of the small sample size with limited statistical power, we focused on variants in these 2 genes (Figure 1A and Table S4) to investigate whether they were associated with cardiac dysfunction.

A

Position*	Reference Allele	Alternative Allele	rsID	Effect†	ClinVar	GERP Score	PolyPhen Score	MAF‡	Subjects
117149147	G	A	rs1800076	R75Q	Y	5.68	1	1.5E-2	1407,1500,1887
117230454	G	C	rs1800098	G576A	Y	4.83	0.907	5.2E-3	1620,1621
117250575	G	C	rs1800111	L997F	Y	2.87	0.999	2.1E-3	1542#

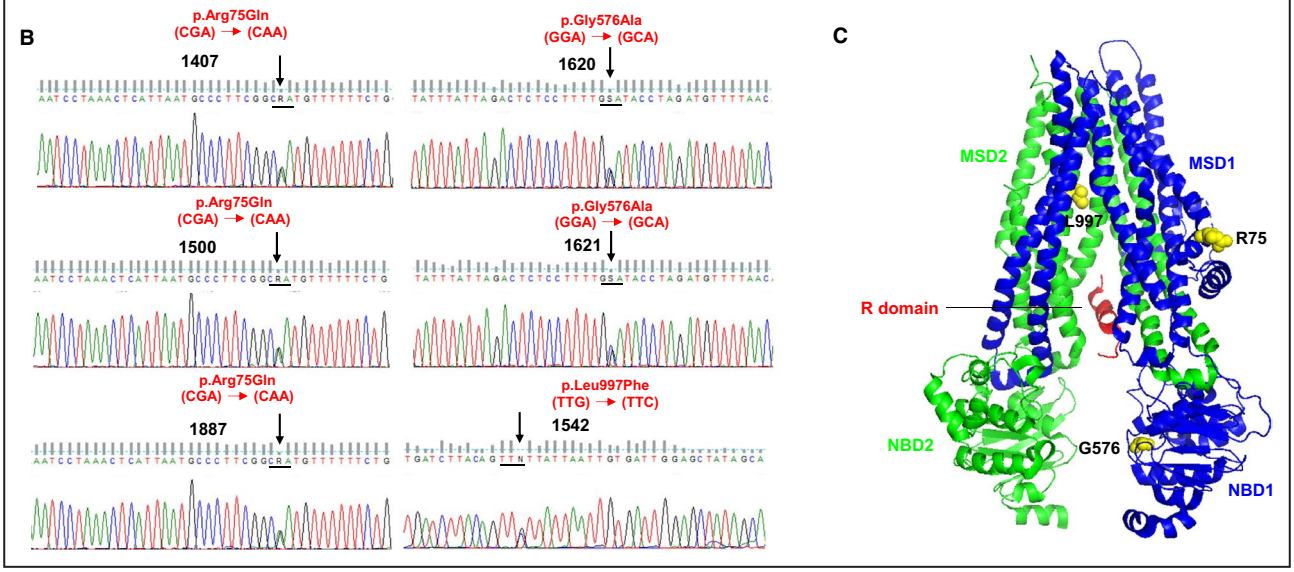

Figure 1. Characterization of *CFTR* risk factor variants identified in patients with DMD.
A, Annotation of 3 *CFTR* potential risk factor variants identified in patients with DMD. (*Mapped to human reference genome b37; †Annotated to transcript NM_000492; ‡Global minor allele frequency in the genome aggregation database; #Cardiac function measured by echocardiography.). **B**, Chromatographs of *CFTR* variants by Sanger sequencing. Black arrows indicate the nucleotide substitutions. **C**, Lateral view of the human CFTR structure in ATP-free conformation. Twelve transmembrane helices and two ATP-binding regions are shown as ribbons in blue (membrane-spanning domain [MSD] 1 and nucleotide-binding domain [NBD] 1) or green (MSD2 and NBD2). The resolved region of regulatory (R) domain is shown in red. The risk factor variant residues identified in this study (Arg^{75}, Gly^{576} and Leu^{997}) are depicted as yellow spheres. (R^{75}=Arg^{75} [or arginine], G^{576}=Gly^{576} [or glycine], and L^{997}=Leu^{997} [leucine]). GERP indicates Genomic Evolutionary Rate Profiling; MAF, minor allele frequency; and PolyPhen, polymorphism phenotyping.

Comparisons of cMRI measures were made between male patients with DMD (n=15) with and without the risk factor variants for both *CFTR* (Figure 2) and *MC1R* (Figure S3). The LVEF was lower in the 5 *CFTR* risk factor variants carriers compared with the 10 noncarriers (Figure 2A; *P*=0.023). The calculated 3-dimensional volume measure LV end-diastolic volume was larger in the carriers (Figure 2B; *P*=0.019). In addition, the NT-proBNP levels, a diagnostic and prognostic biomarker for heart failure, were higher in the carriers (Figure 2C; *P*=0.028). However, there was no difference in the levels of total creatinine kinase, a marker of skeletal muscle injury, or troponin T, a marker of myocardial injury, between the carrier and noncarrier groups (Figure S2). In contrast, there was no difference for any of these variables between the 4 *MC1R* variant carriers and the 11 noncarriers (Figure S3). The results indicated *CFTR*, not *MC1R*, to be a cardiac dysfunction risk factor gene in patients with DMD.

Of 14 independent male patients with DMD of European descent (Table S1), there were 5 carriers of *CFTR* potential risk factor variants and 9 noncarriers. Applying the same filtering criteria to the gnomAD non-Finnish European database, there were 3983 carriers out of 51 361 males. Thus, there was an enrichment of risk factor variants in the *CFTR* gene among the patients with DMD compared with non-Finnish Europeans in gnomAD (*P*=0.0031), which, however, did not attain the Bonferroni corrected significance level (0.05/45=0.0011).

DISCUSSION

Patients with DMD develop progressive skeletal muscle wasting and atrophy, followed by dilated cardiomyopathy. The severity of the cardiomyopathy in patients with DMD varies—some develop progressively worse cardiomyopathy at a faster rate, whereas others develop a more indolent form of cardiomyopathy. This clinical variance raises the question as to whether there exist genetic modifiers underlying the accelerated rate of cardiomyopathy progression

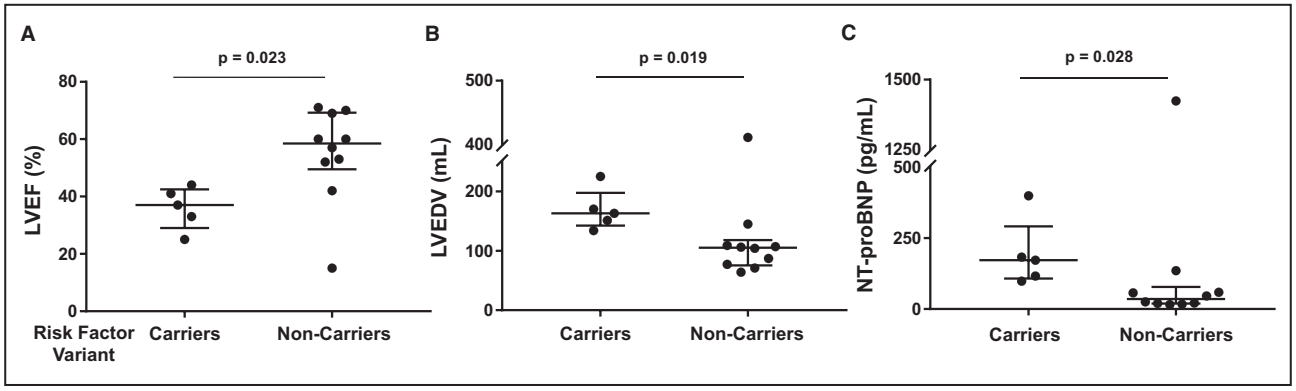

Figure 2. Comparison of prognostic cardiac markers between *CFTR* genotype groups.
Fifteen male patients with DMD whose cardiac function was measured by cardiac magnetic resonance imaging (cMRI) were categorized into 2 groups: 5 patients carrying *CFTR* risk factor variants, and 10 noncarriers, and (**A**) left ventricular ejection fraction (LVEF), (**B**) left ventricular end-diastolic volume (LVEDV), and (**C**) NT-proBNP (N-terminal pro-B-type natriuretic peptide) levels were compared. Each dot represents a unique individual. Median and interquartile range are indicated. Comparison was performed by the Mann–Whitney–Wilcoxon test.

in some of the patients with DMD. The role of genetic modifiers in cardiovascular biology has been identified in several cardiac disorders.[4,5] Current WES data revealed an association between the potential risk factor variants in *CFTR* and low LVEF, large LV end-diastolic volume, and high NT-proBNP levels in patients with DMD, which supports a hypothetical model that *CFTR* may work as a genetic modifier to accelerate maladaptive cardiac remodeling in patients with DMD.

Variants in *CFTR* have been associated with multiple disorders including cystic fibrosis, pancreatitis, and male infertility.[6] Although there is no reported association between *CFTR* variants and the development of cardiomyopathy in human genome-wide association studies yet, there is ample evidence of *CFTR* involvement in cardiac function and myopathy. The *CFTR* gene is expressed in cardiomyocytes.[7] Normal CFTR function has been reported to be necessary for optimal cardiac function in several mammalian species including mice and humans.[8] A study involving the human failing heart revealed a 50% reduction in the expression of the CFTR protein compared with nonfailing control hearts.[9] The loss of function of CFTR was implicated in LV systolic dysfunction in patients with cystic fibrosis, and it is hypothesized to be related to an increase in oxidative stress within the cardiomyocytes.[10] In a murine model of cystic fibrosis, loss of CFTR expression was reported to be involved in maladaptive LV remodeling with altered cardiac function independent of lung disease.[11] Of note, another missense variant I556V cosegregated with hereditary inclusion body myopathy in an extended pedigree, and this variant was further detected in 8 of 101 patients with muscle diseases including 3 patients with DMD.[12]

A limitation of the current study is the lack of an in vitro assay to examine the impact of these missense variants on functionality of CFTR in cardiomyocytes. However, the impact of mutations at residues Arg^{75}, Gly^{576}, and Leu^{997} have been examined in multiple studies.[6,13] They were reported to affect bicarbonate permeation and normal transcript levels of CFTR, though they were not pathogenic variants for cystic fibrosis by the ACMP/AMP guidelines. Based on the crystal structure of human CFTR (Figure 1C), Arg^{75} is located at the hinge region that modulates the collective movements of the nucleotide-binding domains with respect to membrane-spanning domains. Substitution of arginine for glutamine at this position was shown to impair bicarbonate permeation.[6] Gly^{576} is situated at the nucleotide-binding domain homodimer interface. Substitution of glycine with an alanine residue at this position results in a conformational exchange that is linked to dimerization of the nucleotide-binding domains, which may be required to open the ion channel.[14] Leu^{997} is predicted to participate in the formation of the CFTR channel. Replacement of leucine at this position with an amino acid containing larger side chains such as phenylalanine leads to a narrow channel width at the pore region.[6] These results are consistent with the in silico prediction of variant impact and warrant further investigation in human cardiomyocytes. One study showed that the presence of bicarbonate within cardiomyocytes has a stimulatory effect on cardiac contractility, and impaired bicarbonate secretion may account for dysregulation of cardiac function.[15] We speculate the impaired bicarbonate conductance or permeation through the CFTR pore channel may contribute to more severe maladaptive cardiac remodeling in patients with DMD.

Another limitation of the current study is the relatively small sample size. Therefore, to reduce the number of variants to be considered, we defined the *potential risk factor* by applying a hard filter of being submitted as "pathogenic" to ClinVar on at least 1 occasion, which weights the prior knowledge to a great extent. However, there are often conflicting assertions on the same variant from different submitters. Nevertheless, by focusing on genes enriched with qualified variants we were able to show patients with DMD with *CFTR* risk factor variants have lower LVEF with worse cardiomyopathy compared with noncarriers. Future studies with a larger sample size to confirm the genetic modifier effects of *CFTR* are warranted.

Although the data provided in this study are limited regarding the precise effect of the *CFTR* risk factor variants on cardiac function in patients with DMD and the results need to be replicated in another cohort of patients, it does provide a testable hypothesis for further investigation. For example, induced pluripotent stem cells can be generated from patients with DMD carrying *CFTR* risk factor variants, and then functional studies can be undertaken in induced pluripotent stem cell–derived cardiomyocytes. If the current results can be verified in another cohort of patients with DMD, it would provide strong support that *CFTR* variants may accelerate maladaptive cardiac remodeling in patients with DMD. Verification of these results would have important clinical implications, as genetic modifiers would need to be screened in all patients with DMD. If the screening identifies a risk factor variant within the *CFTR* gene, aggressive guideline-directed heart failure medical therapy should to be initiated at an early age. Finally, cardiac-specific strategies to develop gain-of-function therapies in *CFTR* need to be investigated for potential beneficial effects within this population of patients.

ARTICLE INFORMATION

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Affiliations

From the Department of Internal Medicine (X.J., F.G.A., A.A.A., M.H.D., P.P.A.M.), Department of Population & Data Sciences (Y.S., C.X.), Department of Neurology & Neurotherapeutics (B.M.G.), Department of Bioinformatics (C.X.), Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (X.J., P.P.A.M.), Heart Failure, Ventricular Assist Device & Heart Transplant Program (F.G.A., A.A.A., M.H.D., P.P.A.M.), Eugene McDermott Center for Human Growth and Development (C.X.) and Hamon Center for Regenerative Science and Medicine (P.P.A.M.), UT Southwestern Medical Center, Dallas, TX; and Department of Statistical Science, Southern Methodist University, Dallas, TX (Y.S.).

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Disclosures

None.

Supplementary Materials

Tables S1–S4
Figures S1–S3

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SUPPLEMENTAL MATERIAL

Table S1. Demographic characteristics of male DMD patients and female DMD carriers.

Family	Subject	Relationship*	Age	Sex	Race†	DMD mutation‡	Medication				
							ß-blocker	ACEI	ARB	MRA	Digoxin
Male DMD patients with corresponding DMD carrier mothers											
F1	1499	Proband	32	M	EUR	exons 45-50 deletion	N	Y	N	Y	N
	1604§	Mother	59	F	EUR		N	N	N	N	N
F2	1564	Proband	29	M	EUR	exons 40-43 deletion	Y	N	Y	N	N
	1472	Mother	56	F	EUR		N	Y	N	Y	N
F3	1567	Proband	23	M	AMR	p.Arg1051X	Y	Y	N	N	Y
	1566	Mother	46	F	AMR		Y	Y	N	N	N
	1806	Cousin	24	M	AMR		N	Y	N	N	N
F4	1620	Proband	32	M	EUR	exon 51 deletion	Y	Y	N	Y	Y
	1621	Sibling	30	M	EUR		Y	Y	N	N	Y
F5	1680	Proband	17	M	EUR	exon 2 duplication	Y	Y	N	Y	N
	1681	Mother	47	F	EUR		N	N	N	N	N
F6	1927§	Proband	<10	M	EUR	exon 38 deletion	N	N	N	N	N
	1954	Mother	39	F	EUR		N	N	N	N	N
F7	1947	Proband	16	M	EUR	p.Glu1579X	Y	Y	N	Y	N
	1756	Mother	49	F	EUR		Y	N	Y	Y	N
F8	1520#	Proband	39	M	EUR	exon 45 deletion	Y	Y	N	N	N
	1521#	Mother	58	F	EUR		Y	Y	N	N	Y
F9	1542#	Proband	36	M	EUR	exon 9 duplication	Y	Y	N	N	N
	1531#	Mother	65	F	EUR		Y	Y	N	N	Y
Male DMD patients without corresponding DMD carrier mothers											
F10	1398	Proband	23	M	AMR	exons 49-52 deletion	Y	Y	N	Y	N
F11	1407	Proband	26	M	EUR	exon 44 deletion	Y	Y	N	Y	N
F12	1457	Proband	26	M	EAS	exons 45-50 deletion	Y	Y	N	Y	N
F13	1500	Proband	23	M	EUR	exon 51 deletion	Y	Y	N	N	Y
F14	1622	Proband	21	M	EUR	c.831+1G>T	N	Y	N	N	N
F15	1887	Proband	24	M	EUR	exons 18-29 deletion	Y	Y	N	Y	N
F16	1895	Proband	26	M	SAS	exon 45 deletion	Y	Y	N	Y	Y
F17	1926#	Proband	37	M	AMR	exons 49-52 deletion	Y	Y	N	Y	N
F18	1494#	Proband	24	M	EUR	exon 52 deletion	N	Y	N	N	Y
F19	1532#	Proband	30	M	AMR	exon 45 deletion	Y	N	N	Y	Y
F20	1981**	Proband	22	M	EUR	exons 22-34 deletion	Y	N	N	N	N
Female DMD carriers without their corresponding DMD sons											

F21	1772	Mother	48	F	EUR	p.Gln502X	N	Y	N	N	N
F22	1797	Mother	60	F	EUR	exons 46-52 deletion	Y	N	Y	N	N
F23	1525[#]	Mother	41	F	EUR	exon 70 deletion	N	N	N	N	N
F24	1539[#]	Mother	45	F	EUR	exons 53-55 deletion	N	N	N	N	N

[*]Relationship to the proband.

M: Male and F: Female.

[†]Following the 1000 Genomes Project convention:
AMR: Admixed American (i.e., Latinos).
EAS: East Asian.
EUR: European.
SAS: South Asian.

[‡]Annotated to transcript NM_004006.

Cardiac function was assessed by cardiac MRI except where indicated:
[§]No cardiac assessment.
[#]Cardiac function assessed by ECHO.
[**]Cardiac function assessed by cardiac CT scan.

Y: Yes and N: No.

Medications used to treat DMD-associated cardiomyopathy includes:
β blocker: β-adrenergic receptor antagonist.
ACEI: angiotensin-converting enzyme inhibitor.
ARB: angiotensin-II receptor blocker.
MRA: mineralocorticoid receptor antagonist.

Table S2. Primers for Sanger Sequencing of *CFTR* variants.

Variants	Forward Primer	Reverse Primer
p.Arg75Gln	TTAGAAGGAAGATGTGCCTTTCAAAT	CAGGGTCTATGATGGAACTAACAGA
p.Gly576Ala	TTCAGTGAATCGATGTGGTGAC	CAAGGCAATGATACTGCAAAAACT
p.Leu997Phe	AATAAATCACTGACACACTTTGTCCA	TGAATGTCCTGTACACCAACTGT

Table S3. List of 48 potential risk factor variants identified.

rsID	Gene	Effect	MAF*	Subjects
rs142129409	PADI3	p.Leu112His	4.5E-3	1604,**1756,1947**
rs532781899	FCN3	p.Leu117fs	1.6E-2	1927
rs28941785	CTH	p.Thr67Ile	6.3E-3	1398
rs74315342	NPHS2	p.Arg138Gln	5.8E-4	1797
rs121908120	WNT10A	p.Phe228Ile	1.4E-2	1472,1564,**1756,1947**
rs370474706	COL4A4	p.Gly996Arg	3.6E-5	1926#
rs13078881	BTD	p.Asp446His	3.1E-2	1407,**1680,1681**,1887,1895,1981**
rs375683615	PLCD1	p.Ala574Thr	1.8E-4	1539#
rs587777331	QARS	p.Gly45Val	2.4E-5	1981**
rs121965065	F11	p.Phe460Val	4.0E-6	**1499,1604**
rs397507178	RAD50	p.Glu723fs	2.6E-4	1797,1981**
rs62638624	GRM6	p.Gln708*	7.2E-5	1494#
rs34324426	PEX6	p.Arg601Gln	3.0E-3	1887
rs1800076	CFTR	p.Arg75Gln	1.6E-2	1407,1500,1887
rs1800098		p.Gly576Ala	5.0E-3	**1620,1621**
rs1800111		p.Leu997Phe	2.2E-3	1542#
rs41341748	MSR1	p.Arg311*	8.2E-3	1532#
rs148665132	DGAT1	c.751+2T>C	1.3E-4	1926#
rs104894103	APTX	p.Trp293*	1.8E-4	1680
rs117225135	DHTKD1	p.Gly729Arg	1.6E-3	1564
rs35947132	PRF1	p.Ala91Val	2.9E-2	1622,**1680,1681**
rs8192466	BDNF	p.Thr84Ile	1.3E-3	**1620,1621**
rs61731956	NR1H3	p.Arg415Gln	2.2E-4	1887
rs371401403	MYBPC3	p.Pro873His	7.2E-5	1539#
rs104894299	RAPSN	p.Asn88Lys	1.5E-3	**1620,1621**
rs121912638	NDUFS8	p.Arg102His	2.0E-5	1520#
rs201539845	MYO7A	p.Asp218Asn	4.6E-5	1680
rs5742912	SCNN1A	p.Trp552Arg	1.9E-2	1532#,1887,1981**
rs5030861	PAH	c.1315+1G>A	4.0E-4	1494#
rs80359405	BRCA2	p.Val1283fs	5.4E-5	**1756, 1947**
rs35312232	TGM1	p.Val518Met	1.1E-2	**1472,1564**,1622
r s35026927	PYGL	p.Asp634His	3.8E-3	**1927,1954**
rs80338765	FBLN5	p.Gly243Arg	2.6E-4	1887
rs118203962	STRA6	p.Thr360Pro	5.1E-4	1520#
rs11555096	FAH	p.Arg341Trp	1.7E-2	1521#,1797
rs113994095	POLG	p.Ala467Thr	5.1E-4	1494#
rs749969667	CHD2	p.Gln1392fs	4.2E-3	1398
rs190521996	PMM2	p.Phe157Ser	3.2E-4	1499
rs138680796	ACSF3	p.Arg471Trp	2.9E-4	1525#
rs796296176	MC1R	p.Asn29fs	2.0E-3	1407
rs1805009		p.Asp294His	9.2E-3	1564,**1566,1567**,1887
rs121908970	MYO15A	p.Thr2205Ile	4.5E-3	**1531#,1542#**
rs145457535	CCDC103	p.His154Pro	1.2E-3	1797
rs35897051	MPO	c.2031-2A>C	4.5E-3	1564
rs527236149	SCN4A	p.Arg1129Gln	7.9E-5	1532#
rs104886461	MCOLN1	c.406-2A>G	1.9E-4	1525#
rs74315416	PROKR2	p.Leu173Arg	2.2E-3	**1531#,1542#**
rs387907018	TMPRSS6	p.Glu513Lys	1.6E-5	1947

*Global minor allele frequency in the genome aggregation database.

#Cardiac function measured by ECHO.

**Cardiac function measured by cardiac CT scan.

Bold: DMD patients and DMD carriers from the same family.

Table S4. *MC1R* potential risk factor variants identified in DMD patients.

Position*	Reference Allele	Alternative Allele	rsID	Effect†	ClinVar	GERP Score	PolyPhen Score	MAF‡	Subjects
89985750	C	CA	rs796296176	N29fs	Y	NA	NA	2.2E-3	1407
89986546	G	C	rs1805009	D294H	Y	5.27	1	7.8E-3	1564,**1566,1567**,1887

*Mapped to human reference genome b37.

†Annotated to transcript NM_002386.

‡Global minor allele frequency in the genome aggregation database.

Bold: DMD patients and DMD carriers from the same family.

NA: Not Applicable.

Figure S1. The study flow chart.

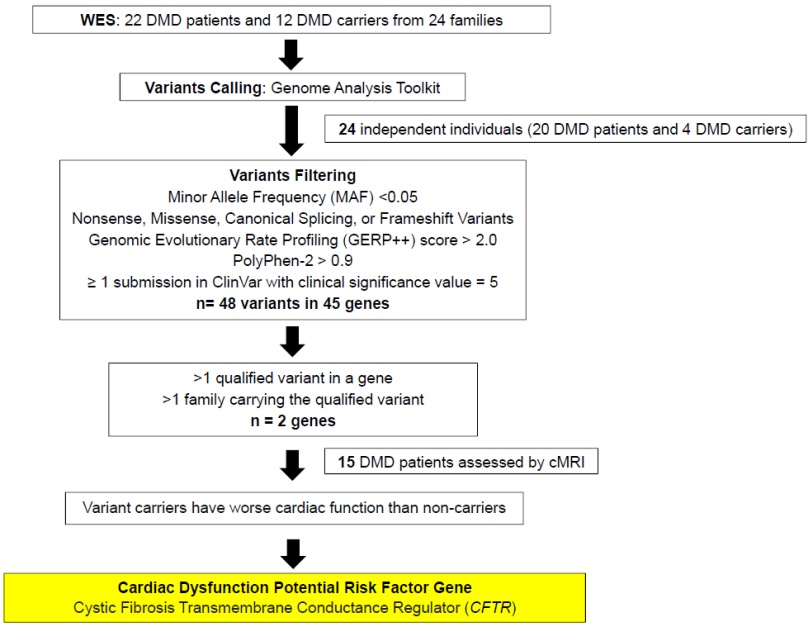
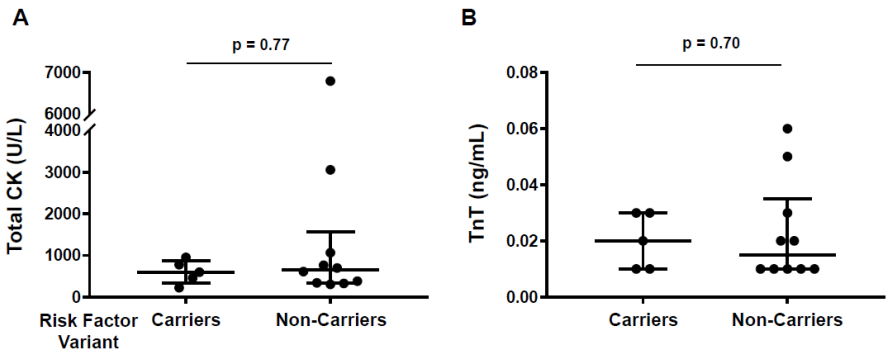
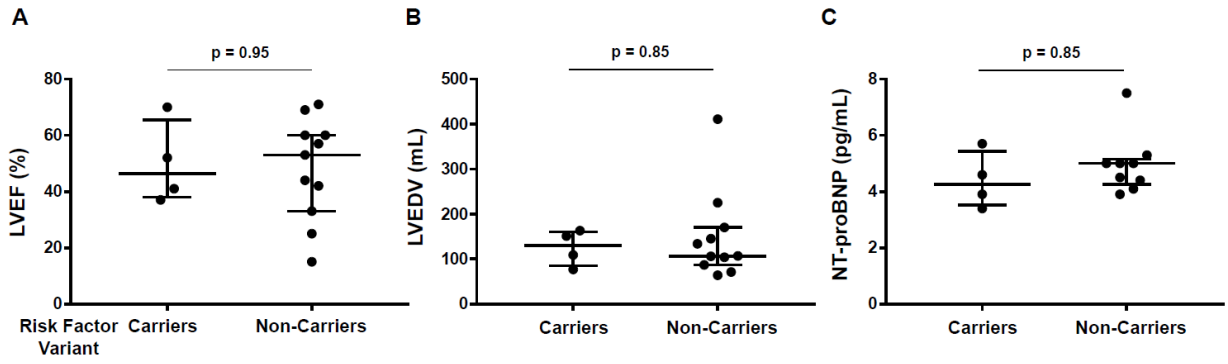


Figure S2. Comparison of circulating biomarkers between *CFTR* genotype groups.



The levels of **(A)** total creatine kinase (CK) and **(B)** Troponin T (TnT) were compared between 5 patients carrying *CFTR* risk factor variants, and 10 noncarriers. Each dot represents a unique individual. *Median* and inter*quartile range are indicated.* Comparison was performed by the Mann–Whitney–Wilcoxon test.

Figure S3. Comparison of prognostic cardiac markers between *MC1R* genotype groups.



The levels of **(A)** left ventricular ejection fraction (LVEF), **(B)** left ventricular end-diastolic volume (LVEDV), **(C)** N-terminal prohormone B-type natriuretic peptide (NT-proBNP) were compared between 4 patients carrying *MC1R* risk factor variants, and 11 noncarriers. Each dot represents a unique individual. *Median* and inter*quartile range are indicated.* Comparison was performed by the Mann–Whitney–Wilcoxon test.