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Rare Variants in Cardiomyopathy Genes Associated With Stress-Induced Cardiomyopathy

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Abstract

Background—Stress-induced cardiomyopathy (SIC) is a poorly understood condition associated with periods of emotional and physical stress. The clinical approaches for management of SIC are supportive and reactive to patient symptoms.

Objective—To utilize next-generation exome sequencing to define genetic variation associated with, and potentially responsible for, this disease.

Methods—We performed exome sequencing of 7 white female patients with SIC. Filtering of the identified variants was performed to limit our investigation to those sequences that passed quality control criteria, were rare or novel, were determined algorithmically to have high impact on the associated protein, and were within regions of high species conservation. All variants were verified using Sanger sequencing.

Results—Exome-sequencing analysis revealed that each patient carried predicted deleterious variants affecting known cardiomyopathy genes. In each case, the identified variant was either not previously found in public human genome data or was previously annotated in a database of clinical variants associated with cardiac dysfunction.

Conclusion—Patients with SIC harbor deleterious mutations in established cardiomyopathy genes at a level higher than healthy controls. We hypothesize that patients at highest risk for SIC likely live in a compensated state of cardiac dysfunction that manifests clinically only after the myocardium is stressed. In short, we propose that SIC is another example of an occult cardiomyopathy with a distinct physiological trigger and suggest that alternative clinical approaches to these patients may be warranted.

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Disclosure

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Keywords

genomics; stress-induced cardiomyopathy; takotsubo

Introduction

Stress-induced cardiomyopathy (SIC), also known as takotsubo cardiomyopathy, is a rare condition associated with periods of intense physical or emotional distress.^{1–3} This cardiomyopathy is characterized by a mild elevation in serum troponins, reduced ejection fraction, and electrocardiographic abnormalities, including QeTc prolongation and T wave and ST segment changes.⁴ The characteristic echocardiographic feature of this disease is apical ballooning that resembles the shape of a Japanese octopus trap, and hence the Japanese name takotsubo “octopus pot.”^{5, 6} To date, the etiology of this disease is not well established. Given the rarity of this unique form of cardiac dysfunction, and the advances in next-generation sequencing technology, we sought to apply whole exome sequencing in order to better delineate the genomic loci associated with this disease. By identifying these loci, identification of this condition may be possible, as well as improved risk assessment of patients afflicted by SIC.

Methods

Patient Population

{Blinded institution}, located in {blinded city}, is the major neurovascular referral center for the state of {blinded}. Between 2008 and 2012, we treated a total of 879 patients with ruptured cerebral aneurysms, averaging 176 patients per year.

From 2005 to 2013, a total of 21 patients were diagnosed with SIC after aneurysmal subarachnoid hemorrhage. Seven white patients consented to be included and were enrolled in this study. The patients' medical histories and diagnostic and therapeutic imaging studies were evaluated. Blood samples were collected for exome sequencing at the time of hospitalization or at follow-up.

Next-Generation Exome Sequencing

Library Preparation and Sequencing—Genomic DNA was extracted from whole blood using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, N.V., Venlo, Netherlands). Exome sequencing was performed on 7 affected individuals using the Illumina platform as follows: 1µg of gDNA was sheared to 100–900 bp fragments with the Covaris E210 (Covaris, Inc., Woburn, MA). Libraries were prepared with the Illumina TruSeq DNA Sample Prep Kit v2 (Illumina, Inc., San Diego, CA) following the manufacturer's protocol for the gel-free method, with the exception that only 8 polymerase chain reaction (PCR) cycles were used instead of 10. Libraries were quantified via Picogreen (Invitrogen, Carlsbad, CA) and 500 ng of each sample was pooled prior to exome enrichment with the Illumina 62 Mb TruSeq Exome Enrichment Kit (Illumina, Inc.) following the manufacturer's protocol. The final library was then validated on a 2100 high-sensitivity for DNA assay Bioanalyzer chip (Agilent Technologies, Inc., Santa Clara, CA) and the library was

quantified by quantitative PCR (qPCR) using the Kapa Library Quantification Kit (Kapa Biosystems, Inc, Wilmington, MA) on the 7900HT (Applied Biosystems, Foster City, CA). The exome libraries were sequenced by 100 bp paired end sequencing on a HiSeq 2500 System (Illumina, Inc.).

Quality Control and Variant Calling—After bar-coded sequencing of the samples, the BCL Converter v. 1.8.3 bcl2fastq module (Illumina, Inc.) was run, allowing one mismatch in the barcode sequence and only outputting passing filter reads in FASTQ format.⁷ A custom Perl script was used to remove the white space in the read name and format to include the barcode and denote whether the sequence is from read one or two. We then used bcbio-nextgen (v. 0.7.9a-6764030) to map reads to the human genome (GRCh37) with the Burrows-Wheeler Aligner, bwa mem (v. 0.7.7)⁸ bcbio-nextgen “gatk-variant.yaml” template (commit 701a5d0fb5d). The bcbio-nextgen gatk-variant pipeline follows the best practices outlined in the Genome Analysis Toolkit (GATK),^{9, 10} and for this work we used Picard v. 1.96 for mark duplicates and GATK v. 3.1-1 BSQR and VSQR recalibration, indel-realignment, and Unified Genotyper. Variants were annotated with SnpEff v. 3.4¹¹ using the ensemble human GRCh37.74 build.

We restricted our initial analysis to protein-coding genes and excluded variants with no impact on protein sequence or splicing efficacy/efficiency (i.e., intronic, synonymous, and intragenic variants were set aside during initial analysis). The output from SnpEff was filtered to include only one line per variant after the previous filters are applied, so genes with multiple transcripts were not duplicated in our downstream analysis. We then used a custom Perl script to parse the VCF and the filtered SnpEff file, and to query each variant against our in-house MongoDB v. 2.6.4 database that includes all variants sequenced in our lab, functional consequences of variants as calculated by dbNSFP,¹² the National Heart, Lung and Blood Institute (NHLBI) Exome Sequencing Project build ESP6500SI-V2, Gene Set Enrichment Analysis,¹³ National Center for Biotechnology Information (NCBI) RefSeq,¹⁴ and NCBI ClinVar.¹⁵ This final in-house database query allows us to annotate variants that may not be in the public database domains due to a myriad of reasons, but which are actual common variants as indicated by their presence in our in-house sequencing database. The Perl script uses the Excel::Writer::XLSX to produce a final variant report for the sequenced individual or cohort.

Variant Filtering—Variants for each patient were filtered using the same schema (see Figures 1–8, Supplemental Digital Content 1). The variant filtering approach was as follows: 1) only protein altering variants were considered; 2) variants must pass the FreeBayes derived haplotypic scoring quality control (arXiv:1207.3907); and 3) variants must be covered to a depth of at least 20 sequencing reads.

The variants were then separated into two analytical streams consisting of those variants that were found to be present in ClinVar (the ClinVar filtration approach) and those that were not (the Research filtration approach). For variants found in ClinVar, we considered only those variants that 1) were previously associated with cardiomyopathy; 2) were found to be pathogenic; and 3) had passed visual inspection in the Integrative Genomics Viewer (IGV) (see Figures 9–15, Supplemental Digital Content 1). Variants in the ClinVar analysis stream

that met those three additional criteria were prioritized for Sanger validation, and any variants that were validated were considered for that patient.

Variants that were not present in ClinVar were only retained if they 1) demonstrated a frequency of less than or equal to 1% in the NHLBI GO ESP European cohort; 2) had less than 20 reported variants within the gene for the patient of interest (in our experience, variants with high “gene events” rates are more likely false positives); 3) correlated to no homozygotes in TGen’s internal database of sequenced genomes and exomes; 4) demonstrated a Combined Annotation Dependent Depletion (CADD)¹⁶ score equal to or greater than 13.65 (which represents the top 50% of scored variants); 5) were expressed in human heart tissue at an FPKM (fragments per kilobase pair of exon per million fragments mapped) of greater than or equal to 1.0; 6) were previously associated with cardiomyopathy; and 7) passed visual inspection in IGV. Variants in this analysis stream that met those seven additional criteria were prioritized for Sanger validation, and any variants that were validated were considered for that patient. Comparisons were made to a cohort of control samples. These samples were derived from deceased donors older than age 80 years who had little to no demonstrable neuropathology at autopsy. This cohort was selected as controls for the SIC cohort, as they had very low likelihood of cardiomyopathy.

Next-Generation RNA Sequencing and Analysis

RNA sequencing was performed using 1.0 ug of total RNA quantified via Nanodrop (Thermo Fisher Scientific, Inc., Pittsburgh, PA). Samples were generated from a healthy donor heart and used for analysis as a control. A sequencing library was prepared with the Illumina TruSeq RNA Sample Preparation Kit v2 (Illumina, Inc.) following the manufacturer’s protocol. In brief, poly-A containing mRNA molecules were purified using poly-T oligo-attached magnetic beads. The mRNA was then thermally fragmented and converted to double-stranded cDNA. The cDNA fragments were end-repaired, a single “A” nucleotide was incorporated, sequencing adapters were ligated, and fragments were enriched with 15 cycles of PCR. Final PCR-enriched fragments were validated on a 2100 Bioanalyzer (Agilent Technologies, Inc.) and quantified by qPCR using the Kapa Library Quantification Kit (Kapa Biosystems, Inc.) on the 7900HT (Applied Biosystems). The final library was sequenced by 50 bp paired-end sequencing on a HiSeq 2000 (Illumina, Inc.).

Raw reads passing Illumina quality filters were converted to FASTQ format in Phred33 scale with CASAVA 1.8.3. RNA-Seq reads were aligned with TopHat (v2.0.8)¹⁷, which first utilizes Bowtie (v2.1.0.0)¹⁸ to map reads with “splice-aware” alignments to the Homo Sapiens build GRCh37 from Ensembl.¹⁹ To estimate the library fragment size for TopHat, we initially mapped a subset of 1 million reads with bwa (v 0.6.1) to the human genome, followed by picard version 1.80 (“<http://picard.sourceforge.net>,” n.d.) module CollectInsertSizeMetrics and provided these values to TopHat options--mate-inner-dist 87--mate-std-dev 86. Additional TopHat flags utilized were --transcriptome-index (to Ensembl GRCh37.70), --no-coverage-search, --b2-sensitive, and--keep-fasta-order. Next, we calculated gene expression values expressed as FPKM using cufflinks version 2.1.1.²⁰ We used the --GTF option in cufflinks to annotate to human gene models GRCh37.70.

Additionally, we used the --multi-read-correct and --frag-bias-correct options in cufflinks and masked tRNAs, rRNAs, and mtRNAs as suggested in the cufflinks documentation.

Splice Site Scoring

Estimates of the strengths of 5' and 3' splice junction variants were formulated using two splice site models. The first is a MaxEntScan model proposed by Yeo and Burge (Web = http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html)²¹ that associates a log₁₀ (O.R.) score with a given splice site sequence motif (9-mer for 5' junctions and 23-mer for 3' junctions). Higher score assignments indicate a higher probability of the sequence acting as a true splice site. Mutant splice sequence scores of patients harboring splice variants were held in comparison to RefSeq-derived wild-type reference sequences. Scores generated from this approach are equal to the difference of MaxEnt wild-type and mutant scores, where the difference is denoted by *ME*.

Sanger Sequencing

All variants were confirmed with Sanger sequencing (see Figures 16–18, Supplemental Digital Content 2 and the Table, Supplemental Digital Content 2). Primers surrounding the identified variants were designed using primer3. PCR was carried out under standard conditions and direct sequencing of the PCR products was performed on an ABI3130XL sequencer (Applied Biosystems).

Study Oversight

The Institutional Review Boards of {blinded} approved the study.

Results

Patient Information

From January 2005 through January 2013, a total of 21 patients with stress-induced cardiomyopathy after aneurysmal subarachnoid hemorrhage were treated at our institute. All patients met the modified Mayo Clinic criteria for a diagnosis of SIC. Of this cohort, 7 white patients provided consent for inclusion in this study (Table 1). All patients were female (mean age, 51.9 years; median, 47 years; range, 42–77 years). Five patients harbored aneurysms in the anterior circulation and two in the posterior circulation. All aneurysms were occluded within 24 hours of admission. Patients were assigned to endovascular (n = 4) or microsurgical treatment (n = 3) of their aneurysms. On the basis of angiographic evaluation and transcranial Doppler (TCD) ultrasonography, 6 patients suffered from severe and 1 patient suffered from moderate cerebral vasospasm with associated neurological decline. These patients were aggressively treated using standard medical and endovascular treatment of vasospasm.^{22, 23} None of the patients had significant prior cardiac history. Stress-induced cardiomyopathy was diagnosed clinically and by echocardiography. The mean time from aneurysm rupture to myocardial dysfunction was 5.4 days (range, 1–8 days). The mean ejection fraction for patients at the time of diagnosis was 18.6% (range, 10%–30%). Patients were treated with intra-arterial balloon pump (IABP) for a mean of 6.6 days (range, 5–11 days). At a mean follow-up of 20.6 months (range, 2–50 months), the mean modified Rankin scale (mRS) score was 2 (range, 1–5).

Rare Genetic Variants Identified by Exome Sequencing

A list of DNA variants identified in patients with SIC is provided in Table 2. Patient A01 was determined to harbor two variants after filtering. The first was a ClinVar variant that is a non-synonymous coding change in DSG2 (rs121913013), the gene for desmoglein 2. This variant is found with a frequency of 0.35% in NHLBI GO-ESP (31 recorded heterozygotes) and has a CADD score of 11.8 and a PhyloP of 0.73. The variant itself is located in the codon for amino acid residue 56, which resides six residues upstream of the most proximal Ca^{2+} binding domain of the first cadherin repeat in desmoglein 2. DSG2 is expressed moderately in our sequenced heart tissue sample (FPKM = 3.5), is a known calcium-binding transmembrane protein, and this same variant was linked previously to arrhythmogenic right ventricular cardiomyopathy by Awad et al.,²⁴ Pilichou et al.²⁵ and Syrris et al.²⁶ and to dilated cardiomyopathy predisposition by Posch et al.²⁷ The second variant in this patient was a non-synonymous coding change in SCN5A (rs1805124), which codes for voltage-gated ion channels. It has a CADD score of only 0.34 and a PhyloP of 0.37. This ClinVar variant has three Clinical Significance determinations, two of which are Benign and one being Pathogenic (as classified by a single submitter). It is present at a frequency of 23% in NHLBI GO-ESP (2300 recorded heterozygotes) and is expressed moderately in the heart (FPKM = 18.3). This variant is located in the codon corresponding to amino acid residue 558 of exon 12 (NM_001160161) of the gene. Mutations in this gene have been associated with several cardiomyopathies, including familial atrial fibrillation, right bundle branch block (Brugada Syndrome 1), dilated cardiomyopathy, progressive (IA) and non-progressive heart block, long QT syndrome, sick sinus syndrome, and familial ventricular fibrillation.^{28–31}

Patient A02 was found to have a ClinVar variant in a gene previously linked to cardiomyopathy. The change is a non-synonymous coding change in MYLK2 (rs34396614, 2% frequency in Europeans in NHLBI GO-ESP, CADD = 14.3). The expression level of MYLK2 in human heart tissue was found to be very low (FPKM = 0.49) and the level of conservation at this variant as measured by PhyloP was found to be only 0.26. Patient 2 was also found to have a second mutation that survived our filtration approach located within the splice site region (–3 bp from an intron/exon boundary) in the LDB3 gene (aka ZASP or CYPHER). LDB3 is a component of the Z-line in both skeletal and cardiac muscle, and mutations in the gene have been linked to dilated cardiomyopathy and left ventricular dysfunction.³² Due to its proximity to the 5' end of the splicing consensus sequence, this variant is predicted to alter LDB3 splicing at exon eight (referencing transcript NM_001171610). Intronic variants like this one are not scored by CADD or PhyloP. Investigation of this variant using the splice site prediction algorithms suggested that the impact of the variant will not significantly alter splicing (Max Entropy score of 11 for the reference allele and 10.47 for the variant). LDB3 is highly expressed in human heart tissue with an FPKM equal to 390.

In Patient A03, we identified three potential variants associated with SIC risk. The first variant was a frame-shift variant in FKTN that is predicted to result in premature truncation of the protein just prior to the transmembrane encoding portion of the transcript. This variant has a 0.01% frequency in NHLBI GO ESP (one heterozygote carrier is present in the

database) and is not scored by CADD (frame shift changes are not assigned a CADD score). FKTN mutations can cause Fukuyama-type congenital muscular dystrophy, a disease characterized by severe muscle wasting from infancy as well as mental retardation,³³ but FKTN variants have also been shown by Murakami et al.³⁴ to be associated with a much wider clinical spectrum of disease, including dilated cardiomyopathy, with little to no muscular dystrophy and normal intelligence. The second variant is a non-synonymous coding change in DSP (rs148147581). The role of DSP in pathogenesis of cardiomyopathy is discussed below under patient A05. The third variant is the same non-synonymous coding change in SCN5A (rs1805124) as identified in patient A01.

Patient A04 was determined to harbor one variant following the implementation of the filtering approach. This variant was the same non-synonymous coding change in SCN5A as was found in Patients A01, A03, and A06 (rs1805124).

Patient A05 was found to harbor two relevant variants post-filtration. The first was a non-synonymous coding change in DSP (rs148147581) that has a frequency of 0.06% in NHLBI GO-ESP (five recorded heterozygotes), a CADD score of 21, and a PhyloP of 0.64. DSP is noted to be expressed moderately in the heart (FPKM = 23), while cases of dilated left and right ventricular cardiomyopathy have been detailed in cases of human DSP mutants by Norgett et al.³⁵ and Rasmussen et al.,³⁶ respectively. This variant is located in the plectin repeat domain of the desmoplakin protein (with IPR001101 as the reference, this would occur at amino acid residue 2103). The second variant found in Patient A05 was a splice site region mutation in RYR2 (rs72751287) that has a frequency of 0.32% in NHLBI GO-ESP (29 recorded heterozygotes). The mutation is an A-to-G substitution that is located -4 bp 5' (upstream) to the intron-exon boundary, and is therefore predicted to affect splicing of exon 72 (referencing transcript NM_001035). Max Entropy scoring of this variant suggested that it should influence splicing (score of 10.38 for reference allele and -1.54 for variant allele). RYR2 is also moderately expressed in the heart (FPKM = 19), while mutations therein have been detailed to result in familial polymorphic ventricular tachycardia by Laitinen et al.³⁷ and catecholaminergic polymorphic ventricular tachycardia (CPVT) by Medeiros-Domingo et al.³⁸ and Priori et al.^{39, 40}

Patient A06 was found to have two variants of interest after filtering. The first was a splice site donor mutation in ME1 (0.8% frequency in NHLBI GO-ESP, CADD = 27.8, and PhyloP = 0.86). This variant is predicted to alter the splice donor sequence by changing a cytosine nucleotide one bp downstream of exon 3 to a thymine. The resulting effect of this variant should be decreased splicing of exon 3 (referencing transcript NM_002395). This variant had a Max Entropy score of 1.61 compared to the reference allele score of 9.79. The second variant found in Patient A06 was the previously discussed non-synonymous coding change in SCN5A (rs1805124).

Patient A07 was determined to harbor one variant of interest post-filtration. This variant was a stop-gained mutation in CD36 (rs201657731), with a frequency of 0.01% in NHLBI GO-ESP (one recorded heterozygote) and a CADD score of 37 (PhyloP = 0.24). CD36 is highly expressed in cardiomyocytes (overall heart FPKM = 247), and mutations of this sequence

were shown by Coburn et al. to reduce fatty acid uptake and subsequent metabolism in the heart by 50%–80%.⁴¹

Rare Cardiomyopathy Genetic Variants in Control Individuals

We also investigated the resulting variants that were identified when the exact same filtration approaches were applied to healthy control individuals. We selected a cohort of 14 Caucasian individuals who lived to at least 80 years of age without a history of cardiac dysfunction and who demonstrated little to no neuropathology at autopsy and obtained brain tissue from this cohort for the analysis. None of these individuals was diagnosed with a cardiomyopathy, and because of their general longevity we hypothesized that they also would not carry risk factors for cardiomyopathy. We investigated the variants in these individuals using the same filtration approach as with our SIC patients and assigned the variants into three classes: 1) ClinVar (for those variants that were present in ClinVar); 2) Research (for those variants identified that were not in ClinVar but survived filtration); and 3) Total (the sum of ClinVar and Research). The resulting mean variants in each of these classes was compared to the same classes of variants in the SIC patients using a two-tailed Mann-Whitney U-test to account for low sample numbers and the potential for non-normal distribution of the data. The results were non-significant for the ClinVar variants ($p = 0.529$, $Z\text{-score} = -0.6341$). We hypothesized that this may be due to the presence of the common variant within SCN5A in both groups; however, after removing just that variant, the ClinVar results were still non-significant ($p = 0.453$, $Z\text{-score} = -0.746$). The Research variant comparisons were statistically significant ($p = 0.01$, $Z\text{-score} = -2.574$), and the Total variant comparisons were also significant ($p = 0.00466$, $Z\text{-score} = -2.835$). Of note, the control cohort carried zero variants that were identified using the Research filtration approach (compared to a mean $\pm$ SD of 1.0 ± 0.82 for individuals in the SIC cohort).

Discussion

Stress-induced cardiomyopathy is a poorly understood and likely underdiagnosed phenomenon. The etiology of this disease is unknown. Although a genetic contribution to this disease has been speculated,^{42–45} no such definitive fingerprint has been described. Clinically, SIC is challenging to treat in the critically ill patient. One of the challenges with SIC is with differentiating this disease from myocardial infarction, as the presenting symptoms of these diseases, chest pain, elevated serum troponins and ST-elevation on echocardiogram, are identical. Cardiac angiography can definitively distinguish between these diseases. Although echocardiography demonstrates the classic apical ballooning and decreased ejection fraction, a blood-based biomarker for this disease has been elusive until recently.⁴⁶

Therapeutically, the impaired cardiac function in the setting of SIC leads to severe pulmonary edema and hypotension requiring inotropic agents such as dobutamine or milrinone and/or vasopressors such as norepinephrine. These agents, in turn, further stress the already malfunctioning myocardium, leading to exacerbation of the disease. In many cases, cardiac malfunction necessitates placement of patients on IABP for support.⁴⁷ Therefore, there are two populations of patients who develop SIC after subarachnoid

hemorrhage: those whose SIC is caused by aneurysm rupture and those who develop SIC secondary to the stressing of the myocardium by triple-H therapy.

Broadly, identification of patients at risk for SIC, based on genetic predispositions, would allow for tailored treatment upon admission of these patients to the intensive care unit and prior to cardiac and neurological decline (Fig. 1). The panel of genes identified by our exome analysis provides a means of identifying patients who may be at risk for developing cardiac malfunction.

Exome analysis of patients with SIC highlights a further interesting peculiarity. Six of the seven patients in our cohort contained heterozygote low-frequency variants in established cardiomyopathy genes. The incidence of individuals in the population who are heterozygote carriers of defects in cardiomyopathy genes is not well-established, but we postulate this situation represents a very minor subset of the general population given the rarity of this disease. Given that the identified variants have previously been associated with various forms of cardiomyopathy, our finding suggests that these patients may live, under non-pathologic conditions, in a compensated state. We postulate that the adrenergic surge associated with extreme stress overwhelms the system, resulting in cardiac dysfunction in this population.³ Therefore, it is possible that more individuals in the population carry such mutations and possibly exist in a compensated state but are never exposed to a significant adrenergic surge to produce SIC. As with other complex diseases, there are likely individuals at high risk for SIC who will avoid the disease all together by avoiding the physiological stressor.

To further examine the genetic findings, we applied the same filtration approaches in a similarly sized cohort of healthy elderly postmortem characterized control samples (Fig. 2). We hypothesized that individuals in this cohort, when processed in the exact same way as the SIC cohort, would carry significantly fewer cardiomyopathy variants. Analysis (by the Mann-Whitney U-test) demonstrated that the SIC cohort was significantly enriched for variants identified by our filtration approach, and specifically for those variants that were highlighted outside of the ClinVar database. A potential criticism of the use of this patient population as a control is the fact that these patients did not harbor aneurysms and that the variants we identified may simply be associated with aneurysm formation. While this is certainly a reasonable assumption, a GWAS have addressed common genetic variants associated with intracranial aneurysm formation. This study⁴⁸ did not identify any of the variants we have discovered as being associated with cerebral aneurysms or enrichment for variants within the genes we report for SIC. Therefore, the variants identified in this study are unlikely to be associated with aneurysms.

Conclusion

Using next-generation sequencing and samples of patients with an ill-understood cardiomyopathy, we have identified a genetic fingerprint associated with this disease that may be used for early identification of patients at risk. With the declining cost and time needed to perform sequencing and analysis, the set of genes identified in this study can be used for early screening and creates an opportunity to affect the clinical care of patients with

this disease. Specifically, we can identify the cohort of patients at risk for developing cardiomyopathy caused by aggressive resuscitation and transition this population to early insertion of balloon pumps to limit iatrogenic induction of SIC. For the patients who present in the immediate post-hemorrhage period with SIC, this screening paradigm is less likely to be useful until the technology becomes much faster and more facile. Identification of deleterious heterozygote mutations in established cardiomyopathy genes suggests a mechanism for cardiac decline in this patient population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Komamura K, Fukui M, Iwasaku T, Hirotani S, Masuyama T. Takotsubo cardiomyopathy: Pathophysiology, diagnosis and treatment. *World J Cardiol.* 2014; 6:602–609. [PubMed: 25068020]
2. Wright PT, Tranter MH, Morley-Smith AC, Lyon AR. Pathophysiology of takotsubo syndrome: Temporal phases of cardiovascular responses to extreme stress. *Circ J.* 2014; 78:1550–1558. [PubMed: 24954393]
3. Coupez E, Eschalier R, Pereira B, Pierrard R, Souteyrand G, Clerfond G, et al. A single pathophysiological pathway in takotsubo cardiomyopathy: Catecholaminergic stress. *Arch Cardiovasc Dis.* 2014; 107:245–252. [PubMed: 24796853]
4. Lee VH, Oh JK, Mulvagh SL, Wijedicks EF. Mechanisms in neurogenic stress cardiomyopathy after aneurysmal subarachnoid hemorrhage. *Neurocrit Care.* 2006; 5:243–249. [PubMed: 17290097]
5. Heggemann F, Hamm K, Brade J, Streitner F, Doesch C, Papavassiliou T, et al. Right ventricular function quantification in takotsubo cardiomyopathy using two-dimensional strain echocardiography. *PLoS One.* 2014; 9:e103717. [PubMed: 25089702]
6. Madias JE. Appropriate implementation of echocardiography in takotsubo syndrome: Earlier and more frequently. *Echocardiography.* 2013; 30:1123–1125. [PubMed: 24579741]
7. Cock PJ, Fields CJ, Goto N, Heuer ML, Rice PM. The sanger fastq file format for sequences with quality scores, and the solexa/illumina fastq variants. *Nucleic Acids Res.* 2010; 38:1767–1771. [PubMed: 20015970]
8. Li H, Durbin R. Fast and accurate long-read alignment with burrows-wheeler transform. *Bioinformatics.* 2010; 26:589–595. [PubMed: 20080505]
9. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, et al. The genome analysis toolkit: A mapreduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 2010; 20:1297–1303. [PubMed: 20644199]
10. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet.* 2011; 43:491–498. [PubMed: 21478889]
11. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, snpeff: Snps in the genome of drosophila melanogaster strain w1118; iso-2; iso-3. *Fly (Austin).* 2012; 6:80–92. [PubMed: 22728672]
12. Liu X, Jian X, Boerwinkle E. Dbnsfp: A lightweight database of human nonsynonymous snps and their functional predictions. *Hum Mutat.* 2011; 32:894–899. [PubMed: 21520341]

13. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A*. 2005; 102:15545–15550. [PubMed: 16199517]
14. Pruitt KD, Tatusova T, Maglott DR. Ncbi reference sequence (refseq): A curated non-redundant sequence database of genomes, transcripts and proteins. *Nucleic Acids Res*. 2005; 33:D501–D504. [PubMed: 15608248]
15. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, et al. Clinvar: Public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014; 42:D980–D985. [PubMed: 24234437]
16. Kircher M, Witten DM, Jain P, O’Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Genet*. 2014; 46:310–315. [PubMed: 24487276]
17. Trapnell C, Pachter L, Salzberg SL. Tophat: Discovering splice junctions with rna-seq. *Bioinformatics*. 2009; 25:1105–1111. [PubMed: 19289445]
18. Langmead B, Salzberg SL. Fast gapped-read alignment with bowtie 2. *Nat Methods*. 2012; 9:357–359. [PubMed: 22388286]
19. Flicek P, Ahmed I, Amode MR, Barrell D, Beal K, Brent S, et al. Ensembl 2013. *Nucleic Acids Res*. 2013; 41:D48–D55. [PubMed: 23203987]
20. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of rna-seq experiments with tophat and cufflinks. *Nat Protoc*. 2012; 7:562–578. [PubMed: 22383036]
21. Yeo G, Burge CB. Maximum entropy modeling of short sequence motifs with applications to rna splicing signals. *J Comput Biol*. 2004; 11:377–394. [PubMed: 15285897]
22. Pandey AS, Elias AE, Chaudhary N, Thompson BG, Gemmete JJ. Endovascular treatment of cerebral vasospasm: Vasodilators and angioplasty. *Neuroimaging Clin N Am*. 2013; 23:593–604. [PubMed: 24156852]
23. Meyer R, Deem S, Yanez ND, Souter M, Lam A, Treggiari MM. Current practices of triple-h prophylaxis and therapy in patients with subarachnoid hemorrhage. *Neurocrit Care*. 2011; 14:24–36. [PubMed: 20838932]
24. Awad MM, Dalal D, Cho E, Amat-Alarcon N, James C, Tichnell C, et al. Dsg2 mutations contribute to arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Am J Hum Genet*. 2006; 79:136–142. [PubMed: 16773573]
25. Pilichou K, Nava A, Basso C, Beffagna G, Bauce B, Lorenzon A, et al. Mutations in desmoglein-2 gene are associated with arrhythmogenic right ventricular cardiomyopathy. *Circulation*. 2006; 113:1171–1179. [PubMed: 16505173]
26. Syrris P, Ward D, Asimaki A, Evans A, Sen-Chowdhry S, Hughes SE, et al. Desmoglein-2 mutations in arrhythmogenic right ventricular cardiomyopathy: A genotype-phenotype characterization of familial disease. *Eur Heart J*. 2007; 28:581–588. [PubMed: 17105751]
27. Posch MG, Posch MJ, Geier C, Erdmann B, Mueller W, Richter A, et al. A missense variant in desmoglein-2 predisposes to dilated cardiomyopathy. *Mol Genet Metab*. 2008; 95:74–80. [PubMed: 18678517]
28. Haas J, Frese KS, Peil B, Kloos W, Keller A, Nietsch R, et al. Atlas of the clinical genetics of human dilated cardiomyopathy. *Eur Heart J*. 2014
29. Beckermann TM, McLeod K, Murday V, Potet F, George AL Jr. Novel scn5a mutation in amiodarone-responsive multifocal ventricular ectopy-associated cardiomyopathy. *Heart Rhythm*. 2014; 11:1446–1453. [PubMed: 24815523]
30. S P. A second case with arrhythmogenic cardiomyopathy, provokable brugada ecg and scn5a mutation. *Int J Cardiol*. 2014; 171:e117–e118. [PubMed: 24388542]
31. Remme CA. Cardiac sodium channelopathy associated with scn5a mutations: Electrophysiological, molecular and genetic aspects. *J Physiol*. 2013; 591:4099–4116. [PubMed: 23818691]
32. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, et al. Mutations in cypher/zasp in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol*. 2003; 42:2014–2027. [PubMed: 14662268]

33. Kobayashi K, Nakahori Y, Miyake M, Matsumura K, Kondo-Iida E, Nomura Y, et al. An ancient retrotransposal insertion causes fukuyama-type congenital muscular dystrophy. *Nature*. 1998; 394:388–392. [PubMed: 9690476]
34. Murakami T, Hayashi YK, Noguchi S, Ogawa M, Nonaka I, Tanabe Y, et al. Fukutin gene mutations cause dilated cardiomyopathy with minimal muscle weakness. *Ann Neurol*. 2006; 60:597–602. [PubMed: 17036286]
35. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, et al. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet*. 2000; 9:2761–2766. [PubMed: 11063735]
36. Rasmussen TB, Hansen J, Nissen PH, Palmfeldt J, Dalager S, Jensen UB, et al. Protein expression studies of desmoplakin mutations in cardiomyopathy patients reveal different molecular disease mechanisms. *Clin Genet*. 2013; 84:20–30. [PubMed: 23137101]
37. Laitinen PJ, Brown KM, Piippo K, Swan H, Devaney JM, Brahmabhatt B, et al. Mutations of the cardiac ryanodine receptor (ryr2) gene in familial polymorphic ventricular tachycardia. *Circulation*. 2001; 103:485–490. [PubMed: 11157710]
38. Medeiros-Domingo A, Bhuiyan ZA, Tester DJ, Hofman N, Bikker H, van Tintelen JP, et al. The ryr2-encoded ryanodine receptor/calcium release channel in patients diagnosed previously with either catecholaminergic polymorphic ventricular tachycardia or genotype negative, exercise-induced long qt syndrome: A comprehensive open reading frame mutational analysis. *J Am Coll Cardiol*. 2009; 54:2065–2074. [PubMed: 19926015]
39. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, et al. Mutations in the cardiac ryanodine receptor gene (hryr2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2001; 103:196–200. [PubMed: 11208676]
40. Priori SG, Napolitano C, Memmi M, Colombi B, Drago F, Gasparini M, et al. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation*. 2002; 106:69–74. [PubMed: 12093772]
41. Coburn CT, Hajri T, Ibrahim A, Abumrad NA. Role of cd36 in membrane transport and utilization of long-chain fatty acids by different tissues. *J Mol Neurosci*. 2001; 16:117–121. discussion 151-117. [PubMed: 11478366]
42. Pison L, De Vusser P, Mullens W. Apical ballooning in relatives. *Heart*. 2004; 90:e67. [PubMed: 15547001]
43. Kumar G, Holmes DR Jr, Prasad A. "Familial" apical ballooning syndrome (takotsubo cardiomyopathy). *Int J Cardiol*. 2010; 144:444–445. [PubMed: 19375184]
44. Subban V, Ramachandran S, Victor SM, Gnanaraj A, Ajit MS. Apical ballooning syndrome in first degree relatives. *Indian Heart J*. 2012; 64:607–609. [PubMed: 23253418]
45. Ikutomi M, Yamasaki M, Matsusita M, Watari Y, Arashi H, Endo G, et al. Takotsubo cardiomyopathy in siblings. *Heart Vessels*. 2014; 29:119–122. [PubMed: 23563753]
46. Jaguszewski M, Osipova J, Ghadri JR, Napp LC, Widera C, Franke J, et al. A signature of circulating micrnas differentiates takotsubo cardiomyopathy from acute myocardial infarction. *Eur Heart J*. 2014; 35:999–1006. [PubMed: 24046434]
47. Ducruet AF, Albuquerque FC, Crowley RW, Williamson R, Forseth J, McDougall CG. Balloon-pump counterpulsation for management of severe cardiac dysfunction after aneurysmal subarachnoid hemorrhage. *World Neurosurg*. 2013; 80:e347–e352. [PubMed: 23022633]
48. Yasuno K, Bilguvar K, Bijlenga P, Low SK, Krischek B, Auburger G, Simon M, Krex D, Arlier Z, Nayak N, Ruigrok YM, Niemelä M, Tajima A, von und zu Fraunberg M, Dóczy T, Wirjatijasa F, Hata A, Blasco J, Oszvald A, Kasuya H, Zilani G, Schoch B, Singh P, Stüer C, Risselada R, Beck J, Sola T, Ricciardi F, Aromaa A, Illig T, Schreiber S, van Duijn CM, van den Berg LH, Perret C, Proust C, Roder C, Ozturk AK, Gaál E, Berg D, Geisen C, Friedrich CM, Summers P, Frangi AF, State MW, Wichmann HE, Breteler MM, Wijmenga C, Mane S, Peltonen L, Elio V, Sturkenboom MC, Lawford P, Byrne J, Macho J, Sandalcioğlu EI, Meyer B, Raabe A, Steinmetz H, Rüfenacht D, Jääskeläinen JE, Hernesniemi J, Rinkel GJ, Zembutsu H, Inoue I, Palotie A, Cambien F, Nakamura Y, Lifton RP, Günel M. Genome-wide association study of intracranial aneurysm identifies three new risk loci. *Nat Genet*. 2010; 42(5):420–5. [PubMed: 20364137]

Figure 1. Proposed clinical paradigm for risk stratification and treatment of patients based on knowledge of genomic status of cardiomyopathy genes. *Used with permission from {Blinded}.*

Figure 2. Mean variants per stress-induced cardiomyopathy (SIC) patients and controls (CONT) after exome sequencing and application of the variant filtering approach. Variants identified using the research filters were significantly greater in the SIC patients ($p = 0.01$, Z-score = -2.574) by the Mann-Whitney U-Test. *Used with permission from {Blinded}*.

Table 1

Clinical characteristics of the patients selected for study.*

Pt #	Age	Sex	Pre-IABP EF (%)	Days with Pump	Aneurysm Location	Fisher Grade	Hunt/Hess Grade	Aneurysm Treatment	Angiographic Cerebral Vasospasm	Follow-up mRS Score	Follow-up (months)
1	42	F	15	6	VA	IV	IV	Flow-diverting Stent	Severe	1	2.5
2	46	F	15	6	PcomA	IV	I	Clipping	Severe	5	13
3	52	F	10	11	AcomA	IV	III	Coil embolization	Severe	1	28
4	47	F	30	5	VA	IV	V	Endovascular parent artery sacrifice	Severe	3	26
5	43	F	10	7	PcomA	IV	IV	Clipping	Moderate	2	50
6	77	F	30	5	AcomA	IV	III	Coil embolization	Severe	1	12
7	56	F	20	6	ACA	IV	IV	Clipping	Severe	1	13

* AcomA = anterior communicating artery; EF = ejection fraction; IABP = intra-arterial balloon pump; mRS =modified Rankin scale;PcomA=posterior communicating artery; VA =vertebral artery.

DNA variants identified in patients with stress-induced cardiomyopathy.*

Table 2

Sample ID	Chr	Position	rsID	ref	alt	Gene	Effect	CADD score	PhyloP	NHLBI GO ESP	lkg MAF	ClinVar	Heart FPKM	Association
A01	18	29099850	rs121913013	G	A	DSG2	NSC	11.81	0.73	0.3527	0	Yes	3.50	arrhythmic_right_ventricular_cardiomyopathy
A01	3	38645420	rs1805124	T	C	SCN5A	NSC	0.349	0.37	23.2092	21	Yes	18.34	dilated_cardiomyopathy
A02	10	88452331		A	G	LDB3	SPLICE_SITE	-9	NA	-9	-9	No	390.73	dilated_cardiomyopathy_with_left_ventricular_noncompaction
A02	20	30408306	rs34396614	C	G	MYLK2	NSC	14.26	0.26	2.0237	1	Yes	0.49	hypertrophic_cardiomyopathy
A03	9	108397538		T	TA	FRAME_SHIFT		NA	NA	0.0121	-9	No	1.40	dilated_cardiomyopathy/hypertrophic_cardiomyopathy
A03	6	7583802	rs149513743	A	G	DSP	NSC	20.6	0.82	0.0349	0	No	23.21	arrhythmic_right_ventricular_cardiomyopathy
A03	3	38645420	rs1805124	T	C	SCN5A	NSC	0.349	0.37	23.2092	21	Yes	18.34	dilated_cardiomyopathy
A04	3	38645420	rs1805124	T	C	SCN5A	NSC	0.349	0.37	23.2092	21	Yes	18.34	dilated_cardiomyopathy
A05	6	7570791	rs148147581	G	A	DSP	NSC	21	0.64	0.0581	0	No	23.21	arrhythmic_right_ventricular_cardiomyopathy
A05	1	237880494	rs72751287	A	G	RYR2	SPLICE_SITE	NA	NA	0.3159	-9	No	18.92	cardiomyopathy
A06	6	84108085	rs78734745	C	T	ME1	SPLICE_SITE	27.8	0.86	0.8488	1	No	5.29	dilated_cardiomyopathy
A06	3	38645420	rs1805124	T	C	SCN5A	NSC	0.349	0.37	23.2092	21	Yes	18.34	dilated_cardiomyopathy
A07	7	80302104	rs201657731	C	T	CD36	STOP_GAINED	37	0.24	0.0116	0	No	247.32	hypertrophic_cardiomyopathy

* CADD = Combined Annotation Dependent Depletion;Chr = chromosome;MAF = minor allele frequency; NHLBI GO ESP = National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project; NSC =non-synonymous coding.