

## Application of Whole Exome Sequencing in the Clinical Diagnosis and Management of Inherited Cardiovascular Diseases in Adults

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**Background**—With the advent of high throughput sequencing, the identification of genetic causes of cardiovascular disease (CVD) has become an integral part of medical diagnosis and management and at the forefront of personalized medicine in this field. The use of whole exome sequencing for clinical diagnosis, risk stratification, and management of inherited CVD has not been previously evaluated.

**Methods and Results**—We analyzed the results of whole exome sequencing in first 200 adult patients with inherited CVD, who underwent genetic testing at the Yale Program for Cardiovascular Genetics. Genetic diagnosis was reached and reported with a success rate of 26.5% (53 of 200 patients). This compares to 18% (36 of 200) that would have been diagnosed using commercially available genetic panels ( $P=0.04$ ). Whole exome sequencing was particularly useful for clinical diagnosis in patients with aborted sudden cardiac death, in whom the primary insult for the presence of both depressed cardiac function and prolonged QT had remained unknown. The analysis of the remaining cases using genome annotation and disease segregation led to the discovery of novel candidate genes in another 14% of the cases.

**Conclusions**—Whole exome sequencing is an exceptionally valuable screening tool for its capability to establish the clinical diagnosis of inherited CVDs, particularly for poorly defined cases of sudden cardiac death. By presenting novel candidate genes and their potential disease associations, we also provide evidence for the use of this genetic tool for the identification of novel CVD genes. Creation and sharing of exome databases across centers of care should facilitate the discovery of unknown CVD genes. (*Circ Cardiovasc Genet.* 2017;10:e001573. DOI: 10.1161/CIRCGENETICS.116.001573.)

**Key words:** sudden cardiac death ■ cardiomyopathy ■ arrhythmia ■ genetics

Since the completion of the Human Genome Project in 2003, researchers have strived to develop fast and inexpensive methods for sequencing large-scale genetic data and from these efforts, next-generation sequencing technology has emerged. An individual's whole exome can now be sequenced at low cost in less than a week. Whole exome sequencing (WES) enables high throughput sequencing of the coding regions (>90%) of ≈20 000 genes in a single analysis. As it is approximated that >85% of mutations in single disease gene disorders reside within the exons/exon–intron boundaries,<sup>1</sup> WES is a high yield and cost effective alternative to whole genome sequencing for monogenic disorders. The use of WES

has the potential to revolutionize the way we practice medicine by generating large-scale personalized genetic information.

### See Clinical Perspective

Cardiovascular diseases (CVDs) comprise the most common causes of death and disability in Western countries. Early twin studies have established the importance of genetic influences on most CVD.<sup>2,3</sup> CVD display both single gene and complex inheritance patterns. Depending on the particular subcategory of CVD, many of the genes contributing to familial forms have been elucidated,<sup>4,5</sup> while in other areas of CVD, the underlying genes remain largely unknown.

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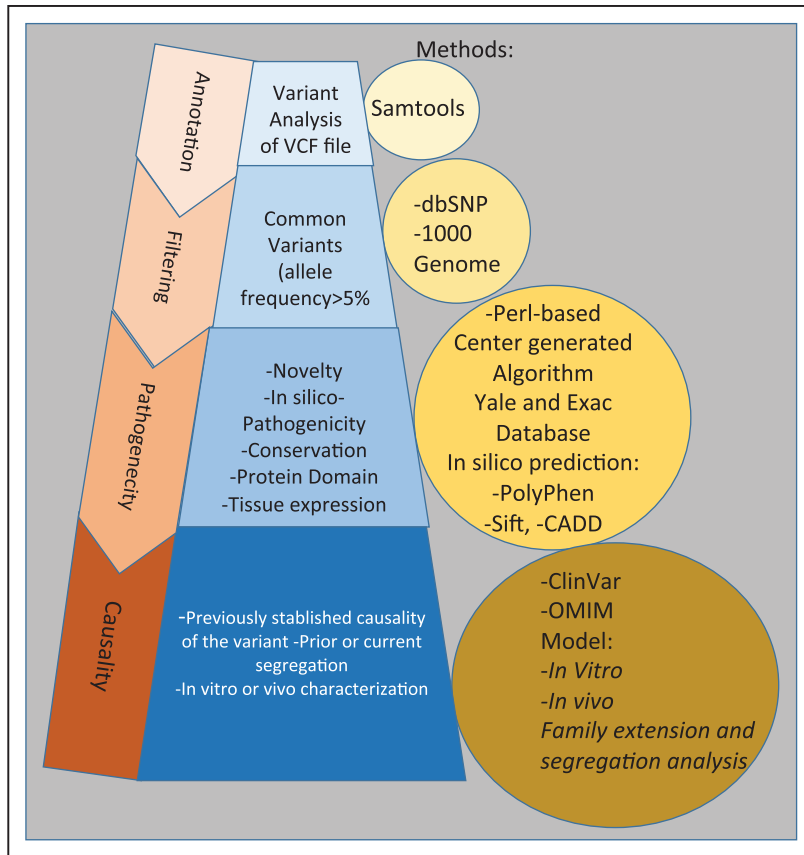
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**Figure 1.** Pipeline to assess the pathogenicity of genetic variants identified in an adult cardiovascular disease genetics clinic.

CVD genetics is a rapidly expanding field and the need for practitioners to diagnose and treat individuals with familial forms of CVD is large. Although there have been several reports suggesting that genetic panels should be used as the first-line evaluation of patients with CVD in the adult genetics clinic,<sup>6,7</sup> there has been no report on the use of WES in this context. Herein represents the first published report of 200 adult patients with familial CVD initially evaluated with proband-only clinical WES. The results will provide an estimate for the success rate of genetic diagnosis of the condition by WES in conjunction with clinical data and its use in identifying novel candidate genes and providing a database for future discovery of novel disease genes.

## Materials and Methods

### Recruitment

Individuals referred to the Yale Program for Cardiovascular Genetics (YPCG) for genetic testing typically had undergone an extensive cardiac work-up and have a working diagnosis. This included, but was not limited to, ECG, echocardiograms, cardiac magnetic resonance imaging, Holter monitoring, and electrophysiological studies. At the YPCG appointment, a board-certified genetic counselor and cardiovascular geneticist, took family and medical histories and evaluated previous cardiac records. Genetic testing through WES was offered to patients, in compliance with the previously published guidelines for inherited CVD (ie, long QT syndrome [LQTS], hypertrophic cardiomyopathy [HCM], etc.) as a first-line test.<sup>8-12</sup> Testing was performed at the Yale Center for Genome Analysis and interpreted by the DNA Diagnostic Laboratory (DNA Laboratory), a College of American Pathologists and Clinical Laboratory Improvement Amendments-certified laboratory with input from YPCG. The Human Investigation Committee of the Yale University School of Medicine approved the study protocol.

Consent was obtained from all subjects. The written consent includes release of information, including incidental findings to the patient and referring physicians and permission to extend the kindred.

### Presenting Diagnosis

We made the best effort to categorize these complex patients into a single presenting diagnosis based on the indication for the referral. Sudden cardiac death (SCD) was defined as witnessed instantaneous circulatory arrest requiring resuscitation in the field for a previously stable subject without structural heart disease. This definition was used when referring physicians were unable to report a more specific diagnosis based on presentation and clinical data. In certain cases, the QRS morphologies, the intervals on ECG or the left ventricular ejection fraction (LVEF) were used for a working diagnosis, but these findings were often not perfectly consistent with the clinical presentation. Dilated nonischemic cardiomyopathy were grouped under dilated cardiomyopathy (DCM). If a clear diagnosis of a subtype of cardiomyopathy (dilated versus hypertrophic) could not be reached, “nonspecific-nonischemic cardiomyopathy” was used.

### Evaluation

The YPCG and the DNA Laboratory evaluate clinical WES in parallel. An algorithm (Figure 1) was created to detect mutations in genes known to cause inherited CVD conditions using a comprehensive internal list of all known published CVD genes. The list is regularly updated using newly published data. In 2012, the list had 88 genes, and currently it has 163 genes.

A genetic diagnosis was considered as established if a variant is classified as likely-pathogenic or pathogenic could be identified based on American College of Medical Genetics (ACMG) 2015 criteria<sup>13</sup> (Tables I and II in the [Data Supplement](#)). Accordingly, mutations were specified as variant of uncertain significance (VUS), if they were novel but potentially pathogenic, that is, were mutations of established disease genes, whereby no segregation analysis or a

functional study had been carried out to confirm their pathogenicity. In cases of a VUS result, every attempt was made to perform family segregation of the variants.

If a pathogenic mutation was not identified, the remainder of genetic data were examined using genomic annotation tools. Mutations were examined for novelty, conservation, the position of the encoded amino acid in relationship to critical domains, potential change in the 3-dimensional structure, the expression of the encoded protein in the heart or relevant tissues, and the protein function annotation. Deleterious mutations in annotated genes were prioritized based on (1) gene expression in the cardiovascular tissues and (2) characterization *in vivo* or *in vitro* with the focus on disease-relevant functions. The exome report created by YPGC was sent to the DNA Laboratory for further evaluation. Sanger sequencing was used to confirm variant from WES until mid-2012. This practice was discontinued as most laboratories, including ours after multiple quality checks deemed it as unnecessary.<sup>14</sup> Sanger sequencing was carried out for segregation analysis. Copy number variation could not be detected by the bioinformatics pipeline available at the time.

The DNA Laboratory reviewed the created reports and released official reports as specified by College of American Pathologists, Clinical Laboratory Improvement Amendments, and ACMG guidelines.<sup>13,15</sup> Cases were reviewed individually in weekly meetings with an interdisciplinary team, established research scientist in the field of cardiovascular genetics, a board-certified cardiologist, internal medicine and cardiovascular medicine fellows, and a board-certified genetic counselor. In certain cases, *in vitro* or *in vivo* analyses were carried out for functional characterization of the candidate genes.

### Sequencing

Genomic DNA was analyzed using WES as previously described.<sup>16</sup> Roche/Nimble-Gen 2.1M Human Exome Array covers 34.0 Mb of genomic sequence and  $\approx$ 180 000 exons of 18 673 protein-coding genes. Briefly, DNA was fragmented and ligated to linkers followed by fractionation by agarose gel electrophoresis. Extracted DNA was polymerase chain reaction (PCR) amplified and hybridized to the capture arrays. Bound genomic DNA was eluted, purified, and amplified by ligation-mediated PCR. The PCR products were purified and subjected to DNA sequencing on the Illumina platform.

Captured libraries were sequenced on the Illumina genome analyzer followed by Image analysis and base calling. Sequence reads were mapped to the reference genome (hg18/hg19) using the Maqprogram SAMtools. Resulting sequence data were processed using Maq software. SAMtools software was used to detect single nucleotide variants and insertion/deletion (indel) were subsequently filtered against reference genome as earlier described.<sup>17</sup> Filters were applied against public databases, initially with 1000 Genomes, dbSNP, and the National Heart Blood and Lung Institute's Exome Variant Server databases, and the Exome Aggregation Consortium's ExAC Browser, after it became available on October 2014. Variants were annotated based on their effects on protein function and structure, *in silico* predication programs, PolyPhen-2 and SIFT, novelty, with a minor allele frequency threshold of  $<0.1$ , conservation, and tissue expression using a perl-based computer script. The results are reported in 2 different data sets—a data set of all exome-wide variants and a list of variants within in the targeted genes designed specifically for the CVD of interest (Figure 1). Statistical comparison of CVD-causing pathogenic variant identified by WES versus those that would have been identified by commercially available panels (defined as standard genes represented by at least 2 of 3 commercial panels as of January 2016) was made using Pearson's  $\chi^2$  test.

### Quantitative Real-Time PCR

Human right ventricle endomyocardial biopsy was obtained fluoroscopically. Total RNA was extracted using RNeasy Kit (QIAGEN, Valencia, CA), and the concentration was measured. Five hundred nanogram of RNA was reversed transcribed using iScript cDNA synthesis kit (Bio-Rad). Real-time PCR was carried out using iQ SYBR Green Supermix kit (Bio-Rad). The relative mRNA expression level

of CACNA1D was calculated using GAPDH as control. The presence of CACNA1D was further confirmed by electrophoresis using real-time PCR products. The following primers were used: CACNA1D-F, 5'-gtgtcaggagtgcccagttt-3'; CACNA1D-R, 5'-ctgggtctcttcagctacg-3'; GAPDH-F, 5'-gagtcacaggatttgctgt-3'; GAPDH-R, 5'-ttgattttggaggatctcg-3'.

## Results

### Reportable Diagnosis

The average age was  $46 \pm 14$  years, and 107 were men (53.5%) and 37 nonwhite (18.5%). The most common indications for referral and the success rate in making a reportable genetic diagnosis or identifying candidate genes are presented in Table.

A minimum depth of 20 reads was achieved for 95% coverage of the reference genome. Overall, reportable genetic diagnosis was reached in 53 of 200 patients (26.5%) with an additional 56 patients (28%) reported as having a VUS. Twenty-nine candidate variants were identified (14.5%) in the remaining cases (Table 1; Tables I and II in the [Data Supplement](#)). In comparison, 36 of our 200 cases (18%) would have been called with definitive diagnosis if we had used commercially available genetic panels, ( $P=0.04$ ). Connective tissue diseases, including Marfan syndrome, Ehlers Danlos syndrome, and familial thoracic aneurysm and dissection (TAA), were the most common reason for referral, comprising 18% of total patients ( $n=37$ ). Genetic diagnosis was reached in 10.8%, with another 29.7% reported as having a VUS, and 18.9% resulting in a candidate variant identification. SCD was the second most common reason for referral, comprising 17.5% of total patients ( $n=35$ ). Genetic diagnosis was reached in 31.4% of patients with SCD, with another 26.7% reported as having VUS, and 17.6% resulting in candidate variant identification; 14% and 12% of patients had HCM and DCM, respectively ( $n=28$  and  $n=24$ ). Genetic diagnosis was reached in 46.4% of HCM cases, whereas in 32.1% of the cases, a VUS was reported. In 7.1% of the HCM cases, a novel candidate gene was identified and documented for future analyses; 16.6% of patients with DCM had an established genetic diagnosis, 37.5% were reported as having a VUS, and in 12.5% a candidate variant was identified. For lipodystrophy/familial hypercholesterolemia ( $n=21$ , 10.5%), genetic diagnosis was reached in 38% of cases, whereas in 4.7% of the cases, a VUS was reported and in roughly 24% a candidate gene was identified.

The working diagnoses and identified gene variants in 200 patients presenting to the YPCG are listed in Table I in the [Data Supplement](#). Classification and supporting evidence based on ACMG 2015 criteria are presented for all gene variants. Candidate genes that are undergoing further analysis at YPCG are denoted by gray blocks.

### Ambiguous Clinical Diagnosis Modified or Altered by the Genetic Diagnosis

A significant advantage of WES was establishing the clinical diagnosis for ambiguous cases, particularly in patients with SCD. We carefully characterized the 35 patients presenting with initial diagnosis of SCD of unspecified causes using WES results and clinical data. Most, if not all these patients had initially abnormal ECGs, often with a prolonged QT duration and

**Table. Reasons for Referral to the Yale Program for Cardiovascular Genetics Clinic and the Genes With Variants Identified as VUS or as Pathogenic**

Clinical Diagnosis	Patients, n (% of Total)	Pathogenic Mutation Identified, n (%)	Likely Pathogenic Mutation Identified, n (%)	VUS Identified, n (%)	Candidate Gene Identified, n (%)	Genes
Connective tissue disease	37 (18.5)	3 (8.1)	1 (2.7)	11 (29.7)	7 (18.9)	<i>COL1A1, COL5A1, *COL5A2, ELN, FBN1, *FBN2, FLNA, *MYH11, MYLK, PTPN11, SKI, SMAD3, TGFB2</i>
Sudden cardiac death	35 (17.5)	8 (22.8)	3 (8.5)	9 (26.4)	6 (17.6)	<i>ACTN2, ANK2, AKAP9, CACNA1D, *DPP6, DSG2, DSP, *GYG1, KCNH2, *LMNA, MYBPC3, *MYH6, MYPN, NEXN, PNN, RBM20, RYR2, SCN5A, *TGFB3, TNNI3, *TTN*</i>
HCM	28 (14)	8 (28.5)	5 (17.5)	9 (32.1)	2 (7.1)	<i>ACTN2, AKAP9, ABCC9, *CALR3, JPH2, *MYBPC3, *MYH6, MYH7, *PRKAG2, *TCAP, TTN, TNNT2, *TPM1, *TRPM4*</i>
DCM	24 (12)	4 (16.6)	0 (0)	9 (37.5)	3 (12.5)	<i>BAG3, DSP, DSG2, HFE, *LMNA, *MYBPC3, MYH6, MYH7, PRDM16, PRKAG2, RBM20, SCN5A, TTN*</i>
FH/Lipodystrophy	21 (10.5)	6 (28.5)	2 (9.5)	1 (4.7)	5 (23.8)	<i>APOB, *APOE, *LDLR, *LMNA, *PLAT, PLIN1</i>
LQTS	15 (7.5)	3 (20)	0 (0)	3 (20)	4 (26.6)	<i>AKAP9, ANK2, *CAV3, CTNNA3, KCNQ1, *RBM20, SCN5A, TTN</i>
Atrial or ventricular arrhythmias	10 (5.0)	0 (0)	0 (0)	4 (40.0)	0 (0)	<i>ABCC9, CACNB2, GPD1L, KCNE2, SYNE2</i>
BrS	9 (4.5)	1 (11.1)	2 (22.2)	3 (33.3)	0 (0)	<i>CACNA1C, DSP, RYR2, *SCN5A*</i>
Family history of SCD or LQTS	9 (4.5)	0 (0)	1 (11.1)	5 (55.5)	1 (11.1)	<i>ANK2, CACNA1C, DES, *DSG2, SCN4B, SCN5A, MYH6, TMEM43, TNNI3, VCL</i>
Nonspecific NICM	6 (3)	2 (33.3)	1 (16.6)	2 (33.3)	0 (0)	<i>AKAP9, LAMP2, LDB3, MIB1, *MYH6, NEXN, PKP2, *RYR2, SCN5A, TNNT2, TTN*</i>
Other	6 (3)	0 (0)	2 (33.3)	0 (0)	1 (20)	<i>DSP, EMD, *NDUFV2, *TNNT2*</i>

BrS indicates Brugada syndrome; FH, familial hypercholesterolemia; HCM, hypertrophic cardiomyopathy; LQTS, long QT syndrome; NICM, nonischemic cardiomyopathy; SCD, sudden cardiac death; and VUS, variant of uncertain significance.

\*Indicates gene variants identified as VUS or pathogenic.

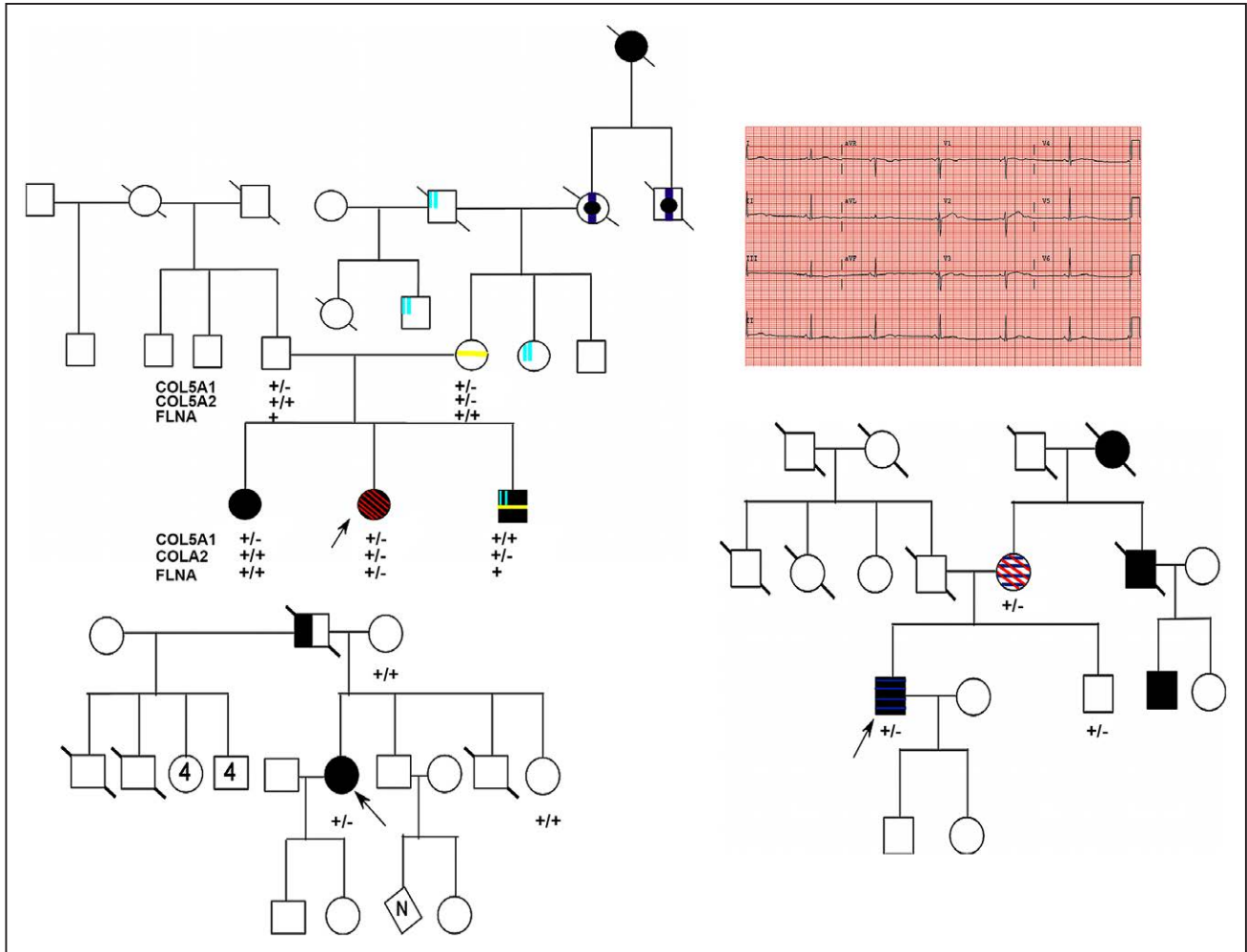
variable degree of left ventricular systolic dysfunction. The working diagnosis by the referring physicians were arbitrarily assigned to either nonischemic cardiomyopathy or LQTS, with the final diagnosis awaiting the genetic results. Primary ventricular arrhythmia was the final diagnosis in 42.8% of cases, followed by LQTS (17.1%), DCM (14.2%), and HCM and Nonspecific-nonischemic cardiomyopathy (8.5% each). Arrhythmogenic right ventricular cardiomyopathy, catecholaminergic polymorphic ventricular tachycardia, and restrictive cardiomyopathy all comprised the final diagnosis in 9% of SCD cases. Genes represented on commercially available panels that can be used to evaluate cardiac arrhythmia and cardiomyopathy are represented in Tables III and IV in the [Data Supplement](#), respectively. It is apparent that the genetic diagnosis in most cases was only possible by using WES data when compared with individual disease-based panels. It is noteworthy that the medical management in many cases were altered based on genetic results by the referring physician.

Following, we present several cases to provide justification for the use of WES to establish a final diagnosis.

Patient 6 (Tables I and II in the [Data Supplement](#); Figure 2A) was a 22-year-old female with a history of

X-linked periventricular heterotopia (PVH), hyperflexibility of the major joints, and familial aneurysm of different vascular beds referred for targeted genetic testing for *FLNA* to confirm the diagnosis of PVH. Family history included a sister with congenital hip dislocation, easy bruising, joint hyperflexibility, carpal tunnel syndrome, a brother with scoliosis, joint hyperflexibility, and pectus excavatum; none had neurological findings of PVH. The index case's mother had mitral valve prolapse and scoliosis. Her maternal aunt had a pectus excavatum and pes planus. Her maternal grandmother had undergone an ascending TAA repair, often seen in PVH, an aneurysm of the renal artery, and no neurological symptoms. The maternal grandmother's brother had died of a TAA rupture at age the 52 years. The maternal great grandmother reportedly had hyperflexible joints.

Given the complexity of the condition, a WES of the index case was carried out, in place of targeted screening for a *FLNA* mutation or Ehlers Danlos syndrome genes. The analysis revealed a frameshift variant in *FLNA* (c.4214delA) and missense variants in *COL5A1* (c.5674G>A; p.D1771N) and *COL5A2* (c.2379C>T; p.G702R) genes. Segregation analysis of the first-degree relatives revealed that the *FLNA*



**Figure 2.** Pedigrees for discussed cases are shown. Circles represent females; squares represent males, and symbols with a slash through them indicate deceased subjects. An arrow indicates the proband/person who had exome sequencing. **A**, Pedigree of case 1 (patient 6). Individuals with hypermobility are indicated by black symbols, individuals not investigated for hypermobility are indicated by a black dot, individuals with aneurysm are indicated by a vertical purple line, individuals with scoliosis are indicated by a yellow vertical line, individuals with pectus are indicated with 2 blue vertical lines, and individuals with periventricular heterotopia are indicated with red diagonal lines. Genotypes of *COL5A1*, *COL5A2*, and *FLNA* are shown below those individuals who underwent genetic testing. **B**, Representative ECG of case 4 (patient 152). Heart rate is 36 bpm and her corrected QTC was 630 ms. **C**, Pedigree of case 4 (patient 152). Individuals with both torsade des pointes and congenital hearing loss are indicated by filled symbols, individuals reported to have permanent pacemakers are indicated by half-filled symbols. **D**, Pedigree of case 5 (patient 97). Individuals with myocardial infarction and surgical intervention (ie, coronary artery bypass grafting, PCI, etc.) are indicated with filled symbols, individuals with deep venous thrombus are indicated by blue horizontal lines, and individual with pulmonary embolus are indicated by red diagonal lines.

variant was denovo, while her mother had the *COL5A2* variant and patient's father with no Ehlers Danlos syndrome had the *COL5A1* variant. The index case's sister had both *COL5A1* and *COL5A2* variants; her brother and her maternal grandmother had only the *COL5A2*. Thus, *COL5A2* (p.G702R) segregated with the hyperflexible joints and with the TAA in the maternal side of the family as a separate entity from *FLNA*.

Patient 4 (Tables I and II in the [Data Supplement](#)) was a 52-year-old female with status post an aborted ventricular fibrillation. An epicardial cardioverter defibrillation patch, a currently retreated device because of a high rate of patch crinkling<sup>18</sup> was implanted in the late 1980s with 2 appropriate shocks identified by device interrogation. Family history was significant for her mother having heart failure. Later her defibrillator was upgraded to an ICD, whereas her patches remained in place. She then developed shortness of breath and

was diagnosed with persistent atrial fibrillation, for which she underwent a radiofrequency ablation without resolution of her symptoms. An echocardiography examination revealed mildly reduced LVEF, severe diastolic dysfunction, mild pulmonary hypertension, and biatrial enlargement.

A WES was carried out to determine whether a forme fruste inherited restrictive cardiomyopathy (in the absence of lower extremity edema) versus the impact from epicardial defibrillation patches were responsible for her shortness of breath. Results revealed a *TNNI3* mutation c.592C>G, p.L198V. In silico L198V creates a splice donor site with splice acceptors predicted in the 3'untranslated region of the gene. This amino acid substitution is found in the C-terminal of the gene, which contains a secondary actin-tropomyosin binding domain necessary for inhibiting cross-bridge cycling during diastole.<sup>19</sup> Mutations in this gene have been associated with restrictive

cardiomyopathy,<sup>20</sup> which was also consistent with echocardiographic findings of diastolic dysfunction and biatrial enlargement. Hence, this diagnosis was considered more plausible; the extremely risky removal of pads was abandoned. Unfortunately, later she developed ventricular arrhythmia and deceased after external defibrillation shocks failed to restore sinus rhythm.

Patient 59 (Tables I and II in the [Data Supplement](#)) was a 20-year-old female with a family history of SCD and an aborted SCD, resuscitated by a defibrillator. She was found to have prolonged QTc (later normalized) and a mildly depressed LVEF (40%–45%). A follow-up echocardiogram showed improvement of her LVEF to near normal (53%), hence, she was referred for genetic testing with a working diagnosis of aborted SCD and possibly LQTS. No genetic variants associated with LQTS but a variant in *MYH6* c.3979 G>A, p.R1270C and a frameshift mutation in *TTN* c.45689delG, p.G8858fs were identified. Her younger sister, who had symptoms of CHF and mildly reduced LVEF by cardiac magnetic resonance imaging but no SCD tested positive for both variants. The index case's father had the *MYH6* variant but no symptom or sign of heart failure on physical examination or by cardiac imaging. These findings led to the final diagnosis of *TTN*-related SCD and mild form of DCM in this patient.

### Identification of Novel Candidate Genes

In cases where no pathogenic variant could be identified, the entire exome was examined for nonconservative and deleterious candidate variants in annotated genes expressed in the cardiovascular tissues. These variants were investigated for disease segregation and occasionally were characterized in an in vitro system. A list of candidate genes was created to serve as a reference for future exome analysis of cases with unknown disease genes. Once independent mutations are identified in a given gene, the data will be made publically available for independent replication before the causality is established. Creation and sharing of large exome databases across the major genomic centers should facilitate establishing causal link between candidate genes and diseases of interest.

Patient 152 (Tables I and II in the [Data Supplement](#)) was a 34-year-old female with a history of congenital hearing loss, who presented with torsade des pointes, bradycardia with heart rates between 30 and 50 bpm, and a prolonged QT interval on ECG (Figure 2B). Patient's father had been diagnosed with bradycardia and had undergone permanent pacemaker implantation and died at the age of 52 years from a "sudden heart condition" (Figure 2C). A WES revealed a heterozygous nonsense variant in the *CACNA1D* gene in the proband. Mutations in this gene had been implicated in bradycardia, but not in LQT.<sup>21–24</sup> In addition, the encoded protein has been shown to be expressed only in mice atrium. To explain the ventricular arrhythmia in the subject, a human ventricular biopsy specimen was obtained from a human control, which revealed high expression levels of both mRNA and protein (Figure I in the [Data Supplement](#)). These findings suggest a possible causal link between this mutation and LQTS. As her unaffected mother and sister did not carry this variant, the variant is likely the cause of bradycardia in her father.

Patient 97 (Table I in the [Data Supplement](#)) was a 44-year-old male athlete, who presented with a second acute

ST-segment–elevation myocardial infarction in the absence of major CAD risk factor. His first ST-segment–elevation myocardial infarction occurred while swimming. An angiography examination had revealed 100% right coronary artery occlusion with a fresh thrombus, for which he underwent thrombectomy, angioplasty (PCI) and placement of a bare-metal stent and diffuse, critical stenosis of the left anterior descending coronary artery, which was grafted. Perioperatively, he developed a popliteal deep venous thrombus. Two years after coronary artery bypass grafting, he developed chest pain after a long run. The coronary angiogram revealed again a fresh thrombus in the right coronary artery at the site of the bare-metal stent, which was treated with a drug-eluting stent and a second critical lesion in the left circumflex that was treated with PCI. His family history was significant for multiple deep venous thrombus in his mother in her 20s and a more recent massive pulmonary embolus, coronary artery bypass grafting in a maternal uncle at the age of 32 years, CAD stenting in a maternal cousin in his thirties, and acute myocardial infarction and death in his maternal grandmother at the age 38 years (Figure 2D). There was no relevant history on the paternal side of the family. A WES revealed no variants in dyslipidemia genes, but a novel nonconservative missense alteration in the *PLAT* gene, encoding tissue-type plasminogen activator. Segregation analysis supported its possible causality by identifying the mutation in his affected mother and its absence in the unaffected brother.

### Incidental Pathological Findings

We identified actionable pathological variants in 11 of 200 (5.5%) study subjects. This included pathogenic mutations in *ATM*, *APC*, *BRCA2*, *BRIP1/FANCI*, *HFE*, *LDLR*, *MSH6*, and *SCNN1B* genes (Table V in the [Data Supplement](#)). In these cases, patients and referring providers were informed of these findings.

### Discussion

In this study, we present our experience in the use of WES in 200 consecutive adult patients with familial CVD. The data provided are evidence for multiple advantages of WES. Utilizing WES, we have been successful at reaching a genetic diagnosis in a patient population with a spectrum of cardiovascular illnesses. Overall, reportable genetic diagnosis (pathogenic or likely-pathogenic variants) was reached in 26.5% of patients, with an additional 28% reported as having a VUS and 14.5% resulting in (not reportable) candidate variant identification. We owe part of this success to the careful selection of those patients by cardiovascular medicine physicians that have pursued expertise in genetics, working alongside with the genetics team, consisted of a cardiovascular geneticist, a genetic counselor and cardiology fellows in training. Not infrequently, patients referred to the YPCG clinic had complex presentations and ambiguous diagnoses. We illustrate how genetic diagnosis aided in establishing a final clinical diagnosis in this group.

### Genetic Diagnosis Assisted the Final Clinical Diagnosis

In our experience with clinical WES, one clear advantage has been in the establishment of clinical diagnosis. Often in SCD, primary diagnosis at the time of incident is unclear and

difficult to determine whether it is a primary arrhythmogenic disorder versus cardiomyopathy. This makes the selection of phenotype-driven panels for genetic testing particularly challenging. In patients with preserved ejection fraction distinguishing the type of arrhythmia, that is, LQTS versus Brugada syndrome, based on post resuscitation ECG is often impossible. Electrophysiological studies using pharmacological challenge have also limited use. The currently available pan-CVD panels offered by different vendors have very different combinations of arrhythmia and cardiomyopathy genes (Tables III and IV in the [Data Supplement](#)) and none fully covers the entire list of known genes for these disorders. In comparison, WES is in a unique position in establishing a final clinical diagnosis when used in conjunction with the clinical data.<sup>25</sup>

In this study, patient 6 with PVH and familial aneurysm and hyperflexibility of the major joints illustrates one such example. Although a priori association between PVH and vascular traits<sup>26</sup> were thought to be explained by a single gene mutation in the *FLNA* gene, a segregation analysis revealed that the classic Ehlers Danlos syndrome phenotypes, including TAA, segregated with a *COL5A2* mutation in her family. The genetic diagnosis entirely changed the clinical management as the *COL5A2* mutation carriers in the family were recommended to undergo echocardiographic screening for TAA.

### Discovering the Pleiotropic Effects of a Known Disease Gene

Another advantage of using WES has been the unraveling of novel traits for known disease genes. Ventricular arrhythmia was a novel trait associated with truncating *TTN* mutations in our patient population with an initially normal structural heart. As shown in the example of patient number 59, these subjects often had prolonged QTc, normal or near normal LVEF, and had been referred to us with a working diagnosis of LQTS. In many cases, segregation analysis could help to establish causal link between *TTN* mutations and SCD. In such cases, the correct diagnosis would not have been reached if a targeted sequencing had been performed using available arrhythmia panels (Table III in the [Data Supplement](#)). In our WES patients, we reported 6 cases of truncating *TTN* mutations (patients number 59, 75, 84, 26, 114, and 193; Tables I and II in the [Data Supplement](#)) in patients with aborted SCD but no history of cardiomyopathy previous to their aborted SCD event. Our finding is consistent with previous reports of high incidence of ventricular arrhythmias in subjects with truncating *TTN* mutations.<sup>25,27</sup>

### Identification of Novel Candidate Genes

An advantage of WES has been the identification of novel candidate genes. One example is a nonsense mutation in *CACNA1D* gene in patient number 152 (Tables I and II in the [Data Supplement](#)), who had documented torsade des pointes, prolonged QT interval, and congenital hearing loss. This gene has been associated with primary aldosteronism, seizures, and neurological abnormalities (gain of function). Homozygous (and in one single case heterozygous) 3-bp insertion in the *CACNA1D* gene had been reported in 2 consanguineous Pakistani kindreds with sinoatrial node dysfunction and deafness.<sup>28</sup> *CACNA1D*-deficient mice are deaf because of the

complete absence of L-type currents in cochlear inner hair cells and degeneration of outer and inner hair cells and have sinoatrial node dysfunction.<sup>29</sup> However, *CACNA1D* mutation has not been previously reported in LQTS and the gene is not reportedly not expressed in mice ventricular myocardium. Our investigation, however, showed that *CACNA1D* is expressed in control human ventricular myocardium and could account for the patient's hearing loss and episode of torsade des pointes.

A second example is the patient number 97, who was a 44-year-old triathlete in peak physical fitness with a history of coronary artery stent and coronary artery bypass grafting at 42 years of age and perioperative deep venous thrombus, who presented with a second ST-segment-elevation myocardial infarction and recurrent thrombi in the native vessel or at the site of his first stent. A novel, likely-pathogenic, nonconservative missense variant in the *PLAT* gene was identified, which segregated with deep venous thrombus/pulmonary embolus in his family. Loss of function *PLAT* variants have been associated with decreased release of the thrombolytic protein tissue-type plasminogen activator and thrombophilia and gain of function variants in this gene have been associated with increased tissue-type plasminogen activator release and hyperfibrinolysis.<sup>30–33</sup> Common variants of *PLAT* gene have been associated with the risk of CVD<sup>34</sup> and myocardial infarction.<sup>35</sup> It is particularly interesting that release of tissue-type plasminogen activator is stimulated by exercise, presumably in response to a physiological need, and both of this patient's ST-segment-elevation myocardial infarctions occurred during or directly after intense exercise. On the basis of the patient's clinical history and genetic finding, indefinite continuation of dual-antiplatelet therapy was opted. The causality of all these candidate genes can be established by independent replication or preferably by sharing of large exome databases between the major genomic centers. Because of their highly speculative nature, most other identified candidate genes were not reported in this article.

### Incidental Pathological Findings

Exome sequencing can reveal variants in genes with actionable findings, as previously reported.<sup>36,37</sup> Five and a half percent of patients referred to our clinic had actionable incidental findings (Table V in the [Data Supplement](#)), many of whom had no personal or family history of cancer. These results were reported to patients as indicated by guidelines. With the identification of incidental findings, patients were referred to proper follow-up for the condition identified. They were encouraged to discuss these results with their family physician and family members if applicable.

### WES Compared With Available Panels

Although we have shown that WES is an effective tool for adult cardiovascular genetic testing, the development of phenotype-based panels has grown. Of the 53 subjects (26.5%) identified with a CVD causing pathogenic variant by WES (Table II in the [Data Supplement](#)), only 36 patients (18%) would have definitively been identified by commercially available panels (defined as standard genes represented by at

least 2 of 3 commercial panels), a difference that is statistically significant ( $P=0.04$ ). It is noteworthy that these numbers are based on panels current as of January 2016. However, the majority of WES were performed at a time when commercially available panels had considerably fewer gene sets with much less power to identify the causal variants. With many disease genes remaining unknown but discovered in an ongoing basis, the current panels will be soon deemed as incomplete.

### Limitations

Our study has several limitations. Most importantly, the success of WES technique is greatly dependent on the current knowledge of disease genes. By the same token, it is the most appropriate screening test for low-yield cases for it to provide a comprehensive database that can be revisited in future as the genetic literature grows or used for the discovery of novel genes. Another limitation is that we performed proband-only WES. Previous reports of the use of this technology in the pediatric populations describe WES in duos and trios. This practice is often unfeasible in an adult CVD clinic, either because affected family members are deceased or geographically dispersed. For cases where a VUS was identified, every attempt was made to perform segregation analysis in the extended families (Table I in the [Data Supplement](#)). This reduces false-positive or false-negative rates to a greater magnitude compared with genetic study of trios. Overall, the determination of pathogenicity in our center was made in accordance with the ACMG 2015 guidelines by using its major criteria. The major criteria for pathogenicity include the presence of a radical or a de novo mutation and supportive in vitro or in vivo studies. A fourth limitation is the requirement for a multidisciplinary infrastructure for analyzing large data. Finally, WES is not useful for the detection of chromosomal rearrangements or mutations in noncoding regions. In addition, our analysis at the time did not include copy number variation. The applicability of copy number variation analysis in clinical cardiovascular genetics, however, remains undetermined.<sup>38</sup> Despite all our efforts, there are always potentials for false-positive results. In this context, we like to state that a genetic diagnosis alone cannot establish the clinical diagnosis and should be strictly used in conjunction with the clinical data.

In conclusion, our study shows many advantages of WES for its capability to establish the correct clinical diagnosis to identify novel traits for known disease genes or novel candidate genes for CVD.

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### Disclosures

None.

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### CLINICAL PERSPECTIVE

High throughput sequencing will soon revolutionize the way we practice medicine by generating large-scale, personalized, and relatively affordable genetic information. Cardiovascular disease (CVD) is the most common cause of death in developed countries and the need for practitioners to diagnose and treat individuals with familial forms of CVD is urgent. Although phenotype-based genetic panels are widely used as the first-line tool for genetic evaluation of patients with CVD in the adult genetics clinic, there has been no report on the use of whole exome sequencing (WES) in this context. This work represents the first published report of patients with familial CVD evaluated with proband-only clinical WES. Using this diagnostic tool, 26.5% of subjects were identified with a disease causing pathogenic variant, compared with only 18% if commercially available genetic panels would have been used ( $P=0.04$ ). We also demonstrate that WES is a valuable screening tool in establishing the clinical diagnosis of poorly defined cases of sudden cardiac death, as well as for the identification of novel CVD genes. Furthermore, we offer a blueprint for how CVD genetics clinics could offer precise and personalized services using WES, including the use of CVD-specific algorithms, in silico prediction tools and publically available resource to help establish WES variant pathogenicity; the use of interdisciplinary team of physicians, researchers, and genetics counselors for collective review of CVD cases and potential identification of causative mutations through WES; current success rate for the genetic diagnosis; and expected frequency and recommendations for communicating incidental findings.