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,[1] 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.[2,3] 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,[4,5] while in other areas of CVD, the underlying genes remain largely unknown.

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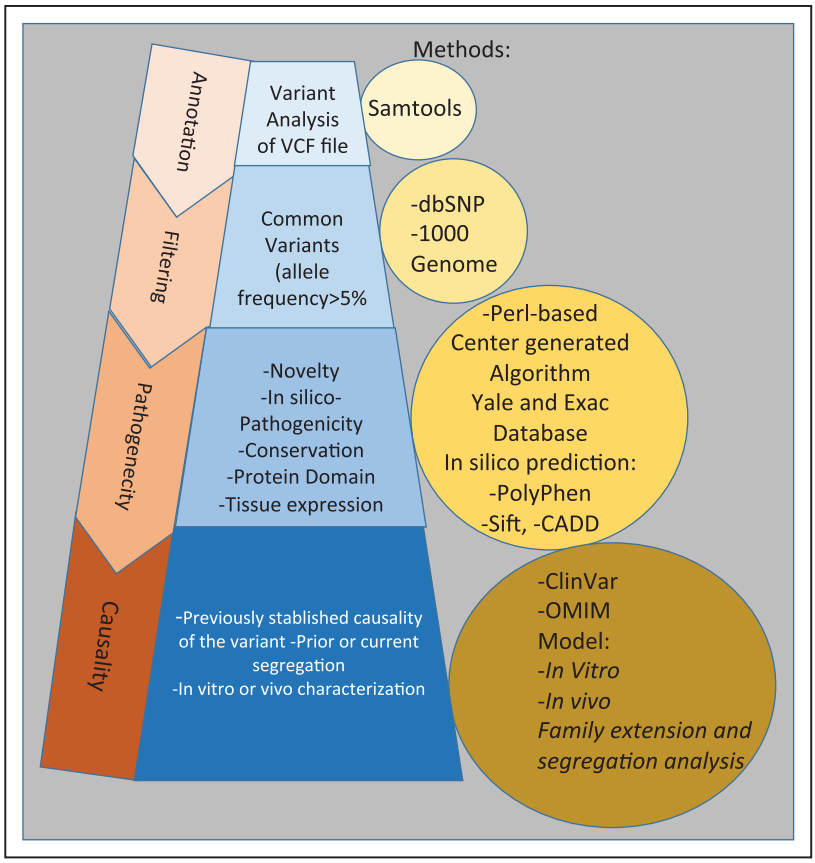

Figure 1. Pipeline to assess the pathogenicity of genetic variants identified in an adult cardiovascular disease genetics clinic.

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,[6,7] 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.[8–12] 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[13] (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.[14] 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.[13,15] 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.[16] Roche/Nimble-Gen 2.1M Human Exome Array covers 34.0 Mb of genomic sequence and ≈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.[17] 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 χ^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′-ctgggtcctcttcagctacg-3′; GAPDH-F, 5′-gagtcaacggatttggtcgt-3′; GAPDH-R, 5′-ttgattttggagggatctcg-3′.

Results

Reportable Diagnosis

The average age was 46±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)	*COL1A1, COL5A1,* COL5A2, ELN, FBN1,* FBN2, FLNA,* MYH11, MYLK, PTPN11, SKI, SMAD3, TGFBR2*
Sudden cardiac death	35 (17.5)	8 (22.8)	3 (8.5)	9 (26.4)	6 (17.6)	*ACTN2, ANK2, AKAP9, CACNA1D,* DPP6, DSG2, DSP,* GYG1, KCNH2,* LMNA, MYBPC3,* MYH6, MYPN, NEXN, PNN, RBM20, RYR2, SCN5A,* TGFB3, TNNI3,* TTN**
HCM	28 (14)	8 (28.5)	5 (17.5)	9 (32.1)	2 (7.1)	*ACTN2, AKAP9, ABCC9,* CALR3, JPH2,* MYBPC3,* MYH6, MYH7,* PRKAG2,* TCAP, TTN, TNNT2,* TPM1,* TRPM4**
DCM	24 (12)	4 (16.6)	0 (0)	9 (37.5)	3 (12.5)	*BAG3, DSP, DSG2, HFE,* LMNA,* MYBPC3, MYH6, MYH7, PRDM16, PRKAG2, RBM20, SCN5A, TTN**
FH/Lipodystrophy	21 (10.5)	6 (28.5)	2 (9.5)	1 (4.7)	5 (23.8)	*APOB,* APOE,* LDLR,* LMNA,* PLAT, PLIN1*
LQTS	15 (7.5)	3 (20)	0 (0)	3 (20)	4 (26.6)	*AKAP9, ANK2,* CAV3, CTNNA3, KCNQ1,* RBM20, SCN5A, TTN*
Atrial or ventricular arrhythmias	10 (5.0)	0 (0)	0 (0)	4 (40.0)	0 (0)	*ABCC9, CACNB2, GPD1L, KCNE2, SYNE2*
BrS	9 (4.5)	1 (11.1)	2 (22.2)	3 (33.3)	0 (0)	*CACNA1C, DSP, RYR2,* SCN5A**
Family history of SCD or LQTS	9 (4.5)	0 (0)	1 (11.1)	5 (55.5)	1 (11.1)	*ANK2, CACNA1C, DES,* DSG2, SCN4B, SCN5A, MYH6, TMEM43, TNNI3, VCL*
Nonspecific NICM	6 (3)	2 (33.3)	1 (16.6)	2 (33.3)	0 (0)	*AKAP9, LAMP2, LDB3, MIB1,* MYH6, NEXN, PKP2,* RYR2, SCN5A, TNNT2, TTN**
Other	6 (3)	0 (0)	2 (33.3)	0 (0)	1 (20)	*DSP, EMD,* NDUFV2,* TNNT2**

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*

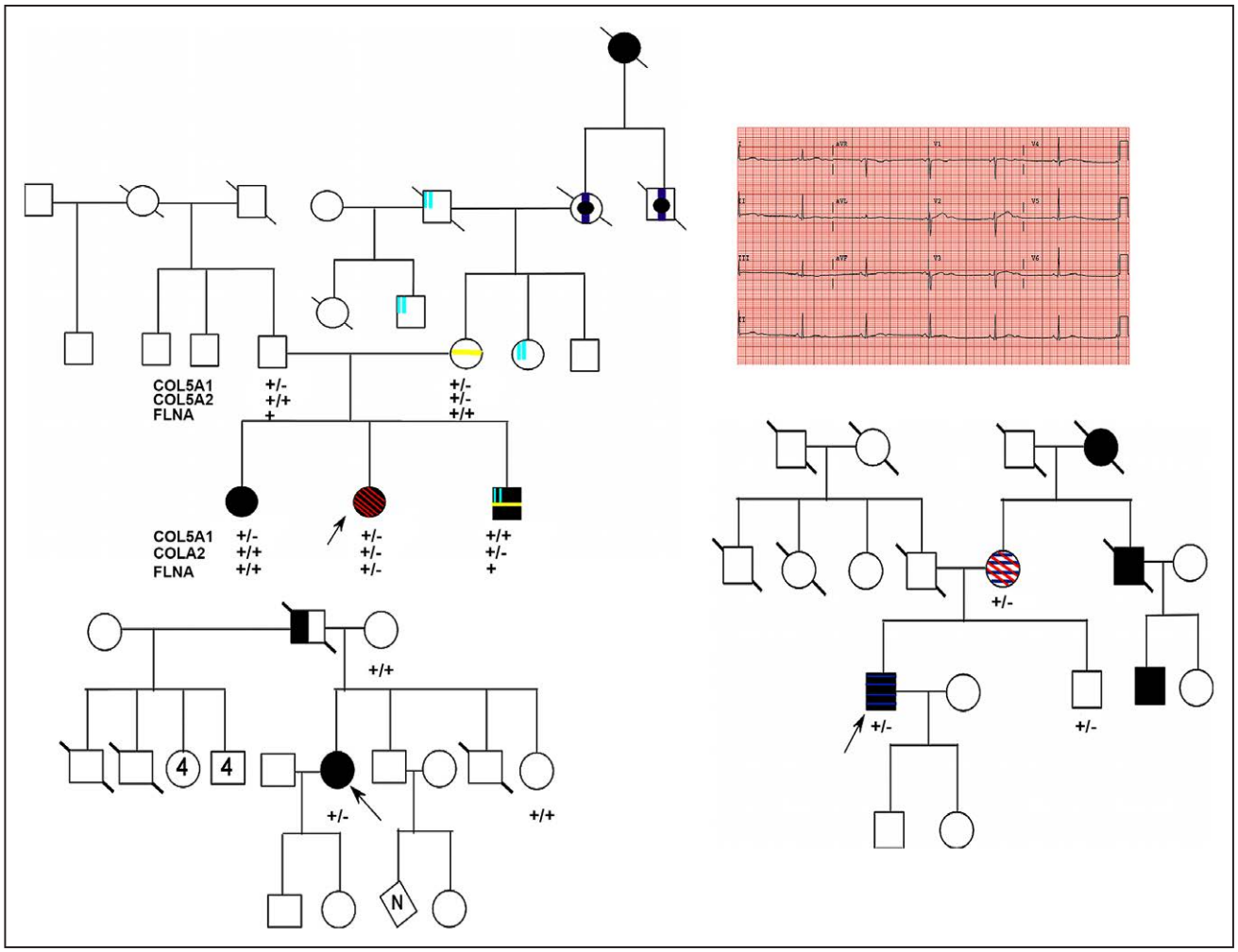

Figure 2. Pedigrees for discussed cases are shown. Circles represent females; squares represent males, and symbols with a slash through them indicate deceased subjects. An arrow indicates the proband/person who had exome sequencing. **A**, Pedigree of case 1 (patient 6). Individuals with hypermobility 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[18] 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.[19] Mutations in this gene have been associated with restrictive

cardiomyopathy,[20] 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.[21–24] 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 descendent 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/FANCJ*, *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.[25]

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[26] 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 Pleotropic 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.[25,27]

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.[28] *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.[29] 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.[30–33] Common variants of PLAT gene have been associated with the risk of CVD[34] and myocardial infarction.[35] 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.[36,37] 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 denovo 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.[38] 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.

References

1. Rabbani B, Tekin M, Mahdieh N. The promise of whole-exome sequencing in medical genetics. *J Hum Genet*. 2014;59:5–15. doi: 10.1038/jhg.2013.114.
2. Mangino M, Spector T. Understanding coronary artery disease using twin studies. *Heart*. 2013;99:373–375. doi: 10.1136/heartjnl-2012-303001.
3. Levine RS, Hennekens CH, Duncan RC, Robertson EG, Gourley JE, Cassady JC, et al. Blood pressure in infant twins: birth to 6 months of age. *Hypertension*. 1980;2(4 pt 2):I29–I33.
4. Hannah-Shmouni F, Seidelmann SB, Sirrs S, Mani A, Jacoby D. The genetic challenges and opportunities in advanced heart failure. *Can J Cardiol*. 2015;31:1338–1350. doi: 10.1016/j.cjca.2015.07.735.
5. Ho CY. Hypertrophic cardiomyopathy. *Heart Fail Clin*. 2010;6:141–159. doi: 10.1016/j.hfc.2009.12.001.
6. Alfares AA, Kelly MA, McDermott G, Funke BH, Lebo MS, Baxter SB, et al. Results of clinical genetic testing of 2,912 probands with hypertrophic cardiomyopathy: expanded panels offer limited additional sensitivity. *Genet Med*. 2015;17:880–888. doi: 10.1038/gim.2014.205.
7. Li Z, Huang J, Zhao J, Chen C, Wang H, Ding H, et al. Rapid molecular genetic diagnosis of hypertrophic cardiomyopathy by semiconductor sequencing. *J Transl Med*. 2014;12:173. doi: 10.1186/1479-5876-12-173.
8. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al; Heart Rhythm Society (HRS); European Heart Rhythm Association (EHRA). HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA). *Europace*. 2011;13:1077–1109. doi: 10.1093/europace/eur245.
9. Ganesh SK, Arnett DK, Assimes TL, Assimes TL, Basson CT, Chakravarti A, et al; American Heart Association Council on Functional Genomics and Translational Biology; American Heart Association Council on Epidemiology and Prevention; American Heart Association Council on Basic Cardiovascular Sciences; American Heart Association Council on Cardiovascular Disease in the Young; American Heart Association Council on Cardiovascular and Stroke Nursing; American Heart Association Stroke Council. Genetics and genomics for the prevention and treatment of cardiovascular disease: update: a scientific statement from the American Heart Association. *Circulation*. 2013;128:2813–2851. doi: 10.1161/01.cir.0000437913.98912.1d.
10. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MR, Towbin JA; Heart Failure Society of America. Genetic evaluation of cardiomyopathy–a Heart Failure Society of America practice guideline. *J Card Fail*. 2009;15:83–97. doi: 10.1016/j.cardfail.2009.01.006.
11. Hiratzka LF, Bakris GL, Beckman JA, Bersin RM, Carr VF, Casey DE Jr, et al; American College of Cardiology Foundation; American Heart Association Task Force on Practice Guidelines; American Association for Thoracic Surgery; American College of Radiology; American Stroke Association; Society of Cardiovascular Anesthesiologists; Society for Cardiovascular Angiography and Interventions; Society of Interventional Radiology; Society of Thoracic Surgeons; Society for Vascular Medicine. 2010 ACCF/AHA/AATS/ACR/ASA/SCA/SCAI/SIR/STS/SVM guidelines for the diagnosis and management of patients with thoracic aortic disease: executive summary. A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines, American Association for Thoracic Surgery, American College of Radiology, American Stroke Association, Society of Cardiovascular Anesthesiologists, Society for Cardiovascular Angiography and Interventions, Society of Interventional Radiology, Society of Thoracic Surgeons, and Society for Vascular Medicine. *Catheter Cardiovasc Interv*. 2010;76:E43–E86.
12. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424. doi: 10.1038/gim.2015.30.
13. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al; American College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15:565–574. doi: 10.1038/gim.2013.73.
14. Strom SP, Lee H, Das K, Vilain E, Nelson SF, Grody WW, et al. Assessing the necessity of confirmatory testing for exome-sequencing results in a clinical molecular diagnostic laboratory. *Genet Med*. 2014;16:510–515. doi: 10.1038/gim.2013.183.
15. Rehm HL, Bale SJ, Bayrak-Toydemir P, Berg JS, Brown KK, Deignan JL, et al.; Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Commitee. ACMG clinical laboratory standards for next-generation sequencing. *Genet Med*. 2013;15:733–747. doi: 10.1038/gim.2013.92.

16. Mani A, Meraji SM, Houshyar R, Radhakrishnan J, Mani A, Ahangar M, et al. Finding genetic contributions to sporadic disease: a recessive locus at 12q24 commonly contributes to patent ductus arteriosus. *Proc Natl Acad Sci U S A*. 2002;99:15054–15059. doi: 10.1073/pnas.192582999.
17. Ng PC, Levy S, Huang J, Stockwell TB, Walenz BP, Li K, et al. Genetic variation in an individual human exome. *PLoS Genet*. 2008;4:e1000160. doi: 10.1371/journal.pgen.1000160.
18. Molina JE, Benditt DG, Adler S. Crinkling of epicardial defibrillator patches. A common and serious problem. *J Thorac Cardiovasc Surg*. 1995;110:258–264. doi: 10.1016/S0022-5223(05)80032-7.
19. Solaro RJ, Van Eyk J. Altered interactions among thin filament proteins modulate cardiac function. *J Mol Cell Cardiol*. 1996;28:217–230.
20. Mogensen J, Kubo T, Duque M, Uribe W, Shaw A, Murphy R, et al. Idiopathic restrictive cardiomyopathy is part of the clinical expression of cardiac troponin I mutations. *J Clin Invest*. 2003;111:209–216. doi: 10.1172/JCI16336.
21. Karnabi E, Qu Y, Mancarella S, Boutjdir M. Rescue and worsening of congenital heart block-associated electrocardiographic abnormalities in two transgenic mice. *J Cardiovasc Electrophysiol*. 2011;22:922–930. doi: 10.1111/j.1540-8167.2011.02032.x.
22. Matthes J, Yildirim L, Wietzorrek G, Reimer D, Striessnig J, Herzig S. Disturbed atrio-ventricular conduction and normal contractile function in isolated hearts from Cav1.3-knockout mice. *Naunyn Schmiedebergs Arch Pharmacol*. 2004;369:554–562. doi: 10.1007/s00210-004-0940-7.
23. Qu Y, Baroudi G, Yue Y, Boutjdir M. Novel molecular mechanism involving alpha1D (Cav1.3) L-type calcium channel in autoimmune-associated sinus bradycardia. *Circulation*. 2005;111:3034–3041. doi: 10.1161/CIRCULATIONAHA.104.517326.
24. Rose RA, Sellan M, Simpson JA, Izaddoustdar F, Cifelli C, Panama BK, et al. Iron overload decreases CaV1.3-dependent L-type Ca2+ currents leading to bradycardia, altered electrical conduction, and atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2011;4:733–742. doi: 10.1161/CIRCEP.110.960401.
25. Bagnall RD, Weintraub RG, Ingles J, Duflou J, Yeates L, Lam L, et al. A prospective study of sudden cardiac death among children and young adults. *N Engl J Med*. 2016;374:2441–2452. doi: 10.1056/NEJMoa1510687.
26. Sheen VL, Jansen A, Chen MH, Parrini E, Morgan T, Ravenscroft R, et al. Filamin A mutations cause periventricular heterotopia with Ehlers-Danlos syndrome. *Neurology*. 2005;64:254–262. doi: 10.1212/01.WNL.0000149512.79621.DF.
27. Campuzano O, Allegue C, Sarquella-Brugada G, Coll M, Mates J, Alcalde M, et al. The role of clinical, genetic and segregation evaluation in sudden infant death. *Forensic Sci Int*. 2014;242:9–15. doi: 10.1016/j.forsciint.2014.06.007.
28. Baig SM, Koschak A, Lieb A, Gebhart M, Dafinger C, Nürnberg G, et al. Loss of Ca(v)1.3 (CACNA1D) function in a human channelopathy with bradycardia and congenital deafness. *Nat Neurosci*. 2011;14:77–84. doi: 10.1038/nn.2694.
29. Platzer J, Engel J, Schrott-Fischer A, Stephan K, Bova S, Chen H, et al. Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca2+ channels. *Cell*. 2000;102:89–97.
30. Petäjä J, Rasi V, Vahtera E, Myllylä G. Familial clustering of defective release of t-PA. *Br J Haematol*. 1991;79:291–295.
31. Ladenvall P, Wall U, Jern S, Jern C. Identification of eight novel single-nucleotide polymorphisms at human tissue-type plasminogen activator (t-PA) locus: association with vascular t-PA release in vivo. *Thromb Haemost*. 2000;84:150–155.
32. Sartori MT, Saggiorato G, Spiezia L, Varvarikis C, Carraro G, Patrassi GM, et al. Influence of the Alu-repeat I/D polymorphism in t-PA gene intron 8 on the stimulated t-PA release after venous occlusion. *Clin Appl Thromb Hemost*. 2003;9:63–69.
33. Stead NW, Bauer KA, Kinney TR, Lewis JG, Campbell EE, Shifman MA, et al. Venous thrombosis in a family with defective release of vascular plasminogen activator and elevated plasma factor VIII/von Willebrand's factor. *Am J Med*. 1983;74:33–39.
34. Kathiresan S, Yang Q, Larson MG, Camargo AL, Tofler GH, Hirschhorn JN, et al. Common genetic variation in five thrombosis genes and relations to plasma hemostatic protein level and cardiovascular disease risk. *Arterioscler Thromb Vasc Biol*. 2006;26:1405–1412. doi: 10.1161/01.ATV.0000222011.13026.25.
35. Guella I, Duga S, Ardissino D, Merlini PA, Peyvandi F, Mannucci PM, et al. Common variants in the haemostatic gene pathway contribute to risk of early-onset myocardial infarction in the Italian population. *Thromb Haemost*. 2011;106:655–664. doi: 10.1160/TH11-04-0247.
36. Yang Y, Muzny DM, Reid JG, Bainbridge MN, Willis A, Ward PA, et al. Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *N Engl J Med*. 2013;369:1502–1511. doi: 10.1056/NEJMoa1306555.
37. Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among patients referred for clinical whole-exome sequencing. *JAMA*. 2014;312:1870–1879. doi: 10.1001/jama.2014.14601.
38. Ceyhan-Birsoy O, Pugh TJ, Bowser MJ, Hynes E, Frisella AL, Mahanta LM, et al. Next generation sequencing-based copy number analysis reveals low prevalence of deletions and duplications in 46 genes associated with genetic cardiomyopathies. *Mol Genet Genomic Med*. 2016;4:143–151. doi: 10.1002/mgg3.187.

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.

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

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SUPPLEMENTAL MATERIAL

Table S1: Clinical Indications and Genetic Testing Results – Grouped by Disease Category

A) Connective Tissue Diseases

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
2	TAA/AAA (both)	65	M	*SMAD3* R243S	No	Yes (Nat Genet 2011;43(2):121-6	0	R243R in EXAC, personal history of TAA, AAA, tall, lanky, family history of TAA, AAA	Unknown Significance	PM2, PP3, PP4
27	TAA	62	M	*MYLK* R1232C	No	Yes (Am J Hum Genet 2010; 87(5):701-7	0	Actin-binding domain of gene, no curated	Unknown Significance	PM2, PP3 ,PP4,
29	TAA	45	M	*MYLK* R55W	No	Yes (Am J Hum Genet 2010; 87(5):701-8)	5.79E-5	Did not segregate in family; hot spot R55R, R55Q, R55L all in EXAC none have any ClinVar correlation	Unknown Significance	BS4
36	TAA	57	M							
47	TAA	46	F							
55	TAA	60	F							
58	TAA/family history TAA	63	M							
70	TAA	32	M							
74	TAA	52	M							
94	TAA; family history TAA	37	M							
139	TAA	61	M							
143	TAA	61	M							
148	TAA	54	M							
156	TAA	62	M							
159	TAA, Family history of TAAD	45	M							
173	TAA	54	M							
176	TAA	61	F	*MYH11 R1805W*	No	Yes (Nat Genet 2006; 38(3):343-9)	1.65E-05	Family history of valve replacement, brain aneurysm, family members unavailable for segregation	Unknown Significance	PP3, PP4
197	TAA	37	M	*SKI R489K*	No	For Sphrpintzen-Goldberg Craniosynostosis, Marfan Syndrome, or Loeys-Dietz (Nat Genet 2012;44(11):1249-54)	8.871E-06	Has aneurysm at Sinus of Valsalva, but no other features of other conditions.	Uncertain Significance	PP3, PP4
6	PVH; EDS	25	F	*FLNA* 4214delA; *COL5A1* D1771N; COL5A2 *G702R*	No; No; No	Yes (Neurology 2005;64(2):254-262;Yes(Am J Med Genet C Semin Med Genet 2005;139C(1):17-23); Yes(J Med Genet 1998;35:846-8)	0; 0; 1.663E-5	Family segregation done - Dad had *COL5A1*, but no phenotype, Mom has *COL5A2* with phenotype, *FLNA* de novo, sister has COL5A1/2 and phenotype, brother has COL5A2 and phenotype, maternal grandmother has COL5A2, history of aneursym; D1771G in EXAC 1 time; predicted benign	Pathogenic; Benign; Likely Pathogenic	PS2, PM2;BS2, BS4; PM2, PP1(family well-characterized), PP3
157	EDS	33	M	*ELN G3V*	No	No for Williams Syndrome (Cell 1993; 73:159-68), Cutis Laxa (Am J Med Genet 2008;146A:977-83), and Supravalvular Aortic Stenois (J Clin Invest 1994;93:1071-77.	0	G3G in EXAC	Unknown Significance	PM2, PP3

158	EDS	35	F							
160	EDS	62	F	*COL1A1 R763H*	No	Yes (Am J Hum Genet 2000;66:1398-1402)	4.954E-05	Sister has similar symptoms, but untested. G764 is a variant reported to cause osteogenesis imperfecta type 2. In triple helical region of gene	Unknown Significance	PP2, PP3, PP4
16	Marfan syndrome	26	F	*FBN1* IVS2+1G>A	Yes (http://www.umd.be/FBN1/4DACTION/W_DMDT1/1)	Yes (Proc Natl Acad Sci USA 1992;89(13):5917-22	0	Family has clinical phenotype: Brother, mother untested	Pathogenic	PVS1, PM2, PM3
20	Marfan syndrome	27	M							
31	Marfan syndrome	55	F	*FBN1* G77X	Yes(http://www.umd.be/FBN1/4DACTION/W_DMDT1/1)	Yes (Proc Natl Acad Sci USA 1992;89 (13):5917-21	0	Family has clinical phenotype, son was given Marfan clinical diagnosis as a child, but no genetic testing	Pathogenic	PVS1, PM2, PM4
34	Marfan syndrome	21	M	*TGFBR2* V387M	Yes (ClinVar, Hum Mutat 2006;27(8):760-9)	Yes(Hum Mutat 2006;27(8):760-9, Hum Mutat 2008; 29(11):E284-95)	0	Mild phenotype - tall lanky	Likely Benign	BS3, BP6
42	Marfan syndrome	64	F	*FBN2* D1485G	No	Yes (Nat Genet 1995; 11(4):456-8)	2.997E-4	D1485N also in EXAC, segregation in family; son has similar phenotype and same genotype	Unknown Significance	PP1, PP3
51	Marfan syndrome	53	F							
85	Marfan syndrome	57	F	*FBN1* R945C	No	Yes (Genomics 1993;17(2):468-75)	0	R954H in ClinVar as Pathogenic, son died of ruptured TAA, prompted testing	Likely Pathogenic	PM2, PM5, PP3, PP4
33	Noonan syndrome	24	M	*PTPN11* I309V; *MYH11* V87L	Yes (ClinVar);No	Yes(Am J Hum Genet 2002; 70(6):1555-63); Yes (Nat Genet 2006; 38(3):343-9	4.119E-4; 0	1 homozygous in EXAC for I309V; V87 in myosin motor domain of MYH11, Dad, sister has some features, but untested	Likely Benign; Unknown Significance	BS3, BP4, PB5, BP6; PM2
38	SCAD	60	F							
98	SCAD	28	M							
103	SCAD	63	M							
178	SCAD	43	F	*COL5A2 R1106W, FBN1 A2025S*	Yes (ClinVar); Yes (ClinVar)	For EDS (J Med Genet 1998;35:846-8); For SCAD (Heart 2016;102:878-881)	0.0004982; 0.0004622	Patient was post-partum with event and is hyperflexible, mother has similar symptoms (coronary stent w/o CAD and hyperflexible), patient also has had cerebral aneurysm <1 year post SCAD	Unknown Significance; Unknown Significance	PP3; PP3
186	SCAD	36	F							
188	SCAD	52	M	*FBN1 D2757N*	No	Yes (J Am Coll Cardiol 2016;67(23):2744-54, Heart 2016;102:878-881)	0	Patient also had splenic infarction, celiac artery aneurysm, renal artery aneurysm, has some flexibility, has family history of stroke and "heart attack". Variant in carboxyl terminal of gene	Uncertain Significance	PM2, PP3
190	SCAD	60	F							

B) Sudden Cardiac Death (SCD)

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
4	SCD (RCM)	54	F	*TNNI3* L198V	Yes(ClinVar)	Yes (Neth Heart J 2011; 19(7-8): 344-51)	0	Causes a change in splice site	Pathogenic	PM2, PM4, PP3, PP5
11	SCD	51	F							
12	SCD	66	F	*KCNH2* N33T	No	Yes (Circulation 2002; 105(7):794-9)	0	2 submissions in ClinVar - Gene DX pathogenic, NHS VUS, this mutation increased rate of deactivation several	Pathogenic	PS3, PM1, PM2, PP3, PP5

								papers; family segregation done unaffected do not have variant		
13	SCD	61	M	*DPP6* D801N	No	Yes (Am J Hum Genet 2009;84(4):468-76)	0	Segregation done on family, but since idiopathic VF, no phenotypic information	Unknown Significance	PM2, PP3
19	SCD (HCM)	58	M	*MYBPC3* IVS28+22T>G	No	Yes (Circulation 2003; 107(17):2227-32)	0	Family segregation done - children without phenotype and do not carry mutation	Pathogenic	PVS1, PM2, PP1, PP3
21	SCD (DCM)	47	F	*LMNA* S595R; *MYH6* R1636C	No; Yes (ClinVar)	Yes (Eur J Heart Fail 2013; 15(6):628-37;Yes (Circulation 2005;112(1):54-9)	0; 4.2E-4	EXAC also has R1636H	Unknown Significance; Likely Benign	PM2, PP3; BP4, BP6
26	SCD (Non-specific NICM)	47	F	*TTN* R18966X; *NEXN* E205K	No; Yes (ClinVar)	Yes (N Engl J Med 2012; 366(7): 619-28);Yes (Am J Hum Genet 2010; 87(5):687-93)	0; 1.9E-4	2 reports in ClinVar both unknown significance for NEXN variant, NEXN variant in Glu rich region.	Pathogenic; Unknown Significance	PVS1, PM2, PM4;PP3
30	SCD	21	M	*DSG2* V56M	Yes (ClinVar)	Yes for ARVC (Eur Heart J 2007; 28(5):581-8)	1.873E-3	Reported not likely pathogenic for ARVC (Nat Clin Cardiovasc Med 2008;5(12):E1)	Likely Benign	BS3, BP6,
32	SCD (HCM)	26	M							
44	SCD, TdP, LQTS	42	F	*SCN5A* L461V	Yes (ClinVar)	Yes (Heart Rhythm 2010;7(1):33-46)	0.01163	L461L in EXAC; Reported Polymorphism in Heart Rhythm 2010;7(1):33-46	Likely Benign	BS1, BP4, BP5
54	SCD (DCM)	47	F	*DSP* Q1672X	No	Yes for ARVC (Can J Cardiol 2014;30(12):1655-61), For DCM (Genet Med 2014; 16(8):601-8)	0		Pathogenic	PVS1, PM2, PM4
59	SCD (Non-Specific NICM)	24	F	*TTN* c.45689delG; *MYH6* R1270C	No; No	Yes (N Engl J Med 2012; 366(7):619-28, Sci Tranl Med 2015; 7(270):270ra6);Yes (Circulation 2005;112(1):54-9)	0; 5.766E-5	R1270H also in EXAC; segregation done on family - Dad carries *MYH6* only without disease; sister carries both mutations	Pathogenic; Benign	PVS1, PM2; BS2, BS4, BP5
63	SCD (Non-Specific NICM)	29	M							
65	SCD	47	F	*SCN5A* S216L; *DSG2* 12-2 A>G	Yes (ClinVar, Clin Transl Sci 2008;1(1):21-26); Yes(Circulation 2006;113:1171-1179)	Yes (Heart Rhythm 2010;7(1):33-46); Yes(Circulation 2006;113:1171-1179)	0.001;0	DSG2 mutation may not be sufficient to cause disease	Likely Pathogenic; Likely Benign	PS3, PP3, PP4; BS3, BP2
68	SCD	60	F							
75	SCD (DCM)	40	F	*TTN* c.29042-2A>C	Yes(Sci Tranl Med 2015; 7(270):270ra 6)	Yes (N Engl J Med 2012; 366(7):619-28, Sci Tranl Med 2015; 7(270):270ra6)	0	VF arrest, family unavailable for testing	Pathogenic	PVS1, PM2, PM4
77	SCD (LQTS)	78	M							
79	SCD (ARVC)	22	M	*SCN5A* V1951L; *PNN* I331(GAGdel)	Yes (ClinVar, Heart Rhythm 2004;1(5):60 0-7); No	Yes for BrS (Heart Rhythm 2010;7(1):33-46);	5.412E-3;0	V1951L has 6 homozygotes in EXAC, reported polymorphism, V1951M in EXAC and Pathogenic in ClinVar, most ClinVar reports for V1951L are benign likely benign;	Benign; Unknown Significance	BS2, BS3, BP4, BP6; PM2
83	SCD (LQTS)	60	F	*ANK2* V3634D; *AKAP9* R1285G; *GYG1* Y282S	Yes(ClinVar) ;No; No	Yes (Nature 2003;421:633-639, Proc Natl Acad Sci USA 2007;104(52):20990-5)	2.738E-4; 1.048e-5; 0	V3646I in EXAC, ClinVar reports V3634I as pathogenic;AKAP9 variant also known as R1246G; GYG1 Y282X in EXAC 8.238e-06, both heterozygous variants	Unknown Significance; Unknown Significance; Unknown Significance	PM5, PP3; BP4; PM2
84	SCD (DCM)	40	M	*TTN* W21280X	No	Yes (N Engl J Med 2012; 366(7):619-28)	0	Family segregation, Dad same phenotype and genotype	Pathogenic	PVS1, PM2, PM4, PP1
90	SCD (DCM)	19	M	*MYPN* L867I, *RBM20* F510S, *ACTN2* D475N	Yes (ClinVar); No; Yes (ClinVar)	Yes(Cardiovasc Res 2008;77(1):118-125); Yes(Clin Tranl Sci 2010; 3(3):90-7); Yes (Circ Cardiovac Genet 2014;7(6):741-50	3.296E-3; 9.39E-5; 7.229E-3	*MYPN* variant has 5 homozygous, 2 reports in ClinVar with no pathogenicity (uncertain significance); 45 homozygotes for *ACTN2* variant and 3 reports in ClinVar all benign	Unknown Significance; Unknown Significance; Likely Benign	PP3; PP3;BP4, BP5, BP6
127	SCD	52	F							
131	SCD (CPVT)	50	F	*RYR2* Y2553C; *AKAP9* G3816V	No; No	Yes(Heart Rhythm 2015;12(7):1636-43); Yes (Proc Natl Acad Sci USA 2007;104(52):20990-5)	0;0	Y2553Y in EXAC, segregation done on children; both have AKAP9 mutation and not RYR2	Unknown Significance; Unknown Significance	PM2, PP3, BS4; PM2, PP1, PP3

132	SCD	37	M	*NEXN* E407Q	No	Yes (Am J Hum Genet 2010; 87(5):687-93	0	In frame deletion in EXAC at E407, no clinical significance	Unknown Significance	PM2, BP4
136	SCD	34	F							
137	SCD (HCM)	40	F							
138	SCD	26	M	*TGFB3* Y390F	No	No for LDS/FTAAD (Am J Med Genet A 2013; 161A(8): 2040-6, J Am Col Cardiol 2015;65(13):1324-36)	0	No genotype/phenotype correlation known	Unknown Significance	PM2
140	SCD (LQTS)	32	F	*ANK2* D2445G	No	Yes (Nature 2003;421:633-639)	8.25E-6		Unknown Significance	PP3, PP4
144	SCD	23	M							
147	SCD	37	F							
152	Congenital hearing loss/TdP	34	F	*CACNA1D* R1902X	No	Homozygous Inframe insertion (Nat Neurosci 2011;14(1):77-84);	0	Possible dominant negative effect	Likely Pathogenic	PM2, PM4, PM6, PP4
163	SCD; TdP	57	M	*CACNA1D c.5827_5829 (F1943del)*	Yes (ClinVar)	Homozygous Inframe insertion (Nat Neurosci 2011;14(1):77-84)	0.002542	Patient also has bradycardia. Sister (unaffected) does not have variant. Reported benign in ClinVar	Likely Benign	BS1, BS4, BP6
165	SCD	18	M	*MYPN R955W*	Yes (ClinVar, Eur J Hum Genet 2013; 21(3):294-300	For cardiomyopathy (Cardiovasc Res 2008; 77(1):118-25 and J Am Col Cardiol 2014;64(25):2765-76).	0.0004287	ClinVar conflicting interpretations – 1 likely benign, 1 likely pathogenic, 2 uncertain significance, segregation in family – Dad has variant, but no cardiomyopathy and/or arrhythmia, patient had normal cMR. Eur J Hum Genet paper R955W suspected of being disease causing, variant in α-actinin binding region of gene	Uncertain Significance	PP3
170	SCD	25	F	*SCN5A A572D*	Yes (ClinVar, Genet Test 2003;7(1):57-61)	Yes (Genet Test 2003;7(1):57-61, Heart Rhythm 2010;7(7):912-9	0.004304	A572V in EXAC, did not segregate in family, ClinVAR 5 likely benign, 1 likely pathogenic	Benign	BS3, BS4, BP4
193	SCD	54	M	*RYR2 S3349L; TTN c.90223delG*	No; No	Yes(Circulation 2002;106:69-74); Yes(N Engl J Med 2012; 366(7): 619-28)	0; 0	Cardiac event was VF arrest, brother had cardiac arrest as well. RYR2 S3349T in EXAC no clinical information; TTN variant found in A band of gene	Uncertain Significance; Pathogenic	PM2, PP3; PVS1, PM2

C) Hypertrophic Cardiomyopathy (HCM)

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
7	HCM	46	M							
8	HCM	60	M	TPM1 *D175N*	Yes (Ann Med. 2013; 45(1):85-90)	Yes (Ann Med. 2013; 45(1):85-90)	0		Pathogenic	PS3, PP3, PP4, PP5
10	HCM (apical variant), syncope, bradycardia, conduction abnormality	73	F	TRPM4 *Q752X*	No	No, only missense mutations reported (J Clin Invest 2009; 119(9):2737-44, Circ Cardiovasc Genet 2010;3(4):374-85)	1.833E-3	Dominant negative mutations exist - D984A (J Biol Chem 2005;280(24):22899-906)	Pathogenic	PVS1, PS3
23	HCM	47	M	*MYH6* I753T	No	Yes (Circulation 2005;112(1):54-9)	3.30E-5	This has been segregated in family, son has variant and some LVH	Unknown Significance	PP3
37	HCM (apical variant)	57	M							
41	HCM	48	M							
46	HCM	57	M	*MYBPC3* c.2554_2555insT	No	Yes (Circulation 2003; 107(17): 2227-32)	1.69E-5	Family segregation done - son has genotype and clinical disease	Pathogenic	PVS1, PM4, PP1
50	HCM (Family history HCM)	33	F	*MYH6* V39M	Yes (ClinVar)	Yes (Circulation 2005;112(1):54-9)	9.06E-5	Family history of SCD with autopsy showing LVH	Unknown Significance	PP3
52	HCM	42	M	*TNNT2* c.477delCTC	No	Yes(N Engl J Med 1995;232(16):1058-64	0	Segregated with disease in NEJM paper	Pathogenic	PVS1, PM2, PM4
53	HCM	58	M	*MYH6* R34C; *MYBPC3* V219L	No; Yes (ClinVar)	Yes (Circulation 2005;112(1):54-9); Yes (Circulation 2003; 107(17):2227-32)	2.471E-5; 0	R34H also in EXAC, V219F in EXAC - considered pathogenic in ClinVar	Unknown Significance; Likely Pathogenic	PP3;PM2, PM5, PP1, PP3,

76	HCM	62	M	*MYBPC3* R820Q	Yes (ClinVar)	Yes (Circulation 2003; 107(17):2227-32, J Am Coll Cardiol 2003l 41(5):781-6)	1.66E-5	R820P and R820W also in ClinVar, both have uncertain significance, R820Q is pathogenic/likely pathogenic in ClinVar R820W also in EXAC	Pathogenic	PS3, PS4, PP5
86	HCM	56	M	*MYBPC3* V598S	No	Yes (Circulation 2003; 107(17):2227-32)	0	V598Sfs is pathogenic in ClinVar single submission with clinical assertion information	Likely Pathogenic	PM2, PP3, PP4
93	HCM	23	M	*TNNT2* A202T; *TCAP* S64L; *CALR3* R356L	No; Yes(ClinVar) ; No	Yes(Cell 1994; 77(5):701-12;Yes(Clin Tranl Sci 2010; 3(3):90-7);Yes(J Mol Cell Cardiol 2007;43(3):337-43)	0; 1.14E-3; 2.207E-	*TCAP* S64 has 2 homozygotes and multiple reports in ClinVar with a benign pathogenicity; R356 in *CALR3* hotspot R/H, R/C, R/G also seen, R365L has 6 homozygotes	Unknown Significance; Likely Benign; Unknown Significance	PM2; BP4, BP6; BP4
104	HCM	56	F	*TNNT2* A202T	No	Yes (Cell 1994; 77(5):701-12	0	Alternate isoform - intronic in canonical common alteration 0.01224 in EXAC (intronic mutation)	Unknown Significance	PM2, BP4
105	HCM	24	M	*MYBPC3* V189I	Yes(ClinVar)	Yes (Circulation 2003; 107(17):2227-32)	2.80E-03	4 homozygotes in EXAC, 4 total reports in ClinVar, 2 likely benign, 2 unknown significance	Likely Benign	BP4, BP6
112	HCM	65	F	*TNNT2* ΔGlu160	Yes(N Engl J Med 1995;232(16) :1058-64	Yes(N Engl J Med 1995;232(16):1058-64	0		Pathogenic	PVS1, PM2, PM4
117	HCM	52	M	*MYH7* T1377M; *MYBPC3* A522V; *AKAP9* D2381G; *TTN* R2262Q	Yes (Circulation 2003; 107(17):2227 -32, J Cardiovasc Med (Hagerstown) 2006;7(8):60 1-17, J Am Coll Cardiol 2004;44(3):6 02-10, J Med Genet 2011; 48(8):572-6); No; No; No	Yes(Circulation 2003; 107(17):2227-32, J Cardiovasc Med (Hagerstown) 2006;7(8):601-17, J Am Coll Cardiol 2004;44(3):602-10, J Med Genet 2011; 48(8):572-6);Yes (Proc Natl Acad Sci USA 2007;104(52):20990-5); No (N Engl J Med 2012; 366(7):619-28)	0; 2.94E-5; 0; 0	T1377M reported multiple cases of HCM without controls having mutations; A522T in ClinVar and called likely benign by all 5 reports	Pathogenic; Likely Benign; Unknown Significance; Likely Benign	PS4, PM2, PP2, PP3, PP4; BP4, BP5; BP5; BP1, BP5
118	HCM	61	M	*TNNT2* R285C	Yes (ClinVar, N Engl J Med 1995;332(16) :1058-64	Yes (N Engl J Med 1995; 232(16):1058-64	0	Did not segregate in family - brother has similar symptoms, but no other mutation found	Unknown Significance	PM2, PP5, BS4
119	HCM	56	F							
121	HCM	69	M							
123	HCM	39	F	*ABCC9* IVS17-1G>A	No	Yes for DCM, (Nat Genet 2004;36:382-387)	1.62E-3	Splice prediction loss of exon 17, radical mutations reported elsewhere, but closer to c-terminal of gene	Pathogenic	PVS1, PM4
145	HCM	53	M	*JPH2* A405T	No	Yes (J Mol Cell Cardiol 2007;42(6):1026-35	6.61E-5		Likely Pathogenic	PS3, PP3, PP4
168	LVH, TAA	48	M	*ACTN2 N170H*	No	For HCM (J Am Coll Cardiol 2010;55:1127-35)	3.29E-05	N170I and N170fs also in EXAC none have clinical correlation in ClinVar, Brother has similar phenotype (TAA with LVH) untested at this time	Uncertain Significance	PP3
177	HCM	28	M	*TPM1 D254G*	Yes (ClinVar)	Yes (Ann Med. 2013; 45(1):85-90)	0	Sister passed away at 13 – known HCM no genetic diagnosis, mother has variant and disease, ClinVar -2 submitters, likely pathogenic	Likely Pathogenic	PM2, PP1, PP3, PP4, PP5
179	HCM, syncope	29	M	*PRKAG2 T174M*	Yes (ClinVar)	Yes (Hum Molec Genet 2001;10:1215-20)	4.12E-05	Also has ventricular arrhythmias, family history of SCD. ClinVar – Uncertain significance 1 submission	Unknown Significance	PP4
182	HCM	54	M	*TNNT2 A28V*	Yes (Eur J Med Genet 2011;54(6):e5 70-5)	Yes for HCM (Cell 1994;77:701-12), For DCM (Eur J Med Genet 2011;54(6):e570-5)	0.0004868	ClinVar – 3 uncertain significance submissions, 1 likely benign submission, 1 pathogenic submission. All reports for are DCM	Unknown Significance	PP5, BP6
183	HCM	50	M							
184	HCM	27	M	*PRKAG2 R302P*	No	Yes (N Engl J Med 2001; 344(24):1823-31)	0	ClinVar has R302L – 5 submission all pathogenic, N Engl J Med reports R302Q as a segregating variant in a large family, reported binding site for ATP/AMP	Likely Pathogenic	PM1, PM2, PM5, PP3

D) Dilated Cardiomyopathy (DCM)

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
18	DCM	38	M							
22	DCM	30	M	DSP R866C	Yes (http://www.arvcdatabase.info/Default.aspx)	Yes for ARVC (Circulation 2011;123(23):2690-2700)	6.096E-4	Hotspot? R886H and R866L in EXAC; in clinical report of this mutation, several other mutations also found in LMNA, DES, MYBPC3, and MYH7, two independent labs call mutation likely benign	Likely Benign	BS3, BP6
39	DCM	32	F	RBM20 S1195Y	Yes (ClinVar)	Yes (Clin Tranl Sci 2010; 3(3): 90-7)	6.661E-4	No segregation, but family DCM	Unknown Significance	PP3, PP4
45	DCM	59	F	DSG2 V239A	Yes (ClinVar)	Yes for ARVC (Circulation 2011;123(23):2701-9)	9.14E-5		Unknown Significance	PP3
66	DCM	28	M							
88	DCM	29	F							
95	DCM	56	M	MYBPC3 V1139I	Yes(ClinVar)	Yes (Circulation 2003; 107(17):2227-32)	8.75E-5	2 reports in ClinVar with unknown significance	Unknown Significance	BP4
100	DCM, Afib, bradycardia	47	M	MYH6 S1917P; MYH6 L24P	No; No	Yes (Circulation 2005;112(1):54-9)	0;0	L24I in EXAC (with 1 homozygote) and ClinVar, Uncertain significance	Unknown Significance; Unknown Significance	PM2, PP4; PM2, PP4
102	DCM, Afib, bradycardia, VT	48	M							
106	DCM	56	F	PRDM16 A34T; BAG3 P23S	No; No	No related to 1p36 deletion (Am J Hum Genet 2013;93(1):67-77, PLoS One 2014;9(1):e85600); Yes (Am J Hum Genet 2011; 88(3):273-82	2.018E-3, 3.598e-5	1 homozygous in EXAC, Hot spot? A34V, A34G, and A34A all in EXAC, some debate about PRDM16 and disease - related to 1p36 deletion syndrome	Unknown Significance; Unknown Significance	PP3;PP3
107	DCM	54	F	HFE H63D	Yes (ClinVar, Gastroenterology 2002; 122(3): 646-51)	Yes (Am J Hum Genet 1997;61(3):762-4	0.1066	Family known to have hemochromatosis	Pathogenic	PS3, PP3, PP4, PP5
108	DCM	38	M	LMNA c.509 insC	No	Yes (Eur J Heart Fail 2013; 15(6):628-36	0	Other frameshifts reported to be deleterious	Pathogenic	PVS1, PM2, PM4
110	DCM	68	F	LMNA IVS3 - 10A>G	No	Yes (Eur J Heart Fail 2013; 15(6):628-37	0	Other frameshifts reported to be deleterious	Pathogenic	PVS1, PM2, PM4
130	DCM	38	F							
133	DCM	52	M							
134	DCM	67	M							
135	DCM	43	F							
150	DCM/family history SCD	57	M							
151	DCM/family history CHF	55	F							
155	DCM/CHF; family history CHF	64	F	TMPO S444C; LAMA4 N89S	Yes (Circ Cardiovasc Genet 2013;6(4):337-46);	Yes (Circ Cardiovasc Genet 2013;6(4):337-46); Yes(Circulation 2007;116(5);515-25)	1.73E-04; 8.242e-06	N89N in EXAC	Likely Benign; Unknown Significance	BS1, BP1; PM2, PP4

164	DCM	58	M	TTN c.48470delG; MYH7 V964L	No; Yes (ClinVar)	Yes (N Engl J Med 2012; 366(7): 619-28); Yes (Clin Transl Sci 2008;1(1):21-6)	0; 8.236E-06	TTN variant in A-band; father had heart transplant for cardiomyopathy; conflicting pathogenicity in ClinVar for MYH7 – 5 uncertain significance, one likely pathogenic; daughter has TTN variant	Pathogenic; Uncertain Significance	IVS1, PM2; PP3, PP5
180	DCM	57	M	PRDM16 I302T; SCN5A S216L	No; Yes(Clin Transl Sci 2008;1(1):21-6)	Yes(Am J Hum Genet 2013; 93:67-77);Yes for DCM (Clin Transl Sci 2008;1(1):21-6)	8.384E-06; 0.001	For SCN5A 1 homozygous person in EXAC, ClinVar – 5 submissions all uncertain significance, from Clin Transl Sci paper – 2 people with the same variant, no segregation, but highly conserved and changes charge, considered possible cause of disease, variant has been associated with LQTS and atrial fibrillation	Unknown Significance; Unknown Significance	BP4; PP3
185	DCM	62	F							
187	DCM	28	F	PRKAG2 R84W	Yes (ClinVar)	Yes (N Engl J Med 2001; 344(24):1823-31)	0.0002535	Family history of cardiomyopathy, sudden death. ClinVar 1 submission uncertain significance, 2 submissions likely benign, R84Q also in ClinVar, uncertain significance	Uncertain Significance	PP3, PP4, BP6

E) Familial Hypercholesterolemia (FH) and Lipo-dystrophy

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
1	FH	38	M							
89	FH	55	F							
91	FH	41	M							
92	FH	27	F	APOB R3500Q	Yes (ClinVar, Hum Biol 2005; 77(5):663-73)	Yes (Hum Biol 2005; 77(5):663-73)	2.311E-4	rs5742904, R3527Q, high total cholesterol, but no history of CAD	Likely Pathogenic	PS3, PP3, PP4, PP5
97	FH, DVT	45	M	PLAT Y413H						
99	FH	42	M							
101	FH	38	M	apoE V254E	Yes (ClinVar, Am J Hum Genet 1993;52(5)937-46)	Yes (Am J Hum Genet 1993;52(5)937-46)	1.35E-03	single submission in ClinVar - pathogenic	Pathogenic	PS3, PP3, PP5
124	FH	64	M							
141	FH/CAD	55	F	LDLR A431T	Yes (Annu Rev Genet 1990;24:133-70)	Yes (Annu Rev Genet 1990;24:133-70)	0	FH- Algeria	Pathogenic	PS3, PM1, PP3
154	FH/CAD	38	F	LDLR c.313+1G>A	Yes(Atherosclerosis 1994;111(2):175-82, Am J Med Genet 1996;65(2):149-54, Arterioscler Thromb Vasc Biol 1995; 15(2):219-27)	Yes (Atherosclerosis 1994;111(2):175-82, Am J Med Genet 1996;65(2):149-54, Arterioscler Thromb Vasc Biol 1995; 15(2):219-27)	4.12E-05	G>T also reported in EXAC	Pathogenic	PS1, PS3, PM1
166	FH, xanthomas	48	F							
169	FH	49	M							
171	FH	25	M							

172	FH	52	F							
191	FH; Family History of SCD	62	M							
192	FH	33	F	LDLR M1fs c.3delG	No	Yes (Cell 1985;41:735-43)	0	Maternal family history of hyperlipidemia without CAD	Pathogenic	PVS1, PM2, PM4
198	FH	22	M	LDLR I764fs c.2292delA	No	Yes (Cell 1985;41:735-43)	0	Paternal family history of premature CAD, hypercholesterolemia	Pathogenic	PVS1, PM2
5	Lipo-dystrophy	56	F							
71	Lipo-dystrophy	41	M	LMNA R644C	Yes (Am J Med Genet A 2008;146A(12):1530-42)	Yes (Am J Med Genet A 2008;146A(12):1530-42)	1.243E-3	Segregation in family, well-established mutation site with variable phenotypes	Likely Pathogenic	PS3, PP3, PP4
87	Lipo-dystrophy	33	M	PLIN1 R23W	No	No radical mutations only (N Engl J Med 2011;364(8):740-8, Diabetes 2015;64(1):299-310)	8.544E-4	Radical mutations only reported so far; brother has similar phenotype	Unknown Significance	BP1
149	Lipo-dystrophy	46	F	LMNA R582C	Yes (Eur J Endocrinol 2012;167(3):423-31)	Yes (Eur J Endocrinol 2012;167(3):423-31)	0	R582R in EXAC	Pathogenic	PS4, PM2, PP3, PP4, PP5

F) Long QT Syndrome

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC	Additional Information	Pathogenicity	ACMG criteria*
24	LQTS	56	F							
28	LQTS, syncope	56	F	AKAP9 Q3520H	No	Yes (Proc Natl Acad Sci USA 2007;104 (52):20990-5)	0	Q3520Q in EXAC	Unknown Significance	PM2, PP3
43	LQTS/AF	57	M							
81	LQTS, syncope	25	F							
82	LQTS, syncope	28	F	ANK2 V3634I	Yes (ClinVar, Heart Rhythm 2005;2(11):1218-23)	Yes (Heart Rhythm 2005;2(11):1218-23, Nature 2003;421:633-639)	3.30E-5	V3634I reported in ClinVar pathogenic -one report, V3634D has conflicting pathogenicity in ClinVar	Unknown Significance	PP4, PP5
96	LQTS, palpitations	35	F	RBM20 D996Y, TTN c.32562_insAGA	Yes(ClinVar) Yes (Eur Heart J 2014;35(32):2165-73)	Yes(Clin Tranl Sci 2010; 3(3):90-7); Yes (N Engl J Med 2012; 366(7):619-28)	0; 0	RBM20 Reported once in ClinVar with unknown significance; TTN reported twice with familial and non-familial DCM/PPCM	Unknown Significance; Pathogenic	PM2, PP3; PVS1, PM2, PM4
126	LQTS/AF/HCM	70	M							
129	LQTS	34	M							
142	LQTS, syncope	49	F							
153	LQTS	23	F							
162	LQTS; Family history of SCD	37	F	CTNNA3 H727R	No	Yes (Eur Heart J 2013;34(3):201-10	0	Brother drowned in ocean – military, patient had normal cMR	Unknown Significance	PM2, PP3
175	LQTS	24	F	CAV3 T78M	Yes (Circulation 2006; 114: 2104-2112).	Yes (Circulation 2006; 114: 2104-2112)	0.003038	1 Homozygote in EXAC, ClinVar 5 benign/likely benign, 2 uncertain significance, 2 pathogenic, no other family members available for testing	Unknown Significance	PP5, BP6
181	LQTS, syncope	52	F	ANK2 E1449G	Yes (Nature 2003;42(6923):634-9)	Yes (Nature 2003;42(6923):634-9 and Proc Natal Acad Sci 2004;101(24):9137-42)	0.0004222	ClinVar – 1 submussion likely benign, 5 submissions likely pathogenic/pathogenic, in Nature paper segregation in family	Pathogenic	PS1, PS3, PP4, PP5
196	LQTS, Family History SCD	67	M							

199	LQTS	57	M	KCNQ1 L266P	Yes (Heart Rhythm 2005;2:507-517, Circulation 2007;115(19):2481-9, Heart Rhythm 2009;6:1297-1303)	Yes (Heart Rhythm 2005;2:507-517; Circulation 2007;115(19):2481-9 Heart Rhythm 2009;6:1297-1303)	0	History of syncope in childhood (near drowning), VF arrest, family history of SCD. L266P is known/established cause of LQTS, transmembrane variant	Pathogenic	PS4, PM2, PP3, PP4, PP5

G) Atrial and/or Ventricular Arrhythmias

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
56	Atrial and Ventricular arrhythmias	43	F	CACNB2 V255L	No	Yes for BrS and SCD (J Mol Cell Cardiol 2009; 46(5):695-703,Heart Rhythm 2010;7(12):1872-82)	0		Unknown Significance	PM2, PP3
60	Atrial Arrhythmia	56	F							
62	Atrial Arrhythmia	66	F							
64	Atrial Arrhythmia	69	M	KCNE2 T8A	Yes (ClinVar)	Yes (Proc Natl Acad Sci U S A 2000;97(19):10613-18)	3.804E-3	May cause long QT when on antibiotic therapy (Proc Natl Acad Sci U S A 2000;97(19):10613-18) T8I in EXAC as well, all reports from ClinVar Benign	Unknown Significance	PS3, PP3, BP6
67	Atrial Arrhythmia (SVT)	41	F	ABCC9 I614T	No	Yes (Nat Clin Pract Cardiovasc Med 2007;4:110-116, Circ Res 2013; 112(7):1059-72)	0		Unknown Significance	PP3
73	Atrial Arrhythmia	22	M							
15	VT/Family History SCD	33	F	SYNE2 E497V	No	Yes (Hum Mol Genet 2007; 16(23): 2816-33)	0	GWAS identified SYNE2 loci for AF (Nat Genet 2012;44(6):670-5). Family history of SIDS, personal history of NSVT	Unknown Significance	PM2, PP3
57	Ventricular arrhythmia	23	F	GPD1L R220H	Yes (ClinVar)	Yes (Hum Mutat 2009;30(9):1256-66	3.484E-4	R220C also in EXAC 2.49E-5, both reports in ClinVar as Likely Benign	Likely Benign	BP4, BP6
167	VT, positive procainamide test, family history of VF	57	F							
189	VT/Family History of SCD	22	M							

H) Brugada syndrome

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
9	BrS	55	F							
48	BrS	63	F							
61	BrS, AF	53	M							
69	BrS	23	F	SCN5A S910L	Yes (Nat Rev Cardiol 2009;6(5):337-48)	Yes(Heart Rhythm 2010;7(1):33-46)	0	S910S in EXAC; known BrS variant	Likely Pathogenic	PS3, PM2, PP3, PP4
72	BrS	37	M	SCN5A I786V	No	Yes(Heart Rhythm 2010;7(1):33-46)	0	I786I in EXAC	Unknown Significance	PP3
78	BrS (Family history of SCD)	40	F	DSP V2388I	No	Yes for ARVC (Circulation 2011;123(23):2690-2700)	1.65E-05	Mom, Sister, Niece all have this variant, no one has cMRI indicative of CM, index case does not have ARVC, "low normal LVEF" on cMRI/echo, family hx of SCD, but autopsy shows no CM	Unknown Significance	PP3

115	BrS	64	F	SCN5A c.2551insTG; MYBPC3 V896M; CACNA1C G2022R	Yes(ClinVar) ; Yes(Am J Hum Genet 1999; 65(5):1308-1320; No	Yes (Heart Rhythm 2010;7(1):33-46); Yes (Proc Natl Acad Sci USA 2005;102(23):8089-96); Yes (Circulation 2003; 107(17):2227-32)	0; 0.1275; 0	F851L in ClinVar - Pathogenic; V896M - homozygote in EXAC	Pathogenic; Likely Benign; Unknown Significance	IVS1; BP5, BP6; PM2
146	BrS	51	M	RYR2 R1482H	Yes(ClinVar)	Yes for LQTS (Heart Rhythm 2005; 2(10):1099-1105); Yes for CPVT with and without Atrial Fibrillation (Can J Cardiol 2013;29(8):993-6, Heart Rhythm 2015;12(7):1636-43).	1.994E-4	R1482C in EXAC	Likely Pathogenic	PS3, PM5, BP4
194	BrS	49	M	RYR2 R1482H	Yes(ClinVar)	Yes for LQTS (Heart Rhythm 2005; 2(10):1099-1105); Yes for CPVT with and without Atrial Fibrillation (Can J Cardiol 2013;29(8):993-6, Heart Rhythm 2015;12(7):1636-43).	0.0001994	R1482C in EXAC. ClinVar – 3 submissions 1 likely benign, 2 uncertain significance	Uncertain Significance	BP4

I) Family History of SCD or LQTS

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
3	Family history SCD	59	M	TMEM43 V364M	No	Yes for ARVC (Eur Heart J 2013; 34(13): 1002-11)	8.24E-5	No ARVC phenotype - normal cMRI. Family history of SCD	Unknown Significance	PP2
14	Family history SCD	45	F							
49	Family history SCD; presyncope/palpitations	55	F	SCN5A R1192Q; SCN4B G8S	Yes (ClinVar);Yes (ClinVar)	Yes(Heart Rhythm 2010;7(1):33-46); Circulation 2007; 116(2):134-42	6.215E-3; 4.742E-4	R1192W and R1192R, R1192Q has 17 homozygotes, reports in ClinVar vary for SCN5A variant, SCN5A called polymorphism in 2 papers; SCN4B reported likely benign by one lab in ClinVar	Likely Benign; Unknown Significance	BP4, BP6; BP4, BP6
80	Family history of LQTS	45	M							
116	Family history of SCD, VT	67	M	DES K109N	No	Yes for myopathy with arrhythmias (Nat Genet 1998;19(4):402-3)	0	1st amino acid in the rod domain, E108K is reported to cause DCM (Circulation 2007;115(10):1244-51)	Likely Pathogenic	PM1,PM2, PP3, PP4
120	Family history SCD	45	M							
128	Family history SCD, TWI on EKG	60	F	CACNA1C W1612C; TNNI3 R141Q	No; Yes (Circulation 2003; 107(17):2227-32)	Yes (Proc Natl Acad Sci USA 2005;102(23):8089-96); Yes (Circulation 2003; 107(17):2227-32)	0; 0	CACNA1C W1612X in EXAC; TNNI3 R141W in EXAC and ClinVar - 1 report, predicted likely pathogenic, unclear if R141Q causes disease	Unknown Significance; Unknown Significance	PM2; PM2, PP3, PP5
161	Family history SCD/LQTS	60	M	ANK2 M3649V; DSG2 A308S	No; No	Yes (Nature 2003;421:634-9); Yes for ARVC (Circulation 2006;113:1171-9)	9.893E-05; 0	Brother has ANK2 variant and had SCD; DSG2 variant in Cadherin 3 domain, next to glycosylation site (N309), patient had normal cMR	Unknown Significance; Unknown Significance	PP1, BP4; PM2, PP3
174	Family history SCD	31	M	MYH6 R1398Q; VCL R502Q	Yes (ClinVar); No	For cardiomyopathy (Circulation 2005;112(1):54-9); For cardiomyopathy (Circulation 2002;105:431-7, Biophys Res Commun 2006;345:998-1003)	0.00003879;8.514E-05	VCL R502W and R502R in EXAC, patient had normal imaging studies	Unknown Significance; Unknown Significance	BP5;BP5

J) Non-specific NICM

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
111	Non-Specific NICM, primary conduction disease CM	58	F	LAMP2 T196S	Yes (ClinVar)	Yes (Neuromuscul Disord 2005;16(5):409-11)	2.393E-4	Two reports in ClinVar - one likely benign, one uncertain significance	Unknown Significance	BP6
114	Non-Specific NICM, VT	34	F	LDB3 c.287insC; TTN IVS100-2A>C; MYH6 A1004S	No; No; Yes(ClinVar)	No (Clin Transl Sci 2008;1(1):21-6); Yes (N Engl J Med 2012; 366(7):619-28); Yes (Circulation 2005;112(1):54-9)	0; 0; 9.801E-4	ClinVar has LDB3 c.287T>C likely benign, only missense reported in the literature. Unclear if radical mutation has an effect. MYH6 mutation reported in OMIM, but conflicting reports in ClinVar	Unknown Significance; Pathogenic; Unknown Significance	PM2; PVS1, PM2, PM4; PP5, BP6

122	Non-Specific NICM, Atrial Arrhythmias, Neuromuscular Disorder	33	M	RYR2 R3260W	No	Yes (Heart Rhythm 2015;12(7):1636-43)	4.15E-5	R3260Q in EXAC (with 1 homozygote)	Unknown Significance	PP4
125	Non-Specific NICM	23	M	MIB1 R676X	No	Yes for LVNC (Nat Med 2013;19(2):193-201	0	EXAC has c.2556delC (same location) and in ClinVar as well - reported pathogenic	Pathogenic	PVS1, PM2, PM4
195	Non-Specific NICM/LQT	45	F	PKP2 IVS6-1C>T(c.1379-1C>T; NEXN M540V; AKAP9 R3734P	Yes (ClinVar); Yes (ClinVar); Yes (ClinVar	Yes for ARVC(Nature Genet 2004;36:1162-4); Yes for DCM (Nature Med 2009;15:1281-8), Yes for HCM(Acta Biochm Biophys Sinica 2001;33:19-24); Yes for LQTS (Proc Nat Acad Sci 2007;104:20990-5)	0.0001499; 0.0004253; 0.0007003	PKP2 – ClinVar single submission uncertain significance, high confidence LoF variant EXAC; NEXN Clin Var – 2 submissions 1 likely benign, 1 uncertain significance; AKAP9 in ClinVar 3 submissions all benign/likely benign, R3742Q also in ClinVar 3 submission all benign/likely benign;	Likely Pathogenic; Uncertain Significance; Likely Benign	PVS1, PM4; BP4; BP4, BP6
200	Non-Specific NICM, VT	32	M	DSG2 L836V	No	Yes (Mol Genet Metab 2008;95:74-80)	0		Unknown Significance	PM2, PP3

K) Other

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
40	ARVC (family history of SCD)	60	M	DSP R1458G	Yes (http://www.arvcdatabase.info/Default.aspx)	Yes for ARVC (Circulation 2011;123(23):2690-2700)	1.737E-3	1 homozygous in EXAC, hotspot R1458R, R1458X, R1458Q all reported at least once in EXAC), Other reports of this mutation have people with other mutations compound heterozygous or other ARVC gene	Likely Benign	BP2, BP4, BP6
25	CPVT	48	F							
17	Heart Block (familial)	64	M							
35	PPCM	40	F	NDUFV2 Q31X	No	Yes for HCM (Hum Mutat 2003; 21(6): 582-6)	0	few reported mutations in ClinVar, similar mutation type with HCM	Likely Pathogenic	PM2, PM4,PP3, PP4
109	PPCM	56	F	EMD c.110_112del AGA	No	Yes for Emery-Dreifuss MD (Nat Genet 1994;8(4):323-327); mild features with DCM (BMC Med Genet 2014;15:77)	2.53E-05	No musculoskeletal features -- potentially very mild?	Likely Pathogenic	PVS1, PM4
113	RCM, VT/AF	15	M	*TNNT2* ΔGlu160; *SCN5A* R1027Q	Yes(N Engl J Med 1995;232(16) :1058-64;No	Yes(N Engl J Med 1995;232(16):1058-64;Yes(Heart Rhythm 2010;7(1):33-46)	0; 5.059E-5		Pathogenic; Unknown Significance	PVS1, PM2, PM4; PP5, PB5

* Abbreviations for the ACMG Criteria: PVS – Pathogenic Very Strong, PS – Pathogenic Strong, PM – Pathogenic Moderate, PP – Pathogenic Supporting, BS – Benign Strong, BP – Benign Supporting

Other Abbreviations: TAA – Thoracic Aortic Aneurysm, AAA – Abdominal Aortic Aneurysm, PVH – Periventricular Heterotopia, EDS – Ehlers Danlos Syndrome, SCAD – Spontaneous Coronary Artery Disease, SCD – Sudden Cardiac Death, RCM – Restrictive Cardiomyopathy, HCM- Hypertrophic Cardiomyopathy, DCM – Dilated Cardiomyopathy, Non-Specific NICM – Non-Ischemic Cardiomyopathy, TdP – Torsade Des Pointes LQTS – Long QT Syndrome, ARVC – Arrhythmogenic Right Ventricular Cardiomyopathy, CPVT – Catecholaminergic Polymorphic Ventricular Tachycardia, LVH – Left Ventricular Hypertrophy, FH – Familial Hypercholesterolemia, CAD – Coronary Artery Disease, AF – Atrial Fibrillation, BrS – Brugada Syndrome, VT – Ventricular Tachycardia, VF- Ventricular Fibrillation, TWI on EKG – T-wave Inversion on Electrocardiogram, PPCM – Peripartum Cardiomyopathy

Table S2: Pathogenic Variants Found with American College of Medical Genetics Data

#	Reason for Referral	Age	Sex	Variant	Variant Previously Reported?	Disease Association	Freq. EXAC (January 2016)	Additional Information	Pathogenicity	ACMG criteria*
4	SCD (RCM)	54	F	TNNI3 L198V	Yes(ClinVar)	Yes (Neth Heart J 2011; 19(7-8): 344-51)	0	Causes a change in splice site	Pathogenic	PM2, PM4, PP3, PP5
6	EDS, PVH	25	F	FLNA 4214delA; COL5A2 G702R	No; No	Yes (Neurology 2005;64(2):254-262; Yes(J Med Genet 1998;35:846-8)	0; 1.663E-5	Family segregation done Mom has COL5A2 with phenotype, maternal grandmother has COL5A2 with phenotype, FLNA de novo, sister has COL5A2 and phenotype, brother has COL5A2 and phenotype	Pathogenic; Likely Pathogenic	PS2, PM2, PM2, PP1(family well-characterized), PP3
8	HCM	60	M	TPM1 D175N	Yes (Ann Med. 2013; 45(1):85-90)	Yes (Ann Med. 2013; 45(1):85-90)	0		Pathogenic	PS3, PP3, PP4, PP5
10	HCM (apical variant), syncope, bradycardia, conduction abnormality	73	F	TRPM4 Q752X	No	No, only missense mutations reported (J Clin Invest 2009; 119(9):2737-44, Circ Cardiovasc Genet 2010;3(4):374-85)	1.833E-3	Dominant negative mutations exist - D984A (J Biol Chem 2005;280(24):22899-906)	Pathogenic	PVS1, PS3
12	SCD	66	F	KCNH2 N33T	No	Yes (Circulation 2002; 105(7):794-9)	0	2 submissions in ClinVar - Gene DX pathogenic, NHS VUS, this mutation increased rate of deactivation several papers; family segregation	Pathogenic	PS3, PM1, PM2, PP3, PP5
16	Marfan syndrome	26	F	FBN1 IVS2+1G>A	Yes (http://www.umd.be/FBN1/4DACTION/W_DMDT1/1)	Yes (Proc Natl Acad Sci USA 1992;89(13):5917-22	0	Family has clinical phenotype… Brother, mother	Pathogenic	PVS1, PM2, PM3
19	SCD (HCM)	58	M	MYBPC3 IVS28+22T>G	No	Yes (Circulation 2003; 107(17):2227-32)	0	Family segregation done - children without phenotype and do not carry mutation	Pathogenic	PVS1, PM2, PP1, PP3
26	SCD (NICM)	47	F	TTN R18966X	No	Yes (N Engl J Med 2012; 366(7): 619-28	0		Pathogenic	PVS1, PM2, PM4
31	Marfan syndrome	55	F	FBN1 G77X	Yes(http://www.umd.be/FBN1/4DACTION/W_DMDT1/1)	Yes (Proc Natl Acad Sci USA 1992;89 (13):5917-21	0	Family has clinical phenotype	Pathogenic	PVS1, PM2, PM4
35	PPCM	40	F	NDUFV2 Q31X	No	Yes for HCM (Hum Mutat 2003; 21(6): 582-6)	0	few reported mutations in ClinVar, similar mutation type with HCM	Likely Pathogenic	PM2, PM4, PP3, PP4
46	HCM	57	M	MYBPC3 c.2554_2555insT	No	Yes (Circulation 2003; 107(17): 2227-32)	1.69E-5	Family segregation done - son has genotype and clinical disease	Pathogenic	PVS1, PM4, PP1
52	HCM	42	M	TNNT2 c.477delCTC	No	Yes(N Engl J Med 1995;232(16):1058-64	0	Segregated with disease in NEJM paper	Pathogenic	PVS1, PM2, PM4
53	HCM	58	M	MYBPC3 V219L	No; Yes (ClinVar)	Yes (Circulation 2003; 107(17):2227-32)	0	V219F in EXAC - considered pathogenic in ClinVar	Likely Pathogenic	PM2, PM5, PP1, PP3
54	SCD (DCM)	47	F	DSP Q1672X	No	Yes for ARVC (Can J Cardiol 2014;30(12):1655-61), For DCM (Genet Med 2014; 16(8):601-8)	0		Pathogenic	PVS1, PM2, PM4
59	SCD (NICM)	24	F	TTN c.45689delG;	No	Yes (N Engl J Med 2012; 366(7):619-28, Sci Tranl Med 2015; 7(270):270ra6);	0	Segregation done on family - Dad does not carry TTN and has no phenotype, sister sister carries and has phenotype	Pathogenic	PVS1, PM2, PM4

65	SCD	47	F	*SCN5A* S216L	Yes (ClinVar, Clin Transl Sci 2008;1(1):21-26);	Yes (Heart Rhythm 2010;7(1):33-46)	0.001		Likely Pathogenic	PS3, PP3, PP4
69	BrS	23	F	*SCN5A* S910L	Yes (Nat Rev Cardiol 2009;6(5):337-48)	Yes(Heart Rhythm 2010;7(1):33-46)	0	S910S in EXAC; known BrS variant	Likely Pathogenic	PS3, PM2, PP3, PP4
71	Lipo-dystrophy	41	M	*LMNA* R644C	Yes (Am J Med Genet A 2008;146A(12):1530-42)	Yes (Am J Med Genet A 2008;146A(12):1530-42)	1.243E-3	Segregation in family, well-established mutation site with variable phenotypes	Likely Pathogenic	PS3, PP3, PP4
75	SCD (DCM)	40	F	*TTN c.29042-2A>C*	Yes(Sci Tranl Med 2015; 7(270):270ra6)	Yes (N Engl J Med 2012; 366(7):619-28, Sci Tranl Med 2015; 7(270):270ra6)	0	VF arrest	Pathogenic	PVS1, PM2, PM4
76	HCM	62	M	*MYBPC3 R820Q*	Yes (ClinVar)	Yes (Circulation 2003; 107(17):2227-32, J Am Coll Cardiol 20031 41(5):781-6)	1.66E-5	R820P and R820W also in ClinVar, both have uncertain significance, R820Q is pathogenic/likely pathogenic in ClinVar R820W also in EXAC	Pathogenic	PS3, PS4, PP5
84	SCD (DCM)	40	M	*TTN W21280X*	No	Yes (N Engl J Med 2012; 366(7):619-28)	0	Family segregation, Dad same phenotype and genotype	Pathogenic	PVS1, PM2, PM4, PP1
85	Marfan syndrome	57	F	*FBN1 R945C*	No	Yes (Genomics 1993;17(2):468-75)	0	R954H in ClinVar as Pathogenic	Likely Pathogenic	PM2, PM5, PP3, PP4
86	HCM	56	M	*MYBPC3 V598S*	No	Yes (Circulation 2003; 107(17):2227-32)	0	V598Sfs is pathogenic in ClinVar single submission with clinical assertion information	Likely Pathogenic	PM2, PP3, PP4
92	FH	27	F	*APOB R3500Q*	Yes (ClinVar, Hum Biol 2005; 77(5):663-73)	Yes (Hum Biol 2005; 77(5):663-73)	2.311E-4	rs5742904, R3527Q, high total cholesterol, but no history of CAD	Likely Pathogenic	PS3, PP3, PP4, PP5
96	LQTS, palpitations	35	F	*TTN* c.32562_insAGA	Yes (ClinVar)	Yes (N Engl J Med 2012; 366(7):619-28)	0	TTN reported twice with familial and non-familial DCM/PPCM	Unknown Significance; Pathogenic	PVS1, PM2, PM4
101	FH	38	M	*apoE V254E*	Yes (ClinVar, Am J Hum Genet 1993;52(5)937-46)	Yes (Am J Hum Genet 1993;52(5)937-46)	1.35E-03	single submission in ClinVar - pathogenic	Pathogenic	PS3, PP3, PP5
107	DCM	54	F	*HFE H63D*	Yes (ClinVar, Gastroenterology 2002; 122(3): 646-51)	Yes (Am J Hum Genet 1997;61(3):762-4	0.1066	Family known to have hemochromatosis	Pathogenic	PS3, PP3, PP4, PP5
108	DCM	38	M	*LMNA c.509 ins C*	No	Yes (Eur J Heart Fail 2013; 15(6):628-36	0	Other frameshifts reported to be deleterious	Pathogenic	PVS1, PM2, PM4
109	PPCM	56	F	*EMD c.110_112del AGA*	No	Yes for Emery-Dreifuss MD (Nat Genet 1994;8(4):323-327); mild features with DCM (BMC Med Genet 2014;15:77)	2.53E-05	No musculoskeletal features -- potentially very mild?	Likely Pathogenic	PVS1, PM4
110	DCM	68	F	*LMNA IVS3 - 10A>G*	No	Yes (Eur J Heart Fail 2013; 15(6):628-37	0	Other frameshifts reported to be deleterious	Pathogenic	PVS1, PM2, PM4

112	HCM	65	F	*TNNT2 ΔGlu160*	Yes(N Engl J Med 1995;232(16):1058-64	Yes(N Engl J Med 1995;232(16):1058-64	0		Pathogenic	PVS1, PM2, PM4
113	RCM, VT/AF	15	M	*TNNT2 ΔGlu160*	Yes(N Engl J Med 1995;232(16):1058-64;No	Yes(N Engl J Med 1995;232(16):1058-64	0		Pathogenic	PVS1, PM2, PM4
114	NICM, VT	34	F	*TTN IVS100-2A>C;*	No	Yes (N Engl J Med 2012; 366(7):619-28	0		Pathogenic	PVS1, PM2, PM4; PP5
115	BrS	64	F	*SCN5A c.2551insTG*	Yes(ClinVar)	Yes (Heart Rhythm 2010;7(1):33-46	0	Pathogenic; V896M - homozygote in EXAC	Pathogenic	PVS1, PM2, PP5
116	Family history of SCD, VT	67	M	*DES K109N*	No	Yes for myopathy with arrhythmias (Nat Genet 1998;19(4):402-3)	0	1st amino acid in the rod domain, E108K is reported to cause DCM (Circulation 2007;115(10):1244-51)	Likely Pathogenic	PM1,PM2, PP3, PP4
117	HCM	52	M	*MYH7 T1377M*	Yes (Circulation 2003; 107(17):2227-32, J Cardiovasc Med (Hagerstown) 2006;7(8):601-17, J Am Coll Cardiol 2004;44(3):602-10, J Med Genet 2011; 48(8):572-6)	Yes (Circulation 2003; 107(17):2227-32, J Cardiovasc Med (Hagerstown) 2006;7(8):601-17, J Am Coll Cardiol 2004;44(3):602-10, J Med Genet 2011; 48(8):572-6)	0	T1377M reported multiple cases of HCM without controls having mutations	Pathogenic	PS4, PM2, PP2, PP3
123	HCM	39	F	*ABCC9 IVS17-1G>A*	No	Yes for DCM, (Nat Genet 2004;36:382-387)	1.62E-3	Splice prediction loss of exon 17, radical mutations reported elsewhere, but closer to c-terminal of gene	Likely Pathogenic	PVS1, PM4
125	NICM	23	M	*MIB1 R676X*	No	Yes for LVNC (Nat Med 2013;19(2):193-201	0	EXAC has c.2556delC (same location) and in ClinVar as well - reported pathogenic	Pathogenic	PVS1, PM2, PM4
141	FH/CAD	55	F	*LDLR A431T*	Yes (Annu Rev Genet 1990;24:133-70)	Yes (Annu Rev Genet 1990;24:133-70)	0	FH- Algeria	Pathogenic	PS3, PM1, PP3
145	HCM	53	M	*JPH2 A405T*	No	Yes (J Mol Cell Cardiol 2007;42(6):1026-35	6.61E-5		Likely Pathogenic	PS3, PP3, PP4
146	BrS	51	M	*RYR2 R1482H*	Yes(ClinVar)	Yes for LQTS (Heart Rhythm 2005; 2(10):1099-1105); Yes for CPVT with and without Atrial Fibrillation (Can J Cardiol 2013;29(8):993-6, Heart Rhythm 2015;12(7):1636-43).	1.994E-4	R1482C in EXAC	Likely Pathogenic	PS3, PM5, BP4

149	Lipo-dystrophy	46	F	*LMNA R582C*	Yes (Eur J Endocrinol 2012;167(3):423-31)	Yes (Eur J Endocrinol 2012;167(3):423-31)	0	R582R in EXAC	Pathogenic	PS4, PM2, PP3, PP4, PP5
152	Congenital hearing loss/TdP	34	F	*CACNA1D R1902X*	No	Homozygous Inframe insertion (Nat Neurosci 2011;14(1):77-84);	0	Possible dominant negative effect	Likely Pathogenic	PM2, PM4, PM6, PP4
154	FH/CAD	38	F	*LDLR c.313+1G>A*	Yes(Atherosclerosis 1994;111(2):175-82, Am J Med Genet 1996;65(2):149-54, Arterioscler Thromb Vasc Biol 1995; 15(2):219-27)	Yes (Atherosclerosis 1994;111(2):175-82, Am J Med Genet 1996;65(2):149-54, Arterioscler Thromb Vasc Biol 1995; 15(2):219-27)	4.12E-05	G>T also reported in EXAC	Pathogenic	PS1, PS3, PM1
164	DCM	58	M	*TTN c.48470delG;*	No	Yes (N Engl J Med 2012; 366(7): 619-28)	0	TTN variant in A-band; father had heart transplant for cardiomyopathy	Pathogenic	IVS1, PM2, PM4
177	HCM	28	M	*TPM1 D254G*	Yes (ClinVar)	Yes (Ann Med. 2013; 45(1):85-90)	0	Sister passed away at 13 – known HCM no genetic diagnosis, mother has variant and disease, ClinVar -2 submitters, likely pathogenic	Likely Pathogenic	PM2, PP1, PP3, PP4, PP5
181	LQTS, syncope	52	F	*ANK2 E1449G*	Yes (Nature 2003;42(6923):634-9)	Yes (Nature 2003;42(6923):634-9 and Proc Natal Acad Sci 2004;101(24):9137-42)	0.0004222	ClinVar – 1 submussion likely benign, 5 submissions likely pathogenic/pathogenic, in Nature paper segregation in family	Pathogenic	PS1, PS3, PP4, PP5
184	HCM	27	M	*PRKAG2 R302P*	No	Yes (N Engl J Med 2001; 344(24):1823-31)	0	ClinVar has R302L – 5 submission all pathogenic, N Engl J Med reports R302Q as a segregating variant in a large family, reported binding site for ATP/AMP	Likely Pathogenic	PM1, PM2, PM5, PP3
192	FH	33	F	*LDLR M1fs c.3delG*	No	Yes (Cell 1985;41:735-43)	0	Maternal family history of hyperlipidemia without CAD	Pathogenic	PVS1, PM2, PM4
193	SCD	54	M	*TTN c.90223delG*	No	Yes(N Engl J Med 2012; 366(7): 619-28)	0	Cardiac event was VF arrest, brother had cardiac arrest as well. TTN variant found in A band of gene	Pathogenic	PVS1, PM2, PM4

195	NICM/LQT	45	F	*PKP2 IVS6-1C>T(c.1379-1C>T*	Yes (ClinVar)	Yes for ARVC(Nature Genet 2004;36:1162-4	0.0001499	PKP2 – ClinVar single submission uncertain significance, high confidence LoF variant EXAC	Likely Pathogenic	PVS1, PM4
198	FH	22	M	*LDLR I764fs c.2292delA*	No	Yes (Cell 1985;41:735-43)	0	Paternal family history of premature CAD, hypercholesterolemia	Pathogenic	PVS1, PM2, PM4
199	LQTS	57	M	*KCNQ1 L266P*	Yes (Heart Rhythm 2005;2:507-517, Circulation 2007;115(19):2481-9, Heart Rhythm 2009;6:1297-1303)	Yes (Heart Rhythm 2005;2:507-517; Circulation 2007;115(19):2481-9 Heart Rhythm 2009;6:1297-1303)	0	History of syncope in childhood (near drowning), VF arrest, family history of SCD. L266P is known/established cause of LQTS, transmembrane variant	Pathogenic	PS4, PM2, PP3, PP4, PP5

* Abbreviations for the ACMG Criteria: PVS – Pathogenic Very Strong, PS – Pathogenic Strong, PM – Pathogenic Moderate, PP – Pathogenic Supporting, BS – Benign Strong, BP – Benign Supporting

Other Abbreviations PVH – Periventricular Heterotopia, EDS – Ehlers Danlos Syndrome, SCD – Sudden Cardiac Death, RCM – Restrictive Cardiomyopathy, HCM-Hypertrophic Cardiomyopathy, DCM – Dilated Cardiomyopathy, NICM – Non-Ischemic Cardiomyopathy, TdP – Torsade Des Pointes LQTS – Long QT Syndrome, FH – Familial Hypercholesterolemia, CAD – Coronary Artery Disease, AF – Atrial Fibrillation, BrS – Brugada Syndrome, VT – Ventricular Tachycardia PPCM – Peripartum Cardiomyopathy

Table S3: Gene represented by various commercially available arrhythmia panels:

Ambry – RhythmFirst and RhythmNext (From www.ambrygen.com on January 21, 2016)

GeneDX – Arrhythmia panel (From www.genedx.com on January 21, 2016)

Invitae – Comprehensive Arrhythmia Panel with Optional Limited Evidence Genes (Marked as O)

(from www.invitae.com on January 21, 2016)

Gene	Ambry	GeneDX	Invitae
ABCC9			X
ACTN2			X
AKAP9	X	X	O
ANK2	X	X	X
ANKRD1			O
CACNA1C	X	X	X
CACNA2D1	X		O
CACNB2	X	X	X
CALM1	X		X
CALM2			X
CALM3			X
CASQ2	X	X	X
CAV3	X	X	X
CTNNA3			O
DES			X
DSC2	X	X	X
DSG2	X	X	X
DSP	X	X	X
EMD			X
GPDL1	X	X	X
HCN4	X	X	X
JUP	X	X	X
KCND3	X		O
KCNE1	X	X	X
KCNE2	X	X	X
KCNE3	X	X	O
KCNE5			O
KCNH2	X	X	X
KCNJ2	X	X	X
KCNJ5		X	O
KCNJ8	X	X	O
KCNQ1	X	X	X
LDB3			O
LMNA	X		X
NKX2.5	X	X	X
PDLIM3			O
PKP2	X	X	X
PLN			X
PRKAG2			X
RANGRF		X	O

RBM20			X
RYR2	X	X	X
SCN1B	X	X	O
SCN2B			O
SCN3B	X	X	O
SCN4B	X	X	O
SCN5A	X	X	X
SCN10A			O
SLMAP			O
SNTA1	X	X	O
TBX5	X		
TGFB3	X		X
TMEM43	X	X	X
TNNI3			X
TNNT2			X
TRPM4	X		O
TRDN	X		X
TTN			X

Table S4: Genes represented by various commercially available cardiomyopathy panels

Ambry – CMNExt (From www.ambrygen.com on January 21, 2016)

GeneDX – Cardiomyopathy panel (From www.genedx.com on January 21, 2016)

Invitae – Comprehensive Cardiomyopathy Panel with Optional Limited Evidence Genes (Marked as O) and RASopathy genes for cardiomyopathy (Marked as R) (from www.invitae.com on January 21, 2016)

Gene	Ambry	GeneDX	Invitae
A2ML1			R
ABCC9	X	X	X
ACTC1	X	X	X
ACTN2	X	X	X
ALMS1			X
ANKRD1	X	X	O
BAG3	X	X	X
BRAF		X	R
CALR3			O
CAV3		X	X
CBL			R
CRYAB	X	X	X
CSRP3	X	X	X
CTF1			O
CTNNA3			O
DES	X	X	X
DMD	X	X	X
DSC2	X	X	X
DSG2	X	X	X
DSP	X	X	X
DTNA		X	O
ELAC2			X
EMD	X	X	X
EYA4	X		X
FHL1			X
FHL2			O
FKRP			X
FKTN	X	X	X
FXN	X		
GATA4			O
GATA6			O
GATAD1	X	X	O
GLA	X	X	X
HCN4			X
HRAS		X	R
ILK		X	O
JPH2	X	X	O
JUP	X	X	X

KRAS		X	R
LAMA4	X	X	O
LAMP2	X	X	X
LDB3	X	X	O
LMNA	X	X	X
MAP2K1		X	R
MAP2K2		X	R
MTND1		X	
MTND5		X	
MTND6		X	
MTTD		X	
MTTG		X	
MTTH		X	
MTTI		X	
MTTK		X	
MTTL1		X	
MTTL2		X	
MTTM		X	
MTTQ		X	
MTTS1		X	
MTTS2		X	
MTO1			X
MYBPC3	X	X	X
MYH6	X		O
MYH7	X	X	X
MYL2	X	X	X
MYL3	X	X	X
MYLK2			O
MYOM1			O
MYOZ2	X	X	O
MYPN	X	X	O
NEBL		X	O
NEXN	X	X	O
NRAS		X	R
NF1			R
NKX2.5	X		
NPPA			O
PDLIM3		X	O
PKP2	X	X	X
PLN	X	X	X
PRDM16			O
PRKAG2	X	X	X
PTPN11	X	X	R
RAF1	X	X	X
RASA1			R
RBM20	X	X	X
RIT1			R
RYR2	X	X	X
SCN5A	X	X	X
SDHA			X
SGCD		X	X
SHOC2			R

SOS1		X	R
SPRED1			R
TAZ	X	X	X
TBX20	X		
TCAP	X	X	X
TGFB3	X		X
TMEM43	X	X	X
TMPO	X	X	O
TNNC1	X	X	X
TNNI3	X	X	X
TNNT2	X	X	X
TPM1	X	X	X
TTN	X**	X	X
TTR		X	X
TXNRD2	X		O
VCL	X	X	X

*****TTN*** optional for Ambry CMNext

Table S5: The Actionable Incidental Pathological findings

Case	Reason for Referral	Incidental Finding	Mutation Previously Reported	Ref	Additional Information	Pathogenicity	ACMG criteria*
33	Noonan Syndrome	*MSH6* c.1352_1353insTCAG	No	1	Mother early onset CRC (40s) - no genetic testing	Pathogenic	PVS1, PM2, PM4
63	NICM	*BRCA2* R2505X	Yes (ClinVar)	2	No family history, but family is mostly males and small structure; index case is male	Pathogenic	PVS1, PP3, PP5
79	SCD (ARVC)	*LDLR* Q254P	Yes	3	LDLR variant - Reggio Emilia -2; family has known history of elevated LDL, not quantified, segregated with those known to have "high cholesterol"	Pathogenic	PS3, PM2, PP1, PP3, PP5
93	HCM	*BRCA2* IVS6-1delG	No	2	No family history, but family is mostly males; index case is male	Pathogenic	PVS1, PM2, PM4
107	DCM	*HFE* H63D	Yes	4	Family history of hemochromatosis	Pathogenic	PS3, PP1, PP3, PP4, PP5
133	DCM	*FBN1* P1009R	Yes (ClinVar)	5	No indication of Marfan syndrome	Likely Pathogenic	PS3, PP3, PP5
142	LQTS, syncope	*BRIP1/FANCJ* IVS3-2A>G	No	6	Maternal family history of disease - mother breast cancer and TAH at 30 years old	Pathogenic	PVS1, PM2, PM4
152	Hearing loss and Torsade	*APC* IVS11-2A>C	No	7	Maternal family history of disease - mother colon polyps and CRC	Pathogenic	PVS1, PM2, PM4
173	TAA	*LDLR G529E*	Yes (ClinVar)	8	Variant known a FH Sicily, known personal/family history of hypercholesterolemia	Pathogenic	PS3, PS4, PP3
183	HCM	*ATM Q1852X*	Yes (ClinVar)	9	Family history of female breast and pancreatic cancers and brain tumor	Pathogenic	PVS1, PS4, PM4
195	NICM	*SCNN1B T594M*	Yes (Lancet 1998;351(9113):1388-92)	10	Known variant for hypertension in individuals of African background	Pathogenic	PS3 PS4,PP3, PP5

* Abbreviations for the ACMG Criteria: PVS – Pathogenic Very Strong, PS – Pathogenic Strong, PM – Pathogenic Moderate, PP – Pathogenic Supporting, BS – Benign Strong, BP – Benign Supporting

References:

1. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med. 2006;354:2751-2763
2. Hakansson S, Johannsson O, Johansson U, Sellberg G, Loman N, Gerdes AM, et al. Moderate frequency of brca1 and brca2 germ-line mutations in scandinavian familial breast cancer. Am J Hum Genet. 1997;60:1068-1078
3. Bertolini S, Cantafora A, Averna M, Cortese C, Motti C, Martini S, et al. Clinical expression of familial hypercholesterolemia in clusters of mutations of the ldl receptor gene that cause a receptor-defective or receptor-negative phenotype. Arterioscler Thromb Vasc Biol. 2000;20:E41-52
4. Melis MA, Cau M, Deidda F, Barella S, Cao A, Galanello R. H63d mutation in the hfe gene increases iron overload in beta-thalassemia carriers. Haematologica. 2002;87:242-245
5. Dietz HC, McIntosh I, Sakai LY, Corson GM, Chalberg SC, Pyeritz RE, et al. Four novel fbn1 mutations: Significance for mutant transcript level and egf-like domain calcium binding in the pathogenesis of marfan syndrome. Genomics. 1993;17:468-475
6. Seal S, Thompson D, Renwick A, Elliott A, Kelly P, Barfoot R, et al. Truncating mutations in the fanconi anemia j gene brip1 are low-penetrance breast cancer susceptibility alleles. Nat Genet. 2006;38:1239-1241
7. Fodde R, Smits R, Clevers H. Apc, signal transduction and genetic instability in colorectal cancer. Nature reviews. Cancer. 2001;1:55-67
8. Hobbs, H. H. , Brown M.S., Goldstein JL. Molecular genetics of the LDL receptor gene in familial hypercholesterolemia. Hum Mutat. 1992;1: 445-66
9. Chen, P.C., Lavin, M.F., Kidson C., Moss, D. Identification of ataxia telangiectasia heterozygotes, a cancer prone population. Nature. 1978;274: 484-6
10. Baker, E.H., Dong, Y.B., Sangella, G.A., Rothwell, M., Onipinla, A.K., Mankandu N.D., *et al.* Association of hypertension with T594M mutation in beta subunit of epithelial sodium channels in black people resident in London. Lancet. 1998; 351:1388-92.

Figure S1: (A) Amplified CACNA1D RNA from a human ventricular biopsy on agarose gel. (B) Relative mRNA expression of CACNA1D compared to housekeeping gene GAPDH.