A Comparison of Whole Genome Sequencing to Multigene Panel Testing in Hypertrophic Cardiomyopathy Patients

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Background—As DNA sequencing costs decline, genetic testing options have expanded. Whole exome sequencing and whole genome sequencing (WGS) are entering clinical use, posing questions about their incremental value compared with disease-specific multigene panels that have been the cornerstone of genetic testing.

Methods and Results—Forty-one patients with hypertrophic cardiomyopathy who had undergone targeted hypertrophic cardiomyopathy genetic testing (either multigene panel or familial variant test) were recruited into the MedSeq Project, a clinical trial of WGS. Results from panel genetic testing and WGS were compared. In 20 of 41 participants, panel genetic testing identified variants classified as pathogenic, likely pathogenic, or uncertain significance. WGS identified 19 of these 20 variants, but the variant detection algorithm missed a pathogenic 18 bp duplication in myosin binding protein C (*MYBPC3*) because of low coverage. In 3 individuals, WGS identified variants in genes implicated in cardiomyopathy but not included in prior panel testing: a pathogenic protein tyrosine phosphatase, non-receptor type 11 (*PTPN11*) variant and variants of uncertain significance in integrin-linked kinase (*ILK*) and filamin-C (*FLNC*). WGS also identified 84 secondary findings (mean=2 per person, range=0–6), which mostly defined carrier status for recessive conditions.

Conclusions—WGS detected nearly all variants identified on panel testing, provided 1 new diagnostic finding, and allowed interrogation of posited disease genes. Several variants of uncertain clinical use and numerous secondary genetic findings were also identified. Whereas panel testing and WGS provided similar diagnostic yield, WGS offers the advantage of reanalysis over time to incorporate advances in knowledge, but requires expertise in genomic interpretation to appropriately incorporate WGS into clinical care.

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Current consensus guidelines recommend the use of genetic testing to establish a molecular cause in patients diagnosed with hypertrophic cardiomyopathy (HCM) and to identify at-risk relatives to target for longitudinal clinical screening.[1,2] Over the past decade, there has been rapid growth in the availability and utilization of HCM genetic testing.[3] With the development of next-generation sequencing technology, HCM multigene panels have expanded from 5 genes in 2004, when genetic testing was first commercially available, to now >100 genes. However, expanding panels to include genes beyond the sarcomere genes has not substantially improved diagnostic yield,[3] as many of these genes have not been definitively established to cause disease and any variants identified in these genes will be of uncertain significance (VUSs).[4] This is a particular limitation when pretest probability for identifying a causal mutation is reduced because of the absence of family history or phenotypic ambiguity.[5–7] Furthermore, regardless of panel size, genetic testing does not yield a molecular cause in 40% to 70% of HCM patients.[3]

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*A list of members of the MedSeq Project is given in the Data Supplement.

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More recently, whole exome sequencing (WES) and whole genome sequencing (WGS) have been increasingly used for molecular diagnosis.[8,9] Initially reserved for complex clinical presentations or as second tier tests after negative targeted genetic testing, decreasing price and wider availability now make such technology more accessible, raising the question of whether these comprehensive tests might replace multigene panels to determine the molecular cause of monogenic conditions such as inherited cardiomyopathies. The breadth of sequence analysis afforded from WES/WGS offers great promise for increased diagnostic yield and the ability to perpetually reexamine the comprehensive sequence data as knowledge emerges, a key advantage over targeted testing. However, their expansive scope also requires careful consideration, particularly on the potential impact of unanticipated secondary findings. The American College of Medical Genetics and Genomics recommends reporting incidentally identified pathogenic variants in 59 genes considered to be medically actionable.[10,11] Learning about secondary findings from WGS has been cited as both a potential advantage and barrier to its use in clinical medicine.[12] In addition, concerns about whether WGS read depth is sufficient to supplant panel testing[13] make WGS sensitivity central to the discussion of its use relative to panel testing, although examination of nonexonic regulatory elements and regions with high guanine-cytosine content may be superior with WGS.

In this study, we compared targeted HCM genetic testing, performed by multigene panel or familial variant test, to WGS in HCM patients to (1) examine the difference in diagnostic yield, (2) quantify the occurrence of secondary findings from WGS, and (3) explore the clinical actions that resulted from additional findings from WGS.

Methods

Study Cohort

The study population for this analysis was drawn from the MedSeq Project, a randomized clinical trial of the incorporation of WGS into clinical practice in adult medicine. The design of this study has been previously reported.[14] In brief, the MedSeq Project cohort included 100 primary care patients and 100 patients with presumptive inherited HCM or dilated cardiomyopathy (DCM). Eligible patients received a study mailing and were approached for participation by telephone or in person during clinic visits. Participants underwent targeted HCM genetic testing before or concurrent with their enrollment and were randomized 1:1 to undergo family history collection and review of targeted HCM genetic testing, or family history collection, review of targeted HCM genetic testing and WGS.

In this report, we limited the analyses to the 41 HCM patients who underwent WGS. This project was approved by the Partners Human Research Committee and all participants provided informed consent.

Genetic Testing

Targeted HCM Genetic Testing

Multigene panel size ranged from 4 to 62 genes depending on year of testing (2004–2016) and clinician panel selection. All but 2 subjects who underwent panel testing had a minimum of 8 sarcomere genes sequenced, including myosin binding protein C (*MYBPC3),* myosin heavy chain *(MYH7),* cardiac troponin T *(TNNT2),* cardiac troponin I *(TNNI3),* α-tropomyosin *(TPM1),* myosin essential and regulatory light chains *(MLY2, MYL3),* and cardiac actin *(ACTC)*. The 2 subjects who had only 4 sarcomere genes sequenced (*MYBPC3, MYH7, TNNT2, and TPM1*) had pathogenic variants identified. Variants were classified as pathogenic, likely pathogenic (LP), VUS, likely benign or benign using the clinical standard of the laboratory at the time of testing.[15–17] The majority of subjects (32/41) had their targeted testing performed by Clinical Laboratory Improvement Amendments-certified Partners Laboratory for Molecular Medicine, Cambridge, MA (methodology is given in the Data Supplement).

Whole Genome Sequencing

The WGS methodology and bioinformatic pipeline used in the MedSeq Project have been previously described.[16,18,19] Genome sequencing was performed by the Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited Illumina Clinical Services Laboratory (San Diego, CA) using paired-end 100 bp reads on the Illumina HiSeq platform between 2013 and 2015. Genomes were sequenced to a minimum of 30× mean coverage, with ≥95% of bases sequenced to at least 8× coverage. Sequencing data were then transferred to the Laboratory for Molecular Medicine for analysis and reporting. The medical exome content evolved with current knowledge throughout the study, but included ≈4000 genes. Noncoding regions outside clinical regions of interest were not interpreted, unless a previously known pathogenic variant was identified. Single-nucleotide variants and small insertions/deletions were identified and assessed. Detection of insertion/deletion variants >10 bp was limited because of the sequencing depth and read length. Larger copy number and structural variants are being investigated separately. Sequence alignment and variant calling information is given in the Data Supplement.

Variants were classified using a 7-tier system: benign, likely benign, VUS favor benign, VUS, VUS favor pathogenic, LP, and pathogenic. Pathogenic, LP, and VUS favor pathogenic were reported. VUSs in cardiomyopathy-associated genes were also reported.[16,17]

WGS results were analyzed independently of targeted HCM panel testing data. Subsequent comparisons of WGS and targeted HCM genetic test results were to assess both the accuracy of WGS and its ability to identify new causal variants.

WGS information reported in the MedSeq Project extended beyond monogenic disease and recessive conditions to include an array of genetic risk information that might impact cardiovascular disease management. The MedSeq Project genome report itself has been described in detail elsewhere.[16,20,21] In brief, it was divided into different categories to report:

- monogenic disease risk, both related and unrelated to the indication for testing (ie, cardiomyopathy);
- carrier variants for recessive conditions;
- selected pharmacogenomic associations;
- comprehensive blood group information[22,23]; and
- a cardiac risk report incorporating predictions based on genomic variation:
  - predicted fasting lipid profile and
  - data from genome-wide association studies on alleles conferring small-to-moderate risk for 8 common phenotypes: atrial fibrillation, hypertension, QT prolongation, abdominal aortic aneurysm, coronary heart disease, type 2 diabetes mellitus, obesity, and platelet aggregation.[21]

Secondary findings were not limited to the genes defined by the American College of Medical Genetics and Genomics guidelines.[10,11] Variants were designated as a secondary finding, by consensus, when there was a lack of moderate, strong, or definitive association with cardiomyopathy, but other potential medical significance. Secondary findings were tallied to determine the burden of such findings in each individual and the cohort.

Clinical Actions Triggered by WGS Results

WGS results were disclosed by the patient's cardiologist after completing a genetics education module. The majority (4/7) of cardiologists had genetics expertise. Physicians completed a postdisclosure survey to indicate whether specific WGS findings resulted in any further action (referrals, additional diagnostic testing, etc.). Medical records were then reviewed a minimum of 1 year after disclosure to determine the outcome of the recommended actions.

Results

Patient Characteristics

Forty-one unrelated participants with HCM underwent WGS and targeted HCM genetic testing (multigene panel [n=38] or familial variant testing [n=3]; Figure). The mean (SD) age was 58 years (12 years); 54% were female and 95% were white (Table 1). A family history of HCM was present in 17 of 41 (42%) subjects. Participants demonstrated the known clinical heterogeneity in HCM, ranging from those who were asymptomatic to those requiring therapy for advanced heart failure (Table 1).

Monogenic Findings Related to Cardiomyopathy

Table 2 shows the variants reported by targeted HCM genetic testing and by WGS. Twenty subjects (49%) had variants identified by targeted HCM genetic testing (10 pathogenic, 3 LP, and 7 VUS). The majority of positive results (pathogenic or LP, n=13, 32% of the cohort) involved *MYBPC3* and *MYH7* (54% and 23% of positive results, respectively). Twenty-one subjects (51%) had no variants identified by targeted HCM testing. Nineteen of 20 variants identified by targeted HCM testing were detected by WGS. One variant, an 18 bp duplication in *MYBPC3* (c.3742_3759dup), was initially missed by the WGS variant detection algorithm. As prior genetic testing by the Laboratory for Molecular Medicine, using a resequencing array, had identified this variant, the WGS data were manually reviewed. The variant occurred in 1 of 12 reads covering the duplication, which was below the threshold for variant detection in the WGS algorithm. It was confirmed by Sanger sequencing. As such, this variant was missed because of a combination of the duplication size and the reduced coverage of this region by WGS.

Three patients had findings identified by WGS in genes that were not analyzed in their prior HCM genetic testing. In 1 subject with prior negative genetic testing, WGS found a pathogenic *PTPN11* variant (c.1403C>T) associated with Noonan syndrome with multiple lentigines, an autosomal dominant condition characterized by lentigines, typical facial features, pulmonic stenosis, and left ventricular hypertrophy among other features.[24] She was diagnosed with HCM at 20 years old because of a murmur and symptoms of effort intolerance. She is 5′1″ tall with mild facial dysmorphism, lentigines on her upper arms and face, left ventricular hypertrophy (maximum wall thickness 15 mm), and outflow tract obstruction. Family history was negative for HCM or left ventricular hypertrophy, but 1 daughter was known to have mild aortic coarctation. Genetic testing in 2009 included 11 genes but did not examine genes associated with Noonan syndrome, often included on current HCM panels. After the initial negative genetic analyses, additional genetic testing and clinical evaluations were deferred because of the family's lack of interest and the patient's perception of limited clinical use. After the identification of the *PTPN11* variant, her 2 adult children were evaluated. Though neither has pursued testing for the *PTPN11* variant, one with aortic coarctation has mild facial dysmorphism and lentigines, consistent with Noonan syndrome with multiple lentigines.

The second new WGS finding was a VUS in the integrin-linked kinase (*ILK)* gene in a patient with a previously known VUS in *MYH7* (p.Arg1344Gln), a definitive HCM gene. Arginine at position 1344 in *MYH7* is highly conserved in evolution. Arg1344Gln has been identified in at least 3 HCM probands but is also reported in 4 samples from the gnomAD database.[25] *ILK* participates in the regulation of cardiomyocyte growth and has been implicated in DCM by studies in mice

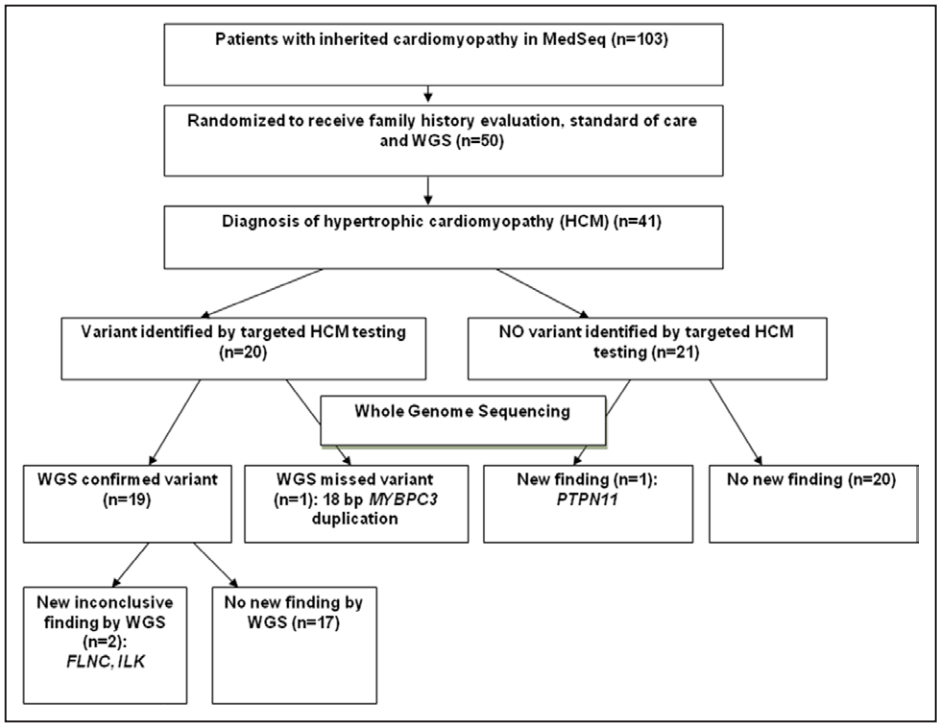

Figure. Subject enrollment and cardiomyopathy-related genetic test results. *FLNC* indicates filamin-C; HCM, hypertrophic cardiomyopathy; *ILK*, integrin-linked kinase; *MYBPC3*, myosin binding protein C; *PTPN11*, protein tyrosine phosphatase, non-receptor type 11; and WGS, whole genome sequencing.

Table 1. Characteristics of HCM Patients Participating in MedSeq who Underwent Multigene HCM Panel Testing and WGS (n=41)

Mean age (SD), y	58 (12)
Female, n (%)	22 (54%)
White, n (%)	39 (95%)
Family history of HCM, n (%)	17 (42%)
Atrial fibrillation, n (%)	10 (24%)
End-stage HCM/HF death/transplant	4 (10%)
Sudden cardiac arrest/death	5 (12%)
Mean maximal left ventricular wall thickness, mm (SD)	17.2 (4.3)
Mean left ventricular ejection fraction, % (SD)	62.2 (14.6)
New York Heart Association functional class	
I	21 (51%)
II	12 (29%)
III	3 (7%)
Unknown	5 (12%)
Sarcomere genes implicated by targeted HCM genetic testing	18 (44%)
MYBPC3, n (%)	10 (56%)
MYH7, n (%)	5 (28%)
TNNI3, n (%)	1 (6%)
MYL2, n (%)	1 (6%)
ACTN2, n (%)	1 (6%)

Age and maximal left ventricular wall thickness are presented as mean and SD, left ventricular ejection fraction as mean percentage and SD and categorical variables as numbers (n) and percentages. *ACTN2* indicates actinin α2; HCM, hypertrophic cardiomyopathy; HF, heart failure; *MYBPC3*, myosin binding protein C; *MYH7*, cardiac β-myosin heavy chain; *MYL2*, myosin light chain 2; *TNNI3*, troponin I; and WGS, whole genome sequencing.

and zebrafish[26] but is not known to cause HCM. The patient had a maximum left ventricular wall thickness of 17 mm with outflow tract obstruction that led to septal myectomy. Clinical evaluations in 3 adult children, all who carry the *ILK* variant and one with the *MYH7* variant, were normal.

The third new finding from WGS was a VUS (p.Ile817Thr) in the filamin-C *(FLNC*) gene, found in a patient with a previously identified VUS in ATP Binding Cassette Subfamily C Member 9 (*ABCC9*), which was included on his panel testing but is not known to cause HCM. *FLNC* variants are primarily associated with adult-onset skeletal myopathy but also occur with cardiomyopathy in some families.[27] Recently, *FLNC* missense variants (but not Ile817Thr) were reported in familial HCM with incomplete penetrance.[28] *FLNC* was not previously analyzed in this patient. He is a 51-year-old male with no family history of HCM and no personal or family history of neuromuscular abnormalities.

Secondary Genetic Findings

Secondary findings, variants identified in several thousand disease genes[14] that are unrelated to the patient's indication for testing, were reported. There is variability in laboratory practices for reporting secondary findings, with some laboratories only reporting findings in genes on the American College of Medical Genetics and Genomics list.[29] The approach for the MedSeq Project was deliberately broad to assess the use of WGS, taking into account all possible genetic results with any clinical significance. For example, variants that are associated with monogenic dominant diseases might identify previously undiagnosed conditions or risk for future disease development, whereas single variants in recessive genes would not cause disease, but variant carriers could incur risk to subsequent generations. In total, 84 secondary variants were identified in 41 subjects (mean=2.05 per person, range=0–6). Monogenic secondary findings and their disease associations are summarized in Table 3. None of the secondary findings reported in the MedSeq Project were in genes on the American College of Medical Genetics and Genomics list.[10,11]

Five subjects (12%) had a variant in one of the following genes, with variably robust disease associations: coagulation factor 5 (*F5*; Factor V Leiden), EYA transcriptional coactivator and phosphatase 4 *(EYA4),* sequestosome 1 *(SQSTM1),* checkpoint kinase 2 *(CHEK2),* and amyloid precursor protein (*APP).* No clinical interventions were initiated based on these secondary findings. Two of these variants may contribute to noncardiac phenotypes in subjects. Factor V Leiden was present in a 44-year old who had subclavian vein thrombosis associated with implantable cardioverter–defibrillator implantation. Although lead-associated venous thrombosis is a known complication of device implantation and only 10% of individuals with Factor V Leiden typically develop a blood clot, the *F5* variant may have been a predisposing factor in this case. The *EYA4* variant is predicted to alter splicing, and similar *EYA4* variants cause dominant postlingual deafness. The subject developed hearing loss around age 50 years that he attributed to excessive noise exposure; however, review of his audiology tracings revealed a pattern more consistent with *EYA4* mutations[30,31] than the 4 kHz notch characteristic of noise-induced hearing loss.[32] He has no family history of hearing loss. Family members have not pursued *EYA4* variant testing or audiological evaluations. An *EYA4* deletion was associated with hearing loss and DCM in 1 family[33] and in 1 proband,[31] but no other *EYA4* variants have been identified in DCM patients with or without hearing loss. As such, the authors considered this a secondary finding with likely association to the patient's hearing loss, but not to HCM.

The other 3 patients with monogenic secondary findings did not exhibit any phenotypic manifestations of the condition, although each condition has reduced penetrance or variable expressivity.[34,35] An LP variant in *SQSTM1* which causes Paget disease of the bone, a dominant late-onset disorder associated with increased bone turnover,[34] was identified in a 55-year-old male without history of orthopedic problems; his cortical bone volume has not been objectively assessed. A pathogenic variant in *CHEK2*, a gene associated with increased risk for various types of cancer,[36] was found in 62-year-old female without a personal or family history of cancer. She declined a referral to a cancer genetics program but will continue age-appropriate cancer screening. A VUS in *APP* was identified in a 24-year-old subject with a grandparent who had Alzheimer disease. Although some *APP* variants are associated with autosomal dominant late-onsat alzheimer disease,[37] the potential clinical relevance is uncertain.

Table 2. Variants Reported by Panel Testing and WGS That May Cause, or Contribute to, Cardiomyopathy

ID	Hypertrophic Cardiomyopathy Targeted Test Result				WGS Result				ExAC Allele Frequency	Reported in Other HCM Probands?
	Gene	DNA Variant	Protein Variant	Classification	Gene	DNA Variant	Protein Variant	Classification		
1	*MYBPC3*	c.2827C>T	p.Arg943X	P	Same				1/16 138 South Asian, 1/64 974 European	Y
2	*MYBPC3*	c.772G>A	p.Glu258Lys	P	Same				3/43 348 European	Y
3	*MYBPC3*	c.3742_3759dup	p.Cys1253_ Arg1254insGlyGlyIleTyrValCys	P	Variant ultimately identified and reported but initially missed by WGS				Absent	Y
18	*MYBPC3*	c.772G>A	p.Glu258Lys	P	Same				3/43 348 European	Y
19	*MYBPC3*	c.2905C>T	p.Gln969X	P	Same				Absent	Y
21	*MYBPC3*	c.103C>T	p.Arg35Trp	VUS	Same				3/50 036 European	Y
27	*MYBPC3*	c.927-9G>A		P	Same				Absent	Y
33	*MYBPC3*	c.2747G>A	p.Trp916X	P	Same				Absent	Y
35	*MYBPC3*	c.3771C>A	p.Asn1257Lys	VUS	Same				Absent	Y
26	*MYBPC3*	c.3005G>A	p.Arg1002Gln	VUS	Variant identified but did not meet MedSeq WGS reporting standards because of insufficient evidence for pathogenicity				4/62 092 European	Y
6	*MYH7*	c.1987C>T	p.Arg663Cys	LP	Same				Absent	Y
11	*MYH7*	c.4031G>A	p.Arg1344Gln	VUS	Same				Absent	Y
					ILK	c.211del	p.Leu71CysfsX26	VUS	Absent	N
15	*MYH7*	c.1357C>T	p.Arg453Cys	P	Same				Absent	Y
22	*MYH7*	c.2717A>G	p.Asp906Gly	P	Same				Absent	Y
38	*MYH7*	c.2609G>A	p.Arg870His	P	Same				1/66 732 European	Y
34	*TNNI3*	c.568G>T	p.Asp190Tyr	LP	Same				Absent	Y
41	*MYL2*	c.484G>A	p.Gly162Arg	LP	Same				Absent	Y
31	*ACTN2*	c.1839+5G>C		VUS	Same				Absent	N
5	*ABCC9*	c.1982G>A	p.Arg661His	VUS	Same				1/11 498 Latino, 1/66 718 European	N
					FLNC	c.2450T>C	p.Ile817Thr	VUS	1/9640 African, 1/16 472 South Asian, 1/65 918 European	N
37	*ABCC9*	c.2238-1G>A		VUS	Variant identified but did not meet MedSeq WGS reporting standards because of insufficient evidence for pathogenicity				118/16 384 South Asian, 70/9748 European, 1/9748 African	N
4	No variant identified*				*PTPN11*	c.1403C>T	p.Thr468Met	Pathogenic	1/6614 European	N

ABCC9 indicates ATP-binding cassette subfamily C member 9; *ACTN2*, actinin α2; *FLNC*, filamin-C; HCM, hypertrophic cardiomyopathy; *ILK*, integrin-linked kinase; LP, likely pathogenic; *MYBPC3*, myosin binding protein C; *MYH7*, cardiac β-myosin heavy chain; *MYL2*, myosin light chain 2; P, pathogenic; *PTPN11*, protein tyrosine phosphatase, non-receptor type 11; *TNNI3*, troponin I; VUS, variant of uncertain significance; and WGS, whole genome sequencing.

*Targeted genetic testing panel did not include *PTPN11*.

Table 3. Monogenic Secondary Findings From WGS

Subject Age, y	Gene	DNA Variant	Protein Variant	Classification	Disease Association
44	*F5*	c.1601G>A	p.Arg534Gln	Risk allele	Factor V Leiden Thrombophilia
63	*EYA4*	c.1739-1G>A		Likely pathogenic	Postlingual deafness
55	*SQSTM1*	c.1175C>T	p.Pro392Leu	Likely pathogenic	Paget disease of the bone
62	*CHEK2*	c.1100del	p.Thr367MetfsX15	Pathogenic	*CHEK2*-related cancer risk
24	*APP*	c.2137G>A	p.Ala713Thr	VUS-FP	Late-onset alzheimer disease

APP indicates amyloid precursor protein; *CHEK2*, checkpoint kinase 2; *EYA4*, EYA transcriptional coactivator and phosphatase 4; *F5*, coagulation factor 5; *SQSTM1*, sequestosome 1; VUS-FP, variant of uncertain significance-favor pathogenic; and WGS, whole genome sequencing.

There were 79 pathogenic/LP recessive carrier variants identified, an average of 2 carrier variants per subject (Data Supplement). Hemochromatosis (*HFE*) carrier variants were most common (16/41 participants; 39%). Approximately 10% to 15% of people of European ancestry in the United States are heterozygote *HFE* variant carriers.[38] The remaining carrier states represented recessive conditions with widely variable, even unknown, carrier frequencies in the general population. Although most participants were beyond their reproductive years, carrier testing in offspring, who each have a 50% chance to carry the variant, would better define risk for future generations.

Clinical Actions Triggered by WGS Findings

In addition to monogenic and recessive carrier variants, MedSeq Project WGS reported genome-wide association studies–based risk predictions for selected common, complex cardiovascular phenotypes.[20] In 5 of 41 (12%) patients, physicians offered referrals to other providers (n=2) or ordered further diagnostic testing (n=3) based on WGS findings (Table 4). The 3 diagnostic tests were prompted by common alleles that suggested an increased risk of either abdominal aortic aneurysms or atrial fibrillation. Follow-up testing was only conducted in a single case. This patient was predicted to have an increased risk for abdominal aortic aneurysm (90th–100th percentile rank of relative risk); however, an abdominal ultrasound revealed normal aortic size. Of note, the physician cited the patient's strong desire for testing as a significant factor in ordering the ultrasound, rather than physician perception of increased risk. For a patient with a predicted increased risk for atrial fibrillation (90th–100th percentile rank of relative risk), ambulatory electrocardiographic monitoring was initially considered, but the cardiologist then opted to examine existing electrocardiographic information from the medical record. The patient continues to be monitored for the development of atrial fibrillation as part of her routine cardiomyopathy care. A patient considering future reproduction was found to have 2 recessive carrier variants and was, therefore, advised to get preconception genetic counseling. Similar prenatal referrals would likely be more common in a younger cohort.

Table 4. Clinical Actions Resulting From WGS Findings Unrelated to Cardiomyopathy

WGS Finding Prompting the Clinical Action	Clinical Testing Ordered	Findings From Clinical Testing
GWAS predicted increased risk for atrial fibrillation	Ambulatory electrocardiographic monitoring, n=1	Test not completed. Existing medical information used instead
GWAS predicted increased risk for aortic aneurysm	Abdominal ultrasound, n=2	No aortic dilatation identified (n=1); imaging not completed (n=1)
CHEK2 variant	Cancer genetics referral, n=1	Declined by patient
2 carrier variants	Preconception genetic counseling recommended, n=1	Not yet completed

CHEK2 indicates checkpoint kinase 2; GWAS, genome-wide association studies; and WGS, whole genome sequencing.

Discussion

In the MedSeq Project, the diagnostic yield of genetic testing in HCM patients was similar using either targeted/multigene panel testing (32%) or WGS (34%). Expanding the scope of genetic testing to interrogate the genome did not trigger substantive additional clinical action for the patients in the study. In this cohort, WGS detected all but 1 variant (95%) previously identified by multigene panels, allaying major concerns about reduced sensitivity and accuracy with WGS. Moreover, the ability to reanalyze the genome sequencing data provides a valuable resource that will be sought as knowledge evolves and new associations between genes and diseases are discovered, allowing WGS to be more dynamic and flexible than panel testing that is inherently constrained to the included genes. However, to achieve the benefit of reanalysis, a realistic workflow is needed to determine how sensitive genomic data would be securely stored and what prompts reanalysis, as well as who would be responsible for testing and communicating results.

Although much of the existing literature on the clinical experience using genomic sequencing in inherited cardiomyopathies consists of case reports describing the use of WES for gene discovery in a proband[39] or small collections of families with severe complex cardiomyopathies of unknown cause,[27,40] data from small cardiomyopathy cohorts have also been reported. Seidelmann at al[41] reported their experience with WES in a variety of inherited cardiovascular conditions, including HCM. In 28 HCM patients, 13 of 28 (46.4%) had pathogenic or LP variants identified; 12 occurred in genes found on current cardiomyopathy panels. Two patients (7.1%) had novel

candidate genes identified. Golbus at al[42] performed WGS in 11 individuals with nonischemic DCM. WGS confirmed a previously identified variant in 3 subjects, identified possible new causal variants in 6 subjects, was negative in 2 subjects, and identified potential disease modifiers in 2 families exhibiting variable disease expression. As such, the MedSeq Project is the only study to date that directly compares targeted testing and WGS in HCM patients while also providing new information on the largely undescribed consequences of secondary findings from WGS in a disease-specific patient population.

Candidate Genes and Genetic Modifiers

The potential for discovering candidate genes or genetic modifiers of disease over time is a major driver for the shift from targeted to comprehensive sequencing. No novel candidate genes for HCM were identified in this study. However, this was not anticipated given the small cohort size and the stringent criteria used for clinical variant reporting. In order to foster gene discovery, larger populations of panel negative patients need to be studied using different bioinformatic pipelines and deeper analysis of potential candidate genes or candidate pathways. Such efforts are underway.

In our cohort, 3 patients had variants identified in genes that have potential associations with different cardiomyopathies (*ILK, EYA4*, *FLNC).*[27,33,43] Although none of these genes has a well-established role in the pathogenesis of HCM, it is conceivable that these variants may contribute to cardiomyopathy in these patients. The *EYA4* variant was found in isolation, whereas the *FLNC* and *ILK* variants were each found in the presence of a VUS in a cardiomyopathy-associated gene (*ABCC9* and *MYBPC3,* respectively). Environmental and genetic modifiers are thought to underlie the substantial clinical heterogeneity of HCM and other cardiomyopathies. It is possible that these variants are modifiers, rather than the primary cause of disease. Additional investigation, including more systematic family evaluation, is required to better understand whether any of the identified variants may be playing a primary or modifier role in the cardiomyopathy phenotype.

Secondary Findings and Clinical Implications

The potential to identify secondary findings may be considered an advantage of WGS by some patients and providers. Indeed, most patients and research participants wish to receive all secondary findings when presented with hypothetical scenarios.[44–46] However, others may raise concerns about what WES/WGS might find, and whether that information would be helpful, particularly if there is no ability to prevent disease expression. In the MedSeq Project, WGS revealed a secondary finding with disease risk in 12% of patients (5/41). Virtually, all patients had carrier variants identified, with an average of 2 carrier variants per patient. Although there are no expected health consequences for the patient, there are reproductive implications for the patient and family. It is important to note that given the broad approach to reporting secondary findings in the MedSeq Project, results may not reflect the typical experience in clinical practice.

With the exception of the potential relationship between the *EYA4* variant and hearing loss in 1 patient and Factor V Leiden in a patient with lead-associated venous thrombosis, secondary findings were not associated with demonstrable clinical features and did not lead to new diagnoses or changes in medical management in this cohort. However, as MedSeq participants had relatively short follow-up and limited phenotyping, clinical features may still emerge. Furthermore, the implications and relevance of a secondary finding to a patient may vary based on context; someone starting a family may be more concerned about a carrier variant than those beyond their reproductive years. Moreover, secondary findings may be largely unexpected by family members if pretest counseling is not appropriately provided. As with all genetic testing, providers should equip patients with information and resources to facilitate family communication about the implications of results. The additional time demands on providers to investigate the potential clinical relevance of new findings and to facilitate family communication may be considered a disadvantage of WGS, which, when coupled with potential increased healthcare utilization, could have important downstream economic impact on the healthcare system.[47] However, although more extensive economic analyses of MedSeq Project data are underway, data derived from physician–participant ordering practices after disclosure indicate that WGS results in patients with established cardiomyopathy had limited clinical impact and, therefore, led to few downstream clinical actions.

Currently, clinical testing laboratories rarely report late-onset diseases with no treatment or cure as secondary findings.[29] By contrast, we took a broader approach to secondary findings and reported an *APP* variant to a young patient, an endeavor that epitomizes the concerns about presymptomatic testing for adult-onset neurodegenerative conditions, such as Alzheimer disease. Joint practice guidelines on genetic counseling and testing for Alzheimer disease suggest adopting the multidisciplinary genetic testing model used for Huntington disease, using both neurological and psychiatric evaluation to minimize adverse psychological outcomes in those considering presymptomatic testing.[48] Given the time and expertise required, this model is challenging to deploy for WGS, particularly for diseases exhibiting variable expression or reduced penetrance, again highlighting the importance of thorough pretest counseling. Standards for pretest counseling have been proposed; ongoing evaluation of the consent process will be important as WES/WGS use increases.[49]

On the basis of this experience using WGS in clinical practice, we highlight the following considerations:

1. Providers and patients should have reasonable expectations about diagnostic yield and the potential for secondary findings and knowledge that our understanding of the genomic sequence data will evolve such that results may need to be revisited.
2. Proper data interpretation is critical and starts with the genetic testing laboratory but often requires careful phenotyping of patients and family members, in specialized clinical programs with the necessary expertise, to allow for deeper understanding of the potential relationship between phenotype and genotype.
3. Given the importance of detailed pre- and post-test counseling, collaboration with providers with specific expertise in cardiovascular genetics is recommended to help achieve the best outcomes for patients and families.

Limitations

Although this is to date the largest study of WGS in HCM, the cohort was small and predominantly of European ancestry. Similar results may not be attainable in a more ethnically diverse population where population data in variant interpretation are limited. The use of WGS as the primary genetic testing strategy requires ongoing study to guide appropriate use in the clinic. Well-recognized limitations of WGS include insensitivity to copy number variation and variants characterized by multinucleotide repeats. Some panel testing is optimized for these in ways that have not yet been applied to WGS.

Conclusions

Clinical WGS in HCM patients has sufficient sensitivity to detect nearly all sarcomere variants identified with multigene panels. Indeed, the overall diagnostic yield of WGS in the MedSeq HCM cohort was similar to that achieved from current and historical multigene HCM panels. Despite the potential to identify important secondary findings WGS resulted in few clinical actions. While recognizing that these findings underscore the difficulties of translating genomic data into clinically useful information and define targeted panel testing as less laborious and more cost-effective, we also highlight that the wealth of information garnered from WGS provided valuable insights that will likely grow with continued discovery of disease genes, risk, and modifiers. Even in this small cohort, WGS reclassified disease based on precise cause (eg, pathogenic *PTPN11* variant) rather than a prespecified phenotype (HCM). We suggest that this may be an important and real impact of genomics: a deeper appreciation of the full spectrum of disease biology that improves medical taxonomy and thereby clinical management. Programs positioned at the interface of clinical care and genetics to properly interpret genomic sequence data and precisely phenotype patients and family members will be best positioned to lead these efforts.

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CLINICAL PERSPECTIVE

Although multigene panel genetic testing for hypertrophic cardiomyopathy (HCM) has been available for over a decade, many HCM patients do not have a molecular cause identified by current testing panels. As whole exome and genome sequencing become more accessible, there has been speculation that these more comprehensive tests may replace multigene panel tests as the preferred strategy for determining the molecular cause in patients with HCM and other inherited cardiomyopathies. However, the efficacy of this approach in the clinical arena has not been carefully assessed. In this study, 41 patients with HCM who had previously undergone genetic testing with either a multigene panel or known familial variant test were randomized to receive whole genome sequencing, allowing direct comparison of the diagnostic yield of multigene panels and whole genome sequencing. Whole genome sequencing and multigene panel testing had comparable diagnostic yield. We also assessed the incidence and consequences of secondary genetic findings—genetic variation associated with diseases unrelated to the testing indication of cardiomyopathy, but identified from genomic sequencing. Through these efforts, we describe that broadening the scope of sequencing to interrogate the genome did not lead to the discovery of new genes associated with HCM, nor did it lead to substantial downstream clinical action as a result of secondary genetic findings.

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A Comparison of Whole Genome Sequencing to Multigene Panel Testing in Hypertrophic Cardiomyopathy Patients

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SUPPLEMENTAL MATERIAL

Genetic testing methodology

Targeted HCM genetic testing

For the panel testing performed by Partners Laboratory for Molecular Medicine, all regions in the assays were either covered by array probes (for CardioChip assays) or a minimum depth of 20x for NGS-based assays. Any base that did not meet the coverage requirements above were sequenced via Sanger sequencing.

WGS

Sequencing reads were aligned to the NCBI reference sequence (GRCh37) using the Burrows-Wheeler Aligner 0.6.1-r104. The aligned reads were sorted and PCR duplicates removed using samtools 0.1.18. Local indel realignment, base quality recalibration, and variant calling were performed with UnifiedGenotyper using Genome Analysis ToolKit (GATK) 2.2.5 and the recommended best practices by the GATK development team at the Broad Institute.

Supplemental table. Carrier variants for recessive conditions

Gene	Variant (Nucleotide)	Variant (Protein)	Disease	Classification
ABCB4	c.959C>T	p.Ser320Phe	Familial progressive intrahepatic cholestasis	Uncertain significance - Favor Pathogenic
ACOX1	c.1851delT	p.Gly618AlafsX24	Peroxisomal acyl-CoA oxidase deficiency	Likely Pathogenic
ASPA	c.854A>C	p.Glu285Ala	Canavan disease	Pathogenic
ATP7B	c.383delG	p.Gly128GlufsX25	Wilson disease	Pathogenic
AURKC	c.94_101dup	p.Met35AlafsX40	Spermatogenic failure 5	Pathogenic
BTD	c.1330G>C	p.Asp444His	Biotinidase deficiency	Pathogenic
BTD	c.1330G>C	p.Asp444His	Biotinidase deficiency	Pathogenic
BTD	c.1330G>C	p.Asp444His	Biotinidase deficiency	Pathogenic
C2	c.841_849+19del		C2 deficiency	Likely Pathogenic
CBS	c.833T>C	p.Ile278Thr	Homocystinuria	Pathogenic
CFTR	c.1521_1523delCTT	p.Phe508del	Cystic fibrosis	Pathogenic
CFTR	c.1521_1523delCTT	p.Phe508del	Cystic fibrosis	Pathogenic
CRTAP	c.471+2C>A		Osteogenesis imperfecta type II	Pathogenic
DNAH11	c.7508_7509insTTG	p.Lys2504X	Primary ciliary dyskinesia	Pathogenic
ESCO2	c.294_297del	p.Arg99SerfsX2	Roberts syndrome	Pathogenic
EYS	c.6416G>A	p.Cys2139Tyr	Retinitis pigmentosa	Uncertain significance - Favor Pathogenic
GJB2	c.109G>A	p.Val37Ile	Hearing loss	Pathogenic
GJB2	c.167del	p.Leu56ArgfsX	Nonsyndromic hearing loss	Pathogenic
GJB2	c.109G>A	p.Val37Ile	Nonsyndromic hearing loss	Pathogenic
GPR56	c.10C>T	p.Gln4X	Bilateral frontoparietal polymicrogyria	Pathogenic
HEXA	c.745C>T	p.Arg249Trp	HEXA pseudodeficiency	Pseudodeficiency allele
HFE	c.845G>A	p.Cys282Tyr	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary Hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.845G>A	p.Cys282Tyr	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary	Pathogenic

			hemochromatosis	
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.845G>A	p.Cys282Tyr	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE	c.187C>G	p.His63Asp	Hereditary hemochromatosis	Pathogenic
HFE2	c.959G>T	p.Gly320Val	Hemochromatosis type 2	Pathogenic
IFT172	c.112C>T	p.Arg38X	Short-rib thoracic dysplasia	Likely Pathogenic
LAMA2	c.5563-2A>G		Congenital muscular dystrophy type 1A	Likely Pathogenic
LIFR	c.2074C>T	p.Arg692X	Stuve-Wiedemann syndrome	Likely Pathogenic
LIPA	c.253C>T	p.Gln85X	Lysosomal acid lipase A deficiency	Pathogenic
LOXHD1	c.4714C>T	p.Arg1572X	Nonsyndromic hearing loss	Pathogenic
LOXHD1	c.4480C>T	p.Arg1494X	Hearing loss	Pathogenic
LTBP4	c.254delT	p.Leu85ArgfsX15	Cutis laxa, autosomal recessive, type IC	Pathogenic
MMAB	c.700C>T	p.Gln234X	Methylmalonic acidemia	Likely Pathogenic
MUTYH	c.536A>G	p.Tyr179Cys	MUTYH-associated polyposis	Pathogenic
MUTYH	c.934-2A>G		MUTYH-associated polyposis	Likely Pathogenic
MYH2	c.3002delA	p.Glu1001GlyfsX26	Myopathy with external ophthalmoplegia	Likely Pathogenic
MYO7A	c.5648G>A	p.Arg1883Gln	Usher syndrome type I	Likely Pathogenic
NPHS2	c.868G>A	p.Val290Met	Idiopathic steroid-resistant nephrotic syndrome,	Likely Pathogenic
PAH	c.842+5G>A	p.(?)	Phenylketonuria (PKU)	Likely Pathogenic
PARK2	c.1289G>A	p.Gly430Asp	Parkinson disease	Likely Pathogenic
PHYH	c.766_767delGT	p.Val256PhefsX14	Refsum disease	Likely Pathogenic

PINK1	c.620del	p.Arg207Glnfs*14	Parkinson disease	Likely Pathogenic
POLG	c.2209G>C	p.Gly737Arg	POLG-related mitochondrial disorder	Uncertain significance: Favor pathogenic
PRX	c.2289delT	p.Asp765ThrfsX10	Charcot-Marie-Tooth disease type 4F	Likely Pathogenic
RAPSN	c.264C>A	p.Asn88Lys	Congenital myasthenic syndrome	Pathogenic
SERAC1	c.262_265dupCATG	p.Gly89AlafsX32	3-methylglutaconic aciduria with deafness, encephalopathy, and Leigh-like syndrome	Likely Pathogenic
SERPINA1	c.1096G>A	p.Glu366Lys	Alpha-1 Antitrypsin Deficiency Disorder	Pathogenic
SGCG	c.525delT	p.Leu85ArgfsX15	Limb girdle muscular dystrophy type 2C	Pathogenic
SLC12A3	c.2221G>A	p.Gly741Arg	Gitelman syndrome	Uncertain significance: Favor pathogenic
SLC26A4	c.1003T>C	p.Phe335Leu	DFNB4/Pendred syndrome	Likely Pathogenic
SLC35C1	c.464_466del	p.Phe155del	Congenital disorder of glycosylation, type Iic	Likely Pathogenic
SLC52A2	c.916G>A	p.Gly306Arg	Brown-Vialetto-Van Laere syndrome	Pathogenic
SPG11	c.1951C>T	p.Arg651X	Spastic paraplegia	Pathogenic
TALDO1	c.516dupC	p.Ala173ArgfsX23	Transaldolase deficiency	Pathogenic
TCIRG1	c.1674-1G>A		Infantile malignant osteopetrosis	Pathogenic
TCTN2	c.1877T>A	p.Leu626X	Joubert syndrome	Pathogenic
TMCO1	c.240_243delGGTT	p.Val81ThrfsX9	Cerebrofaciothoracic dysplasia	Pathogenic
TMEM5	c.1018C>T	p.Arg340X	Congenital muscular dystrophy-dystrophoglycanopathy with brain and eye anomalies	Pathogenic
TRDN	c.613C>T	p.Gln205X	Catecholaminergic polymorphic ventricular tachychardia	Likely Pathogenic
TREX1	c.341G>A	p.Arg114His	Aicardi-Goutieres syndrome	Pathogenic
TSHR	c.545+2_545+3del		Hypothyroidism	Likely Pathogenic
TTC8	c.489G>A	p.Thr163Thr	Bardet Biedl syndrome	Uncertain significance: Favor pathogenic
TYR	c.1118C>A	p.Thr373Lys	Oculocutaneous albinism type 1	Pathogenic
TYRP1	c.1057_1060del	p.Asn353ValfsX31	Oculocutaneous albinism	Pathogenic

			type III	
USH2A	c.1214delA	p.Asn405fs	Usher syndrome type II	Pathogenic
VWF	c.2561G>A	p.Arg854Gln	von Willebrand disease type 2 N	Pathogenic

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