Published in final edited form as:

Circ Cardiovasc Genet. 2017 October ; 10(5): . doi:10.1161/CIRCGENETICS.117.001768.

A Comparison of Whole Genome Sequencing to Multi-Gene Panel Testing in Hypertrophic Cardiomyopathy Patients

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Abstract

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

Methods and Results—Forty-one patients with hypertrophic cardiomyopathy (HCM) who had undergone targeted HCM genetic testing (either multi-gene 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 (VUS). WGS identified 19 of these 20 variants but the variant detection algorithm missed a pathogenic 18-base pair duplication in

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Clinical Trial Registration: https://clinicaltrials.gov; Unique Identifier: NCT01736566.

Disclosures: RCG receives compensation for speaking or consultation from AIA, GenePeeks, Helix, Illumina, Prudential and Veritas; and is co-founder and advisor to Genome Medical. CES is a founder and owns shares in Myokardia Inc. HKR directs a fee-for service laboratory (Laboratory for Molecular Medicine) and is on the scientific advisory board for Genome Medical. ML and KM and employed by a fee-for service laboratory (Laboratory for Molecular Medicine). CAM receives funding from American Heart Association, Verily and AstraZeneca and consults for Personome.

MYBPC3 due to low coverage. In 3 individuals, WGS identified variants in genes implicated in cardiomyopathy but not included in panel testing: a pathogenic *PTPN11* variant and VUSs in *ILK* and *FLNC*. WGS also identified 84 secondary findings (mean=2/person, range= 0–6), which mostly defined carrier status for recessive conditions.

Conclusions—WGS detected nearly all variants identified on panel testing, provided one new diagnostic finding, and allowed interrogation of posited disease genes. Several variants of uncertain clinical utility and numerous secondary genetic findings were also identified. While panel testing and WGS provided similar diagnostic yield, WGS offered the advantage of re-analysis over time to incorporate advances in knowledge, but necessitated expertise in genomic interpretation to appropriately incorporate WGS into clinical care.

Keywords

hypertrophic cardiomyopathy; genetic testing; whole genome sequencing

Journal Subject Terms

Genetics; Functional Genomics; Cardiomyopathy

Introduction

Current consensus guidelines recommend the use of genetic testing to establish a molecular etiology 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 multi gene-panels have expanded from 5 genes in 2004, when genetic testing was first commercially available, to now over 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[4]. This is a particular limitation when pretest probability for identifying a causal mutation is reduced due to the absence of family history or phenotypic ambiguity.[5–7] Furthermore, regardless of panel size, genetic testing does not yield a molecular etiology in 40–70% of HCM patients.[3]

More recently whole exome and genome sequencing (WES and WGS) have been increasingly utilized for molecular diagnosis.[8, 9] Initially reserved for complex clinical presentations, or as second tier tests following 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 multi-gene panels to determine the molecular etiology of monogenic conditions such as inherited cardiomyopathies. The breadth of sequence analysis afforded from WES/WGS offers great promise for increased diagnostic yield as well as 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 regarding the potential impact of unanticipated secondary findings. The American College of Medical Genetics and Genomics

(ACMG) 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 non-exonic regulatory elements and regions with high GC content may be superior with WGS.

In this study, we compared targeted HCM genetic testing, performed by multi-gene 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] Briefly, the MedSeq Project cohort included 100 primary care patients and 100 patients with presumptive inherited hypertrophic or dilated cardiomyopathy (DCM). Eligible patients received a study mailing and were approached for participation by telephone or in person during clinic visits. Participants had targeted HCM genetic testing, prior to 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—Multi-gene panel size ranged from 4–62 genes depending on year of testing (2004–2016) and clinician panel selection. All but two 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)*, alpha-tropomyosin *(TPM1)*, myosin essential and regulatory light chains *(MLY2, MYL3)*, and cardiac actin *(ACTC)*. The two subjects who had only four sarcomere genes sequenced (*MYBPC3, MYH7, TNNT2, and TPM1*) had pathogenic variants identified. Variants were classified as pathogenic, likely pathogenic (LP), uncertain significance (VUS), likely benign or benign using the clinical standard of the laboratory at the time of testing.[15,16,17] The majority of subjects (32/41) had their targeted testing performed by CLIA-certified Partners Laboratory for Molecular Medicine (LMM), Cambridge, MA (see supplemental material for methodology).

WGS—The WGS methodology and bioinformatic pipeline used in the MedSeq Project have been previously described.[16,18,19] Genome sequencing was performed by the CLIA-

certified, CAP-accredited Illumina Clinical Services Laboratory (San Diego, CA) using paired-end 100 base pair reads on the Illumina HiSeq platform between 2013–2015. Genomes were sequenced to minimum of 30X mean coverage, with ≥ 95% of bases sequenced to at least 8X coverage. Sequencing data were then transferred to the LMM for analysis and reporting. The medical exome content evolved with current knowledge throughout the study, but included ~4000 genes. Non-coding 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 due to the sequencing depth and read length. Larger copy number and structural variants are being investigated separately. See supplemental material for sequence alignment and variant calling information.

Variants were classified using a seven-tier system: benign, likely benign, uncertain significance – favor benign (VUS-FB), uncertain significance (VUS), uncertain significance – favor pathogenic (VUS-FP), likely pathogenic (LP), and pathogenic. Pathogenic, LP, and VUS-FP 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] Briefly, it was divided into different categories to report:

- Monogenic disease risk, both related and unrelated to the indication for testing (i.e. cardiomyopathy).
- Carrier variants for recessive conditions
- Selected pharmacogenomic associations
- Comprehensive blood group information[22,23]
- A cardiac risk report incorporating predictions based on genomic variation:
    - Predicted fasting lipid profile
    - Data from genome wide association studies (GWAS) on alleles conferring small to moderate risk for eight common phenotypes: atrial fibrillation, hypertension, QT prolongation, abdominal aortic aneurysm, coronary heart disease, type 2 diabetes, obesity, and platelet aggregation[21]

Secondary findings were not limited to the genes defined by the ACMG 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 post-disclosure 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 one 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 (multi-gene panel (n=38) or familial variant testing (n=3)) (Figure). The mean (standard deviation) age was 58 (12) years; 54% were female and 95% were Caucasian (Table 1). A family history of HCM was present in 17/41 (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 L P, 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-base pair duplication in *MYBPC3* (c.3742_3759dup), was initially missed by the WGS variant detection algorithm. As prior genetic testing, using a resequencing array, had identified this variant, the WGS data was 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 due to 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 one subject with prior negative genetic testing, WGS found a pathogenic *PTPN11* variant (c.1403C>T) associated with Noonan syndrome with multiple lentigines (NSML), an autosomal dominant condition characterized by lentigines, typical facial features, pulmonic stenosis, and left ventricular hypertrophy (LVH) among other features.[24] She was diagnosed with HCM at 20-years-old due to a murmur and symptoms of effort intolerance. She is 5′1″ tall with mild facial dysmorphism, lentigenes on her upper arms, LVH (maximum wall thickness 15mm) and outflow tract obstruction. Family history was negative for HCM or LVH, but one daughter was known to have mild aortic coarctation. Genetic testing in 2009 included 11 genes but did not examine RAS/MAPK pathway genes, often included on current HCM panels. After the initial negative genetic analyses, additional genetic testing and clinical evaluations were deferred due to the family's lack of interest and the patient's perception of limited clinical utility. Following the identification of the *PTPN11*

variant, her two adult children were evaluated. Though neither has pursued testing for the *PTPN11* variant, one with aortic coarctation has mild facial dysmorphism and lentigenes, consistent with NSML.

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 three HCM probands, but is also reported in four samples from the gnomAD database.[25] *ILK* participates in the regulation of cardiomyocyte growth and has been implicated in DCM by studies in mice and zebrafish[26], but is not known to cause HCM. The patient had a maximum left ventricular wall thickness of 17mm with outflow tract obstruction that led to septal myectomy. Clinical evaluations in three 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 *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 ACMG list.[29] The approach for the MedSeq Project was broad in order to assess the utility of WGS, taking into account all possible genetic results with any clinical significance. Hence, 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/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 ACMG 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 non-cardiac phenotypes in subjects. Factor V Leiden was present in a 44-year-old who had subclavian vein thrombosis associated with implantable cardioverter-defibrillator implantation. While 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 post-lingual 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 4kHz 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 one family[33] and in one 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] A likely pathogenic 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-onset Alzheimer disease[37], the potential clinical relevance is uncertain.

There were 79 pathogenic/likely pathogenic recessive carrier variants identified; an average of 2 carrier variants/subject (see supplemental material). Hemochromatosis (*HFE*) carrier variants were most common (16/41 participants; 39%). Approximately 10–15% 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. While 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 GWAS-based risk predictions for selected common, complex cardiovascular phenotypes.[20] In 5/41 (12%) patients, physicians offered referrals to other providers (n=2) or ordered further diagnostic testing (n=3) based on WGS findings (Table 4). The three 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 (90^{th}–100^{th} 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(90^{th}–100^{th} 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 two recessive carrier variants and was, therefore, advised to get preconception genetic counseling. Similar prenatal referrals would likely be more common in a younger cohort.

Discussion

In the MedSeq Project, the diagnostic yield of genetic testing in HCM patients was similar using either targeted/multi-gene 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 one variant (95%) previously identified by multi-gene panels, allaying major concerns about reduced sensitivity and accuracy with WGS. Moreover, the ability to re-analyze 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 re-analysis, a realistic workflow is needed to determine how sensitive genomic data would be securely stored and what prompts re-analysis, as well as who would be responsible for testing and communicating results.

While 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 etiology,[27,40] data from small cardiomyopathy cohorts have also been reported. Seidelmann et al[41] reported their experience with WES in a variety of inherited cardiovascular conditions, including HCM. In 28 HCM patients, 13/28 (46.4%) had pathogenic or likely pathogenic variants identified; twelve occurred in genes found on current cardiomyopathy panels. Two patients (7.1%) had novel candidate genes identified. Golbus et 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 two 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 regarding 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 are major drivers 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, illustrating the need for studying larger populations of panel negative patients using different bioinformatic

pipelines and deeper analysis of potential candidate genes or candidate pathways to foster gene discovery. Such efforts are underway.

In our cohort, three 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 one or more of these variants may contribute to cardiomyopathy in these patients. The *EYA4* variant was found in isolation, while 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.[44,45, 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 two carrier variants per patient. While 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 one 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 pre-test 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, while more extensive economic analyses of MedSeq Project data are underway, data derived from physician-participant ordering practices following 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 employed for Huntington disease, using both neurologic 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 pre-test counseling. Standards for pre-test counseling have been proposed; ongoing evaluation of the consent process will be important as WES/WGS use increases.[49]

Based on 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 is 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 multi-nucleotide 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 multi-gene panels. Indeed, the overall diagnostic yield of WGS in the MedSeq HCM cohort was similar to that achieved from current and historical multi-gene

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 conclude 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 etiology (e.g., 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank the MedSeq Project participants and acknowledge Sarah Kalia and Danielle Azzariti for their contributions to this work.

Sources of Funding: The MedSeq Project is funded by the National Human Genome Research Institute U01-HG006500.

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Clinical Perspective

Although multi-gene panel genetic testing for hypertrophic cardiomyopathy (HCM) has been available for over a decade, many HCM patients do not have a molecular etiology 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 multi-gene panel tests as the preferred strategy for determining the molecular etiology 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 multi-gene panel or known familial variant test were randomized to receive whole genome sequencing (WGS), allowing direct comparison of the diagnostic yield of multi-gene panels and whole genome sequencing. WGS and multi-gene 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.

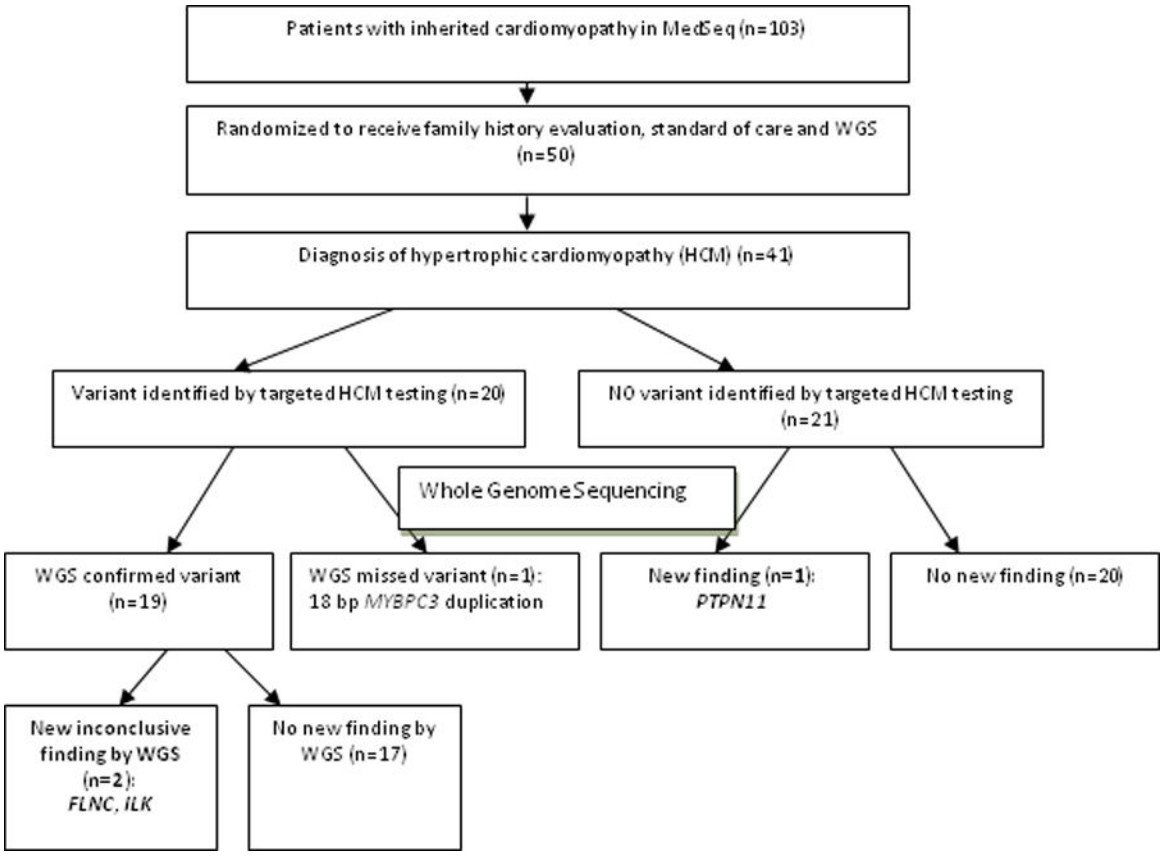

Figure.

Subject enrollment and cardiomyopathy-related genetic test results

ILK, integrin-linked kinase; *FLNC*, Filamin-C; *PTPN11*, Protein Tyrosine Phosphatase, Non-Receptor Type 11

Table 1

Characteristics of HCM patients participating in MedSeq who underwent multi-gene HCM panel testing and WGS (n=41)

Mean Age (SD), years	58 (12)
Female, n (%)	22 (54%)
Caucasian, 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	5/41 (12.2%)
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	19 (63%)
II	11 (36%)
III	1 (3%)
Sarcomere genes implicated by targeted HCM genetic testing	18/41 (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 standard deviation (SD), left ventricular ejection fraction as mean percentage and standard deviation (SD) and categorical variables as numbers (n) and percentages. HCM, hypertrophic cardiomyopathy; *MYBPC3*, Myosin binding protein C; *MYH7*, Cardiac β-myosin heavy chain; *TNNI3*, Troponin I; *MYL2*, Myosin Light Chain 2; *ACTN2*, Actinin Alpha 2

Table 2

Variants reported by panel testing and WGS that may cause, or contribute to, cardiomyopathy

	HYPERTROPHIC CARDIOMYOPATHY TARGETED TEST RESULT				WGS RESULT					
ID	**Gene**	**DNA variant**	**Protein Variant**	**Classification**	**Gene**	**DNA variant**	**Protein Variant**	**Classification**	**ExAC allele frequency**	**Reported in other HCM probands?**
1	*MYBPC3*	c.2827C>T	p.Arg943X	P	Same				1/16138 South Asian, 1/64974 European	Y
2	*MYBPC3*	c.772G>A	p.Glu258Lys	P	Same				3/43348 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				3/43348 European	Y
19	*MYBPC3*	c.2905C>T	p.Gln969X	P	Same				Absent	Y
21	*MYBPC3*	c.103C>T	p.Arg35Trp	VUS	Same				3/50036 European	
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 due to insufficient evidence for pathogenicity				4/62092 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				1/66732 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				1/11498 Latino, 1/66718 European	N
					FLNC	c.2450T>C	p.Ile817Thr	VUS	1/9640 African, 1/16472 South Asian, 1/65918 European	N

	HYPERTROPHIC CARDIOMYOPATHY TARGETED TEST RESULT				WGS RESULT					
ID	**Gene**	**DNA variant**	**Protein Variant**	**Classification**	**Gene**	**DNA variant**	**Protein Variant**	**Classification**	**ExAC allele frequency**	**Reported in other HCM probands?**
37	*ABCC9*	c.2238-1G>A		VUS	Variant identified but did not meet MedSeq WGS reporting standards due to insufficient evidence for pathogenicity				118/16384 South Asian, 70/9748 European, 1/9748 African	N
4	*No variant identified*[*]				*PTPN11*	c.1403C>T	p.Thr468Met	Pathogenic	1/6614 European	N

WGS indicates whole genome sequencing; P, Pathogenic; LP, likely pathogenic; *MYBPC3*, Myosin binding protein C; *MYH7*, Cardiac β-myosin heavy chain; *TNNI3*, Troponin I; *MYL2*, Myosin Light Chain 2; *ACTN2*, Actinin Alpha 2; *ABCC9*, ATP Binding Cassette Subfamily C Member 9; *ILK*, integrin-linked kinase; *FLNC*, Filamin-C; *PTPN11*, Protein Tyrosine Phosphatase, Non-Receptor Type 11

[*] targeted genetic testing panel did not include *PTPN11*

Table 3

Monogenic secondary findings from WGS

Subject age, years	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

F5 indicates Coagulation Factor 5; *EYA4*, EYA Transcriptional Coactivator And Phosphatase 4; *SQSTM1*, Sequestosome 1; *CHEK2*, Checkpoint Kinase 2; *APP*, Amyloid Precursor Protein

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
Two carrier variants	Preconception genetic counseling recommended, n=1	Not yet completed