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A systematic approach to the reporting of medically relevant findings from whole genome sequencing

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Abstract

Background: The MedSeq Project is a randomized clinical trial developing approaches to assess the impact of integrating genome sequencing into clinical medicine. To facilitate the return of results of potential medical relevance to physicians and patients participating in the MedSeq Project, we sought to develop a reporting approach for the effective communication of such findings.

Methods: Genome sequencing was performed on the Illumina HiSeq platform. Variants were filtered, interpreted, and validated according to methods developed by the Laboratory for Molecular Medicine and consistent with current professional guidelines. The GeneInsight software suite, which is integrated with the Partners HealthCare electronic health record, was used for variant curation, report drafting, and delivery.

Results: We developed a concise 5–6 page Genome Report (GR) featuring a single-page summary of results of potential medical relevance with additional pages containing structured variant, gene, and disease information along with supporting evidence for reported variants and brief descriptions of associated diseases and clinical implications. The GR is formatted to provide a succinct summary of genomic findings, enabling physicians to take appropriate steps for disease diagnosis, prevention, and management in their patients.

Conclusions: Our experience highlights important considerations for the reporting of results of potential medical relevance and provides a framework for interpretation and reporting practices in clinical genome sequencing.

Keywords: Clinical genome sequencing, Incidental findings, MedSeq Project, Clinical report formatting

Background

Whole exome and genome sequencing, hereafter referred to as genomic sequencing (GS) are rapidly expanding into the clinical arena [1,2]. As the cost of GS declines and the performance and clinical utility of the technologies improve [3-6], it is likely that most clinical sequencing tests will be replaced by next generation sequencing of exomes and genomes in the near future, especially for indications with extensive genetic heterogeneity.

While the expansion of GS into clinical care is promising for the diagnosis and treatment of patients with genetic disorders, and eventually the screening of healthy individuals, GS produces an extensive amount of sequencing data which must be analyzed, filtered, interpreted, and reported upon by the clinical laboratory. In contrast to traditional genetic tests which typically report back a limited set of variants conditioned on prior clinical or family data and with relatively clearly defined supporting evidence, clinical reports resulting from GS analyses contain many variants each with disparate supporting evidence associated with a broad range of diseases, and a wide set of pretest

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probabilities. Physicians receiving GS reports must digest this complex information and determine the relevant inferences for the care of their patient. This requires a nuanced understanding of the underlying rationale for GS, technical aspects of the testing and its primary interpretation, and knowledge of how to define (or refine) the probabilistic nature of the genotype in the context of the individual patient or family. In many instances, this understanding is also dependent on mechanistic insight into the relevant human biology. Unfortunately, many physicians in non-genetic specialties lack fundamental genetic knowledge and are uncomfortable interpreting genetic test results, which may result in misinterpretation and inaccurate patient counseling [7-10]. Similarly, these non-specialists are often unaware of the phenotypic nuances and pathobiological knowledge necessary to optimize the interpretation of genotypes and realize the full potential of GS in clinical care. Together, these limitations increase the risk that physicians may over-interpret GS results, leading to unnecessary followup testing and/or inaccurate diagnoses; or under-interpret GS results, leading to inadequate patient management.

In order to facilitate the communication of results of potential medical relevance to a broad range of physicians, laboratories must decide upon the level of evidence required for variants to be returned on the report. Some laboratories may choose to report all classified variants, even variants of uncertain significance with little evidence supporting pathogenicity, resulting in massive reports. In this situation, physicians may be overwhelmed by lengthy reports and experience difficulty identifying which variants are most important with respect to the care of their patient. In contrast, laboratories may restrict reports to pathogenic variants with a definitive association with disease and a narrow definition of actionability. However few variants meet this level of evidence and this approach risks the omission of many variants whose relevance is dependent on the clinical context. Indeed, some variants may turn out to be clinically relevant as further clinical data are gathered. Thus, an intermediate approach may be required to facilitate the communication of results of potential medical relevance to a broad range of physicians. Moreover, it will be vital to communicate the requisite information on potential disease or risk associations for each reported variant to allow physicians to interpret GS test results in the relevant clinical context, to orchestrate additional phenotyping, or to refer the patient to an appropriate specialist.

To promote the understanding and utility of GS data, we have developed a Genome Report (GR) for the return of findings of potential medical relevance for individuals participating in the MedSeq Project, a randomized clinical trial assessing the impact of GS in two patient populations — healthy primary care patients and patients with cardiomyopathy of suspected genetic etiology. The GR is a concise 5–6 page genome-scale report featuring a succinct front page summary of findings of potential medical relevance with additional pages containing structured variant, gene, and disease information as well as supporting evidence and disease and health impact summaries.

Methods

MedSeq Project rationale and study design

The MedSeq Project is a randomized clinical trial that is testing approaches for evaluating and reporting of GS data and assessing the impact of integrating GS into primary care and cardiology settings. To achieve these goals, the MedSeq Project is recruiting 10 primary care physicians, each with 10 of their generally healthy patients and 10 cardiologists, each with 10 of their cardiomyopathy patients, for total of 200 participants. Half of the participants are being randomized to the GS arm. The physicians of each patient enrolled in the GS arm receive a GR communicating genetic findings of potential medical relevance. Interviews and survey instruments for both physicians and patients are being used to determine the impact of GS on attitudes, behaviors, healthcare utilization, and decision-making. A detailed report of the goals, protocol, and methods in the MedSeq Project is summarized elsewhere [11].

Genome sequencing

GS is 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 [12]. Genomes are sequenced to at least 30X mean coverage and ≥ 95% of bases are sequenced to at least 8X coverage. Lossless BAM files containing sequence alignment and variant calling data are returned to the Laboratory for Molecular Medicine (LMM) via an encrypted portable hard drive for further analysis.

Clinical bioinformatics pipeline

Lossless BAM files are converted to FASTQ format to obtain sequence read data and reads are realigned to the NCBI reference sequence (GRCh37) using the Burrows-Wheeler Aligner 0.6.1-r104 [13]. Variant calls are made using the Genomic Analysis Tool Kit (GATK) version 2.3-9-gdcdccbb [14] for all positions with ≥8X coverage. Variant annotation is derived from ALAMUT HT version 1.1.2, Variant Effect Predictor version 2.6 and the LMM's GeneInsight laboratory database. Annotated variants are subsequently filtered to identify: (1) variants with a minor allele frequency (MAF) <5% in European American (EA) or African American (AA) chromosomes from the NHLBI Exome Sequencing Project (ESP; http://evs.gs.washington.edu/EVS/) classified as disease causing (DM) or possible disease causing mutations

(DM?) in the Human Gene Mutation Database [15] or as Pathogenic or Likely pathogenic by the LMM; (2) nonsense, frameshift, and canonical splice-site (+/−1,2) variants with a MAF <1% in EA or AA chromosomes from the NHLBI ESP from a list of 4,631 disease-associated genes curated by expert review of many sources of gene-disease relationships (Online Mendelian Inheritance in Man (OMIM),ClinVar, etc.; http://www.iccg.org/iccg-member-toolbox/databases-tools/medial-exome-gene-list) and (3) pharmacogenomic variants for metformin (C11orf65 rs11212617), clopidogrel (CYP2C19 rs12248560, rs4244285, rs4986893, rs28399504, rs41291556, rs72552267, rs72558186, rs56337013), warfarin (CYP2C9 rs1057910, rs1799853, rs7900194, rs9332131, rs28371685, rs28371686 and VKORC1 rs9923231), simvastatin (SLCO1B1 rs4149056), and digoxin (ABCB1 rs1045642) metabolism. The disease-associated gene list is iteratively refined as gene-disease association information is curated. In addition, blood group antigens are predicted through a parallel pipeline as noted below.

Variant classification

The principles we follow for the classification of potential Mendelian disease-causing variants have been previously described [16] and involve analysis of multiple lines of evidence including allele frequency, genetic and functional evidence from peer-reviewed scientific literature, and computational analysis (nucleotide and amino acid conservation, domain localization, missense pathogenicity prediction algorithms, and splice site prediction algorithms). Predicted loss-of-function (LOF) variants are evaluated in the context of review of known gene-disease associations and a determination of whether LOF is an established mechanism of disease for the gene in question. Each variant is classified according to American College of Medical Genetics and Genomics (ACMG) recommendations [17] and LMM criteria [16]. *Several variants required deliberation by MedSeq Project team members (including clinical geneticists, molecular geneticists, genetic counselors, and bioinformaticians) in order to assign a final classification.* The LMM uses a five tier classification system which includes the following classifications: benign, likely benign, uncertain significance, likely pathogenic, and pathogenic. In addition, a subset of uncertain significance variants are further subclassified into "uncertain significance: favor benign" or "uncertain significance: favor pathogenic". With the exception of pharmacogenomic alleles and blood group antigens, only those variants with substantial evidence for causing or contributing to Mendelian genetic disease are reported. This includes all pathogenic, likely pathogenic, and uncertain significance: favor pathogenic variants. All reported disease-associated variants are confirmed via Sanger sequencing before reporting. Variants are fully reassessed when identified in a new case and the last assessment was completed over 1 year ago for pathogenic variants or over 6 months ago for likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants are not fully reassessed unless requested by a clinician. However, all variants in the categories of likely pathogenic, uncertain significance, and likely benign are briefly reevaluated before each reporting through a quick search of online variant databases (ClinVar, HGMD, ESP, 1000G, ExAC) to ensure no new data has been reported.

Pharmacogenomic analysis

A set of 18 variants associated with the metabolism of 5 drugs (see above) commonly used in the treatment of primary care and cardiology patients were selected for inclusion in the GR from PharmGKB Clinical Annotation Levels of Evidence Class I and Class II variants [18]. Additional PharmGKB Class I variants are also made available for validation and reporting if requested by the physician. All pharmacogenomic variant bases were genotyped using GATK version 2.3-9-gdcdccbb [14] and confirmed via Sanger sequencing or Illumina HumanOmni2.5 array (San Diego, CA) before reporting.

Blood group prediction and serological confirmation

Red blood cell (RBC) and human platelet antigens (HPAs) are predicted using GS data. For all 45 RBC and 6 HPA genes, GATK is used to genotype each exon along with the first and last 10 bases of each intron. A custom prediction algorithm is then used to semi-automatically predict RBC and platelet antigens, followed by manual verification in the BAM alignment files for all antigens listed on the GR [19].

Variant curation and reporting

Variant information, including classifications, interpretations, and associated references are stored in an internal laboratory knowledge base using the GeneInsight software suite [20]. Approved variant classifications with supporting evidence descriptions are submitted to the ClinVar database to support community knowledge sharing [21]. GRs are drafted and finalized using GeneInsight Lab and electronically delivered to physicians participating in the MedSeq Project via GeneInsight Clinic. Changes to variant classification in GeneInsight Lab resulting from new data are automatically communicated to participating physicians via an email notification that links them to a patient report update within the GeneInsight Clinic application for subsequent decision support [22].

Development of the genome report

The GR presented herein was developed by a team of MedSeq Project investigators including physicians from genetic and non-genetic specialties, genetic counselors, molecular geneticists, and bioinformaticians. Prior to

implementation with physicians and patients, the GR was evaluated by a multi-disciplinary advisory committee also consisting of physicians from genetic and non-genetic specialties, genetic counselors, molecular geneticists, bioinformaticians, bioethicists, patient advocates, and biotechnology industry leaders. Revised versions of the GR were subsequently reviewed with primary care physicians who were considering participation in MedSeq Project and underwent further changes intended to maximize clarity and utility (Additional file 1).

Results

Analysis and interpretation of genomes

Each patient enrolled in the sequencing arm of the MedSeq Project receives a GR detailing findings of potential medical relevance including monogenic disease risk, carrier risk for recessive disorders, pharmacogenomic associations for commonly used medications, and a blood group antigen summary. The MedSeq Project has adopted an approach for returning findings of potential medical relevance that attempts to strike a balance between over and under reporting of variants. Instead of restricting reports to a very narrow set of disease-associated variants such as those found in the 56 genes recommended by the American College of Medical Genetics and Genomics (ACMG) [23], we have chosen to include additional analysis of many genes of potential medical relevance. This includes reporting of any variant with evidence supporting a causal role in a Mendelian genetic disease or representing carrier status for such diseases. The average genome-wide coverage ≥8X for the first 20 sequenced MedSeq Project cases (12 primary care arm and 8 cardiomyopathy arm) was 95.5% and patients had 3.4 to 5.3 million variants compared to the reference genome. Variants are filtered and analyzed via a comprehensive variant assessment process evaluating allele frequency, computational predictions, and genetic and functional evidence from peer-reviewed scientific literature (Figure 1) [16]. Each variant is classified according to LMM criteria [16], consistent with ACMG recommendations [17].

A total of 381 unique variants were manually assessed and classified for the first 20 MedSeq Project cases (251 ascertained via the HGMD filter, 110 ascertained via the LOF filter, 20 ascertained via both filters). After assessment, 168 (44%) of these variants were classified as benign or likely benign, 168 (44%) were classified as uncertain significance (including 5 classified as uncertain significance: favor pathogenic), and 45 (12%) were classified as likely pathogenic or pathogenic (Figure 2a). Similar to previously published studies [6,24,25], we found that very few variants with a purported disease association in HGMD met our criteria for pathogenicity. Out of 271 variants assessed that were categorized as DM or DM? in HGMD, only 22 (8%) were classified as likely pathogenic or pathogenic, and all but 1 of these had a DM categorization (Figure 2b).

A total of 80 unique variants from the first 20 MedSeq Project cases were selected for Sanger confirmation. Of these, 68 (85%) were confirmed, 1 (1%) was confirmed but with differing zygosity, and 11 (14%) were determined to be false positive. All false positive variants were either indels with low genotyping quality scores, or variants residing

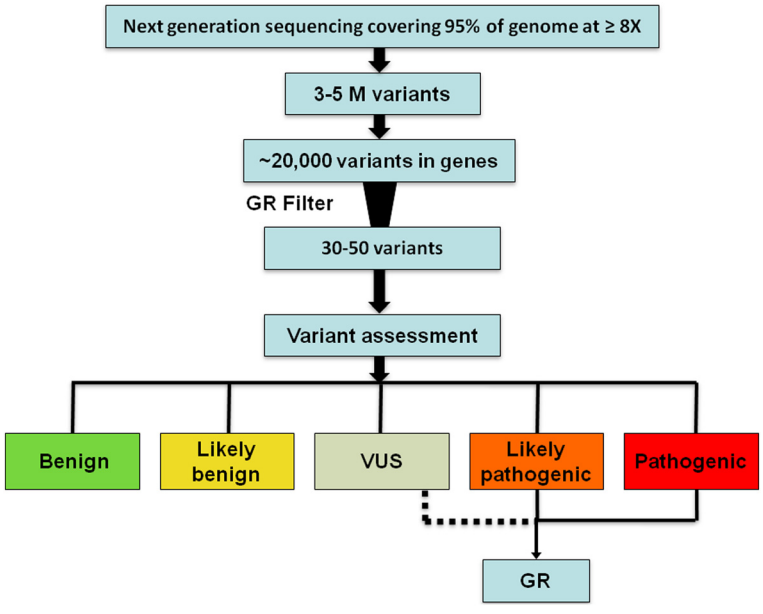
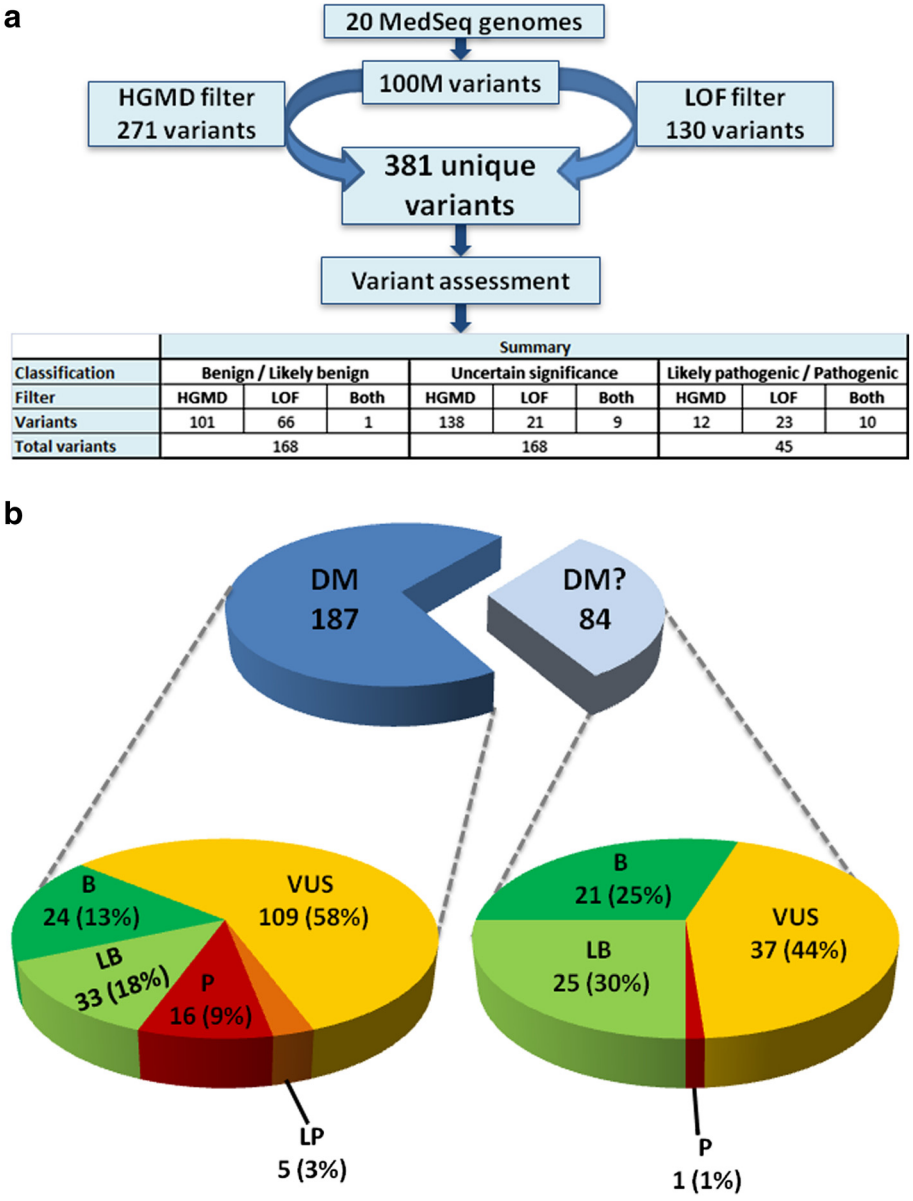


Figure 1 MedSeq Project variant analysis workflow. M (million), Genome Report (GR), variant of uncertain significance (VUS).



	Summary								
Classification	Benign / Likely benign			Uncertain significance			Likely pathogenic / Pathogenic		
Filter	HGMD	LOF	Both	HGMD	LOF	Both	HGMD	LOF	Both
Variants	101	66	1	138	21	9	12	23	10
Total variants	168			168			45		

Figure 2 Variant classifications from 20 genomes (a). A total of 381 unique variants were assessed and classified after filtration **(b)** HGMD classification comparison. Disease causing mutation (DM), likely disease causing mutation (DM?), loss-of-function (LOF).

next to repetitive polynucleotide stretches. After final deliberation by the MedSeq Project team, 48/69 (69%) of the unique Sanger-confirmed variants were returned to the first 20 MedSeq Project participants (Table 1). These included 39 variants associated with carrier status for recessive disorders, 8 variants for monogenic disease risk (5 of which were identified in individuals from the cardiomyopathy arm and assumed to be responsible for these individual's cardiomyopathy pending definitive analysis within the family) and 1 variant that conferred both carrier status and monogenic disease risk. The 21 variants that were Sanger confirmed but not reported were pursued for confirmation because the primary reviewer thought there was potential for being reported but after review by a board-certified laboratory geneticist and/or the full MedSeq Project committee, these variants did not meet the pathogenicity evidence level and/or clinical relevance threshold for return.

An entire genome summarized on a single page

In order to communicate findings of potential medical relevance to MedSeq Project physicians, we developed a GR designed to convey complex genomic data in a succinct and effective manner to the non-genetic specialist physician

Table 1 Reported findings of potential medical relevance for the first 20 MedSeq genomes

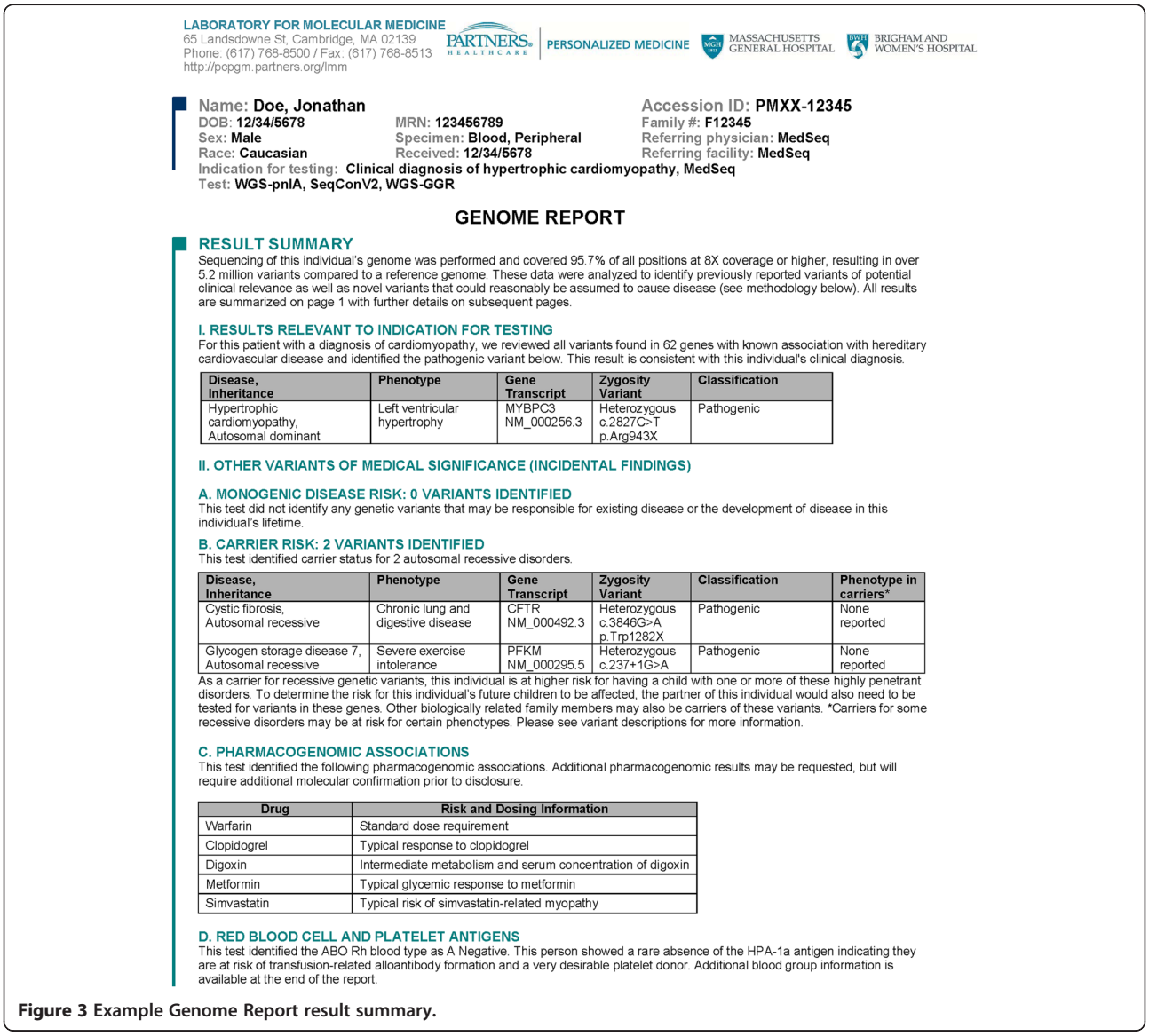
Gene	Nucleotide	Protein	Disease	Classification	Inheritance	Report section	Filter
SPATA7	c.94 + 2 T > C	p.?	Leber congenital amaurosis	LP	AR	Carrier Risk	LOF
ERCC5	c.3238C > T	p.Arg1080X	Xeroderma pigmentosum	LP	AR	Carrier Risk	LOF
COL7A1	c.7557 + 1G > T	p.?	Epidermolysis bullosa dystrophica	LP	AR	Carrier Risk	LOF
C2	c.841_849 + 19del	p.Val281_Arg283del	C2 deficiency	LP	AR	Carrier Risk	LOF
MYO7A	c.5648G > A	p.Arg1883Gln	Usher syndrome type I	LP	AR	Carrier Risk	HGMD
NAGA	c.479C > G	p.Ser160Cys	Alpha-N-acetylgalactosaminidase deficiency	LP	AR	Carrier Risk	HGMD
KCNQ1	c.826delT	p.Ser276ProfsX13	Jervell and Lange-Nielsen syndrome	LP	AR	Carrier Risk	LOF
LAMA2	c.5563-2A > G	p.?	Congenital muscular dystrophy type IA	LP	AR	Carrier Risk	LOF
SP110	c.877A > T	p.Lys293X	Hepatic veno-occlusive disease with immunodeficiency	LP	AR	Carrier Risk	LOF
ARSB	c.1450A > G	p.Arg484Gly	Mucopolysaccharidosis type VI	LP	AR	Carrier Risk	HGMD
BEST1	c.602 T > C	p.Ile201Thr	Autosomal recessive bestrophinopathy	LP	AR	Carrier Risk	HGMD
ACOX1	c.1851delT	p.Gly618AlafsX24	Peroxisomal acyl-CoA oxidase deficiency	LP	AR	Carrier Risk	LOF
LIFR	c.2074C > T	p.Arg692X	Stuve-Wiedemann syndrome	LP	AR	Carrier Risk	LOF
PAH	c.842 + 5G > A	p.?	Phenylketonuria	LP	AR	Carrier Risk	HGMD, LOF
MMACHC	c.271dupA	p.Arg91LysfsX14	Methylmalonic aciduria and homocystinuria cblC type	P	AR	Carrier Risk	LOF
CFTR	c.3846G > A	p.Trp1282X	Cystic fibrosis	P	AR	Carrier Risk	HGMD, LOF
PFKM	c.237 + 1G > A	p.?	Glycogen storage disease 7	P	AR	Carrier Risk	HGMD, LOF
CUBN	c.6928_6934del	p.Glu2310CysfsX3	Imerslund-Gräsbeck syndrome	P	AR	Carrier Risk	LOF
DUOX2	c.3847 + 2 T > C	p.?	Hypothyroidism	P	AR	Carrier Risk	LOF
ABCA4	c.5882G > A	p.Gly1961Glu	Stargardt disease	P	AR	Carrier Risk	HGMD
MPO	c.2031-2A > C	p.?	Myeloperoxidase deficiency	P	AR	Carrier Risk	HGMD
SERPINA1	c.1096G > A	p.Glu366Lys	Chronic obstructive pulmonary disease	P	AR	Carrier Risk	HGMD
USH2A	c.1214del	p. Asn405IlefsX3	Usher syndrome type II	P	AR	Carrier Risk	LOF
CLRN1	c.528 T > G	p.Tyr176X	Usher syndrome type III	P	AR	Carrier Risk	HGMD, LOF
CYP1B1	c.171G > A	p.Trp57X	Primary congenital glaucoma	P	AR	Carrier Risk	LOF
NLRP7	c.337_338insG	p.Glu113GlyfsX7	Recurrent hydatidiform mole	P	AR	Carrier Risk	LOF
BTD	c.1330G > C	p.Asp444His	Biotinidase deficiency	P	AR	Carrier Risk	HGMD
SPG7	c.1529C > T	p.Ala510Val	Spastic paraplegia type 7	P	AR	Carrier Risk	HGMD
PYGL	c.25_44dup	p.Ser15ArgfsX21	Glycogen storage disease 6	P	AR	Carrier Risk	LOF
WFS1	c.124C > T	p.Arg42X	Wolfram syndrome	P	AR	Carrier Risk	LOF
CYP1B1	c.1103G > A	p.Arg368His	Primary congenital glaucoma	P	AR	Carrier Risk	HGMD
TCIRG1	c.1674-1G > A	p.?	Infantile malignant osteopetrosis	P	AR	Carrier Risk	HGMD, LOF
LTBP4	c.254delT	p.Leu85ArgfsX15	Cutis laxa, autosomal recessive, type IC	P	AR	Carrier Risk	LOF

Table 1 Reported findings of potential medical relevance for the first 20 MedSeq genomes *(Continued)*

RAPSN	c.264C > A	p.Asn88Lys	Congenital myasthenic syndrome	P	AR	Carrier Risk	HGMD
TCTN2	c.1877 T > A	p.Leu626X	Joubert syndrome	P	AR	Carrier Risk	LOF
DUOX2	c.2895_2898del	p.Phe966SerfsX29	Congenital hypothyroidism	P	AR	Carrier Risk	LOF
HFE	c.845G > A	p.Cys282Tyr	Hereditary hemochromatosis	P	AR	Carrier Risk	HGMD
GJB2	c.109G > A	p.Val37Ile	Hearing loss	P	AR	Carrier Risk	HGMD
RAB27A	c.259G > C	p.Ala87Pro	Familial hemophagocytic lymphohistiocytosis	VUS:FP	AR	Carrier Risk	HGMD
CNGA3	c.1669G > A	p.Gly557Arg	Achromatopsia	VUS:FP	AR	Carrier Risk	HGMD
KCNQ1	c.826delT	p.Ser276ProfsX13	Romano Ward syndrome	LP	AD	Monogenic	LOF
MYBPC3	c.3742-3759dup	p.Gly1248_Cys1253dup	Hypertrophic cardiomyopathy	LP	AD	Monogenic	HGMD
MYBPC3	c.2827C > T	p.Arg943X	Hypertrophic cardiomyopathy	P	AD	Monogenic	HGMD, LOF
MYBPC3	c.772G > A	p.Glu258Lys	Hypertrophic cardiomyopathy	P	AD	Monogenic	HGMD
LHX4	c.452-2A > C	p.?	Combined pituitary hormone deficiency	P	AD	Monogenic	LOF
PTPN11	c.1403C > T	p.Thr468Met	LEOPARD syndrome	P	AD	Monogenic	HGMD
PPOX	c.199delC	p.Leu67X	Variegate porphyria	P	AD	Monogenic	HGMD, LOF
MYH7	c.1987C > T	p.Arg663Cys	Hypertrophic cardiomyopathy	P	AD	Monogenic	HGMD
ARSE	c.410G > C	p.Gly137Ala	Chondrodysplasia punctata	VUS:FP	XL	Monogenic	HGMD

Uncertain significance: Favor pathogenic (VUS:FP), Likely pathogenic (LP), Pathogenic (P), autosomal dominant (AD), autosomal recessive (AR), X-linked (XL), loss-of-function (LOF), Human Gene Mutation Database (HGMD).

(Figure 3 and Additional file 2, Additional file 3 and Additional file 4). The first page of the GR summarizes all findings including findings related to the indication for testing (for cardiomyopathy patients), monogenic disease risk, carrier risk for recessive disorders, pharmacogenomic results, and a blood group antigen summary on a single page (Figure 3). The GR result summary includes a description of genome coverage and the total number of variants identified compared to a reference genome, providing the physician with a high-level overview of the quality and complexity of their patient's GS data. Results relevant to indication for testing (for cardiomyopathy patients) and other variants of medical significance (incidental findings) are clearly delineated and each is supplemented with the disease name, inheritance, a brief phenotype description, the Human Genome Organization (HUGO)-approved gene name, a variant description in Human Genome Variation Society (HGVS) nomenclature, and variant classification in a simple tabular format. When applicable, milder and/or low penetrant phenotypes that have been reported in association with carrier status for recessive disorders are also noted. For example, individuals who are carriers for pathogenic variants in the Wolfram syndrome 1 (*WFS1*) gene, may exhibit low frequency sensorineural hearing loss and/or diabetes mellitus [26-28]. A summary of pharmacogenomic associations for five commonly administered drugs are also displayed in a tabular format, describing predicted dose requirements, drug response, or the risk of adverse events in straightforward qualitative language. Finally, predicted ABO Rh blood types are returned along with any red blood cell (RBC) or human platelet antigens



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Name: **Doe, Jonathan**
DOB: **12/34/5678**
Sex: **Male**
Race: **Caucasian**
MRN: **123456789**
Specimen: **Blood, Peripheral**
Received: **12/34/5678**
Accession ID: **PMXX-12345**
Family #: **F12345**
Referring physician: **MedSeq**
Referring facility: **MedSeq**
Indication for testing: **Clinical diagnosis of hypertrophic cardiomyopathy, MedSeq**
Test: **WGS-pnlA, SeqConV2, WGS-GGR**

GENOME REPORT

RESULT SUMMARY

Sequencing of this individual's genome was performed and covered 95.7% of all positions at 8X coverage or higher, resulting in over 5.2 million variants compared to a reference genome. These data were analyzed to identify previously reported variants of potential clinical relevance as well as novel variants that could reasonably be assumed to cause disease (see methodology below). All results are summarized on page 1 with further details on subsequent pages.

I. RESULTS RELEVANT TO INDICATION FOR TESTING

For this patient with a diagnosis of cardiomyopathy, we reviewed all variants found in 62 genes with known association with hereditary cardiovascular disease and identified the pathogenic variant below. This result is consistent with this individual's clinical diagnosis.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification
Hypertrophic cardiomyopathy, Autosomal dominant	Left ventricular hypertrophy	MYBPC3 NM_000256.3	Heterozygous c.2827C>T p.Arg943X	Pathogenic

II. OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

A. MONOGENIC DISEASE RISK: 0 VARIANTS IDENTIFIED

This test did not identify any genetic variants that may be responsible for existing disease or the development of disease in this individual's lifetime.

B. CARRIER RISK: 2 VARIANTS IDENTIFIED

This test identified carrier status for 2 autosomal recessive disorders.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification	Phenotype in carriers*
Cystic fibrosis, Autosomal recessive	Chronic lung and digestive disease	CFTR NM_000492.3	Heterozygous c.3846G>A p.Trp1282X	Pathogenic	None reported
Glycogen storage disease 7, Autosomal recessive	Severe exercise intolerance	PFKM NM_000295.5	Heterozygous c.237+1G>A	Pathogenic	None reported

As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's future children to be affected, the partner of this individual would also need to be tested for variants in these genes. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain phenotypes. Please see variant descriptions for more information.

C. PHARMACOGENOMIC ASSOCIATIONS

This test identified the following pharmacogenomic associations. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
Warfarin	Standard dose requirement
Clopidogrel	Typical response to clopidogrel
Digoxin	Intermediate metabolism and serum concentration of digoxin
Metformin	Typical glycemic response to metformin
Simvastatin	Typical risk of simvastatin-related myopathy

D. RED BLOOD CELL AND PLATELET ANTIGENS

This test identified the ABO Rh blood type as A Negative. This person showed a rare absence of the HPA-1a antigen indicating they are at risk of transfusion-related alloantibody formation and a very desirable platelet donor. Additional blood group information is available at the end of the report.

Figure 3 Example Genome Report result summary.

(HPAs) that are known to be rare in the population, enabling risk prediction for blood transfusion complications and/or awareness of desirable blood donor status for rare blood types. Physicians are directed to subsequent pages of the report to obtain more detailed information supporting the result summary.

Supplementing reports with detailed variant, gene, and disease information

Due to the vast array of diseases, genes, and variant types that could be returned from GS, we sought to balance the need for a high level summary that enables quick viewing, with the importance of providing sufficient detail and evidence to support reported results. Therefore, we expand upon the single page GR summary with a detailed variant information section featuring structured tables with RefSeq transcript, variant frequency, disease prevalence, and if applicable, carrier frequency (Additional file 2, Additional file 3 and Additional file 4). Providing transcript information and a clear variant description is essential for an unequivocal definition of the variant. Reporting the population carrier frequency for recessive disorders, if known, allows physicians to counsel individuals on the risk of having an affected child with an existing or future reproductive partner. Variant interpretations are included for each variant describing the evidence collected during the variant assessment process and providing a rigorous rationale for the variant classification. These evidence-based variant summaries are then submitted to the ClinVar database to provide transparent rationales for variant interpretations. Disease information summaries outlining common phenotypes and natural history associated with the disease are extracted from GeneReviews and Orphanet abstracts [29,30]. Uniform resource locators (URLs) for relevant online resources are also provided, allowing physicians to consult appropriate materials. Familial risk information is also described to provide physicians and patients with an explanation of the inheritance pattern and potential risk implications for offspring and biological family members. Literature references are cited for each variant with a full list of references at the end of the report.

Pharmacogenomic results are supplemented with tabular supporting information (Additional file 2, Additional file 3 and Additional file 4). The drug and indication is provided along with a summary of the dose requirement or risk of adverse effects. For each drug, variants evaluated are described using HGVS nomenclature, rsID, and PharmGKB haplotype, when applicable. A detailed interpretation is provided for each drug and genotype frequencies are provided to allow physicians to contextualize the prevalence of the patient's pharmacogenetic diplotype compared to the general population. Literature references are cited for each pharmacogenomic association with a full reference list at the end of the report.

The ABO Rh blood type and rare antigens summary are supplemented by additional blood group information provided in a tabular format (Additional file 2, Additional file 3 and Additional file 4). The presence or absence of a series of a selective number of 57 RBC antigens and all 33 platelet antigens are provided for each patient. The discussion also highlights when an individual is predicted to lack a highly prevalent antigen, making them at risk for transfusion-related events, when an individual lacks an antigen that may impact susceptibility to certain diseases, or when an individual lacks a highly immunogenic antigen, making them a good candidate for whole blood or platelet donation.

Conveying methodology and limitations of genome sequencing

Laboratories offering GS employ a multitude of strategies for their bioinformatics, interpretation, and reporting pipelines [31,32]. In addition to providing required test methodology on the report, we also include a concise description of our analysis and interpretation process to promote understanding of the sequencing and interpretation pipeline, thereby allowing physicians to compare approaches among GS tests.

While our GS test currently offers at least 30X mean coverage across the genome and ≥ 95% of bases are sequenced to at least 8X coverage, there are some limitations to GS that the MedSeq Project team was tasked with conveying to physicians. First, certain types of variation are currently not reliably detected via GS including structural variants, triplet repeat expansions, copy number variants, uniparental disomy, and epigenetic changes. Therefore, the definitive absence of a pathogenic variant in certain disease-associated genes cannot always be reliably inferred depending on the spectrum of causative variation. Secondly, coverage of disease-associated genes may be insufficient to detect all variants. To address this limitation, we have opted to provide physicians with coverage information for any genes upon request and to include coverage information for established indication-associated genes for individuals from the cardiomyopathy arm of the study (Additional file 2 and Additional file 3). Finally, not all disease-associated genes have been identified and the clinical significance of variants in many genes, even those already associated with disease, remains elusive. The limitations described above are clearly listed on the first page and on the limitations section of each MedSeq Project GR.

Delivery of genome reports

Our GeneInsight software suite [20] is used to store variant classifications, interpretations, diseases, and associated references for each reported variant. Reports are drafted using a custom GR reporting template and finalized reports

are electronically delivered in portable document format (PDF) to physicians participating in the MedSeq Project via GeneInsight Clinic (GIC), a physician interface that facilitates report viewing and download as well as updates to reports over time [22]. Electronically transmitted reports also contain structured variant data in an XML packet that is represented in the patient's electronic health record to enable clinical decision support in the future.

Discussion

The reporting of findings of potential medical relevance from GS is rapidly expanding into the clinical arena. However, little attention has been focused on how to effectively communicate GS results to physicians. The MedSeq Project has adopted an experimental approach for the return of results of potential medical relevance to study the impact of introducing a variety of different categories of variant data into the clinical care setting. To convey this range of results to the MedSeq Project participants, we have created a physician-oriented genomics report featuring a concise single-page summary of genome-wide findings of potential medical relevance with clearly delineated sections for highly-penetrant monogenic disease risk, carrier status for recessive disorders, pharmacogenomic associations, and blood group antigens. Preliminary analysis of the first 18 audio-recorded GR disclosure sessions indicates that genetic and primary care physicians are generally able to synthesize essential report information and effectively communicate genetic disease risk to their patients [33-36].

The variant interpretation process required for clinical analysis of whole genome sequence is an arduous endeavor that begins with automated filtration, but always requires manual curation of publications and careful synthesis of available data. Members of the MedSeq Project team, including clinical geneticists, molecular geneticists, genetic counselors, and bioinformaticians, gather weekly to discuss variant evidence. We have found these discussions invaluable for reaching a consensus on the interpretation of difficult variants. Each genome analyzed thus far has contained pathogenic variants in genes and diseases unfamiliar to our clinical laboratory, further adding to the challenge of genomic interpretation. Our experience reinforces the notion that broad data sharing, including gene and variant interpretations, will be a prerequisite to effectively curate thousands of genes and the variation within them in order to improve the interpretation process and achieve consensus on the classification of variants. Community efforts supporting the deposition of variant data into centralized locations such as the ClinVar database [21] will be critical to the successful incorporation of GS into clinical medicine. The MedSeq Project will further support these efforts by depositing all generated variant classifications into the ClinVar database.

The GR reporting template presented here will continue to evolve as the clinical genomics community strives to promote the understanding and utility of GS data. Moving forward, we plan to incorporate hyperlinks to interfaces with supporting resources such as the Online Mendelian Inheritance in Man (OMIM), GeneReviews, PubMed, and disease-specific resources. Future iterations may include the addition of ancestry information and common disease risk alleles. In addition, the scope of genome analysis for each patient may evolve as an individual patient's medical history and clinical situation develop. For example, additional pharmacogenomic results may be added and reinterpretation of genome data may be warranted if new symptoms arise or previously unappreciated familial risk is uncovered.

In the current genetic testing paradigm, clinical laboratories provide physicians with genetic results and physicians are in turn responsible for incorporating these results into care plans for their patients. One of the greatest barriers to integrating GS into clinical care is the limited amount of objective data regarding the best course of action for almost any genetic finding in the context of sequence from an entire genome. Physicians may grapple with ordering additional diagnostic tests for their patients to uncover potential disease-related phenotypes, and whether additional family members should be evaluated [2]. While this is not dissimilar to the problems encountered when any form of clinical testing moves from a narrow indication to broad clinical use, the sheer scale of the uncertainty is many orders of magnitude greater than for any prior scenario. In the future, it is clear that collaboration on a massive scale between clinical laboratories, clinical geneticists, genetic counselors, bioinformaticians, and physicians will be necessary to deconvolute the relationships between genotype and health or disease. A comprehensive redesign of the iterative process of test ordering, result reporting, and secondary interpretation will be necessary to provide integrated guidance and care recommendations for patients with genetic findings warranting further investigation. These steps will be critical as the results found in genome sequence reports will routinely cross the boundaries between existing silos of professional expertise.

Conclusions

In order to advance the incorporation of GS data into clinical care, we have created a concise clinical GR that outlines findings of potential medical relevance, enabling physicians to counsel patients regarding the health and reproductive implications of their genome sequences. Our experience highlights important considerations in the reporting of findings of potential medical relevance and provides a framework for evolving interpretation and reporting practices in clinical GS.

Additional files

Additional file 1: Development of the Genome Report.

Additional file 2: Example Genome Report 1. Cardiomyopathy patient with pathogenic variant.

Additional file 3: Example Genome Report 2. Cardiomyopathy patient with variant of uncertain significance.

Additional file 4: Example Genome Report 3. Primary care patient with risk allele.

Competing interests

The GeneInsight technology has been licensed to a company named GeneInsight Inc., the stockholders of which are Sun Quest and Partners Health Care System. HMM, OCB, MSL, KM, DM, and HLR work at the Laboratory for Molecular Medicine which offers fee-for-service genetic testing and is affiliated with Partners Healthcare. HLR is compensated for advisory board roles for Complete Genomics, Knome, and Ingenuity. RCG has uncompensated collaborative research agreements with Pathway and 23 and Me, and has received speaking compensation an unrestricted research grant from Illumina.

Authors' contributions

HMM wrote the manuscript and all authors critically revised and approved the version submitted for publication. HMM, OC-B, and KM performed the GS data analysis and variant interpretation. JK performed the pharmacogenomic variant interpretation. WJL performed the blood group variant interpretation. HMM, KDC, ISK, JK, WJL, DL, MSL, CM, DM, MFM, CES, JLV, HLR, and RCG developed and implemented the GR reporting format. DL and DM provided project coordination and facilitated report delivery. MSL developed the bioinformatics pipeline. HM, MSL, and HLR reviewed and finalized the reports. HLR and RCG provided oversight on all components of the project and approved the final manuscript. All authors read, edited, and approved the final manuscript.

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Additional file 1. Development of the Genome Report

The GR has undergone iterative revisions and improvements during the study based upon early experiences in returning GR results and assessing physician understanding and feedback. Below are important issues and solutions raised during the development of the GR.

Result summary sections

There was considerable discussion surrounding how to combine versus separate diagnostic findings and secondary findings. Initially we had planned to have two separate reports; however, there are occasions when the distinction between incidental versus diagnostic findings is less clear, making separation difficult. In addition, having multiple reports can risk one getting lost. Therefore, we chose to focus on one report and began reporting all indication-specific and incidental findings in the Monogenic disease risk section of the report However, after receiving feedback from physicians and subsequent discussions amongst the MedSeq Project team, we ultimately decided to separate "Results Relevant to Indication for Testing" from "Other Variants of Medical Significance (Incidental Findings)" when the distinction is clear.

Variant inclusion

The MedSeq Project chose to report variants falling into pathogenic, likely pathogenic, and uncertain significance: favor pathogenic (VUS:FP) categories. This process differs from our routine clinical service offering which does not generally include variants in the VUS:FP category. Inclusion of VUS:FP variants in the MedSeq study was permitted given the controlled study environment and has allowed us to explore what happens when results of less certainty are returned to patients. In addition we felt that subsequent medical evaluation could aid in the determination of variant pathogenicity in some cases and our study had the resources to pursue certain additional studies. We chose to exclude benign, likely benign, uncertain significance: favor benign, and uncertain significance variants because we felt that including these variants would result in undesirably lengthy reports which may obscure variants of potential medical relevance. We also felt that absent the prior probability of family history or clinical features the reporting of variants falling below the classification of VUS:FP was unjustified.

Disease inclusion

We choose to go beyond the "minimum list" of 56 genes recommended by the American College of Medical Genetics and Genomics (ACMG) and expand our analysis to any gene previously associated with a Mendelian disease in order to have the highest yield of incidental findings with which to explore the aims of the study. In addition, we felt that systematic evaluation of genes claimed to be associated with Mendelian disease would allow ongoing improvement of the literature associated with gene-disease relationships that are used in diagnostic evaluations.

Blood group antigen inclusion

To our knowledge, GS data has not been previously used to determine blood group antigens for the purposes of clinical reporting. Including blood group antigens on MedSeq Project reports allowed us to assess the feasibility and utility of providing such information for determination of rejection risk from transfusions as well as determination of desirability for blood donation, a

critical clinical need in healthcare. Details of this effort and the validation of findings are being reported in an additional manuscript in preparation led by Dr. William Lane.

Gene-specific coverage metrics

In the initial version of the GR we choose to highlight coverage levels for any genes relevant to the indication for testing (*e.g.,* MedSeq patients from the cardiomyopathy cohort) that fell below 95% coverage. However, we later realized that additional information would be helpful to contextualize this information given that reduced coverage for a gene contributing 40% yield for HCM versus a gene contributing less than 1% yield should be considered differently. Therefore, each GR for cardiomyopathy cohort patients includes a table that lists the average coverage >8X for each gene with a known association with cardiomyopathy along with information regarding the relative contribution of each gene to the patient's specific type of cardiomyopathy (see Additional files 2-3). In addition, all patients may receive coverage information for any gene upon request.

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Name: Doe, Jonathan
DOB: 12/34/5678 **MRN: 123456789**
Sex: Male **Specimen: Blood, Peripheral**
Race: Caucasian **Received: 12/34/5678**

Accession ID: PMXX-12345
Family #: F12345
Referring physician: MedSeq
Referring facility: MedSeq

Indication for testing: Clinical diagnosis of hypertrophic cardiomyopathy, MedSeq
Test: WGS-pnlA, SeqConV2, WGS-GGR

GENOME REPORT

RESULT SUMMARY

Sequencing of this individual's genome was performed and covered 95.7% of all positions at 8X coverage or higher, resulting in over 5.2 million variants compared to a reference genome. These data were analyzed to identify previously reported variants of potential clinical relevance as well as novel variants that could reasonably be assumed to cause disease (see methodology below). All results are summarized on page 1 with further details on subsequent pages.

I. RESULTS RELEVANT TO INDICATION FOR TESTING

For this patient with a diagnosis of cardiomyopathy, we reviewed all variants found in 62 genes with known association with hereditary cardiovascular disease and identified the pathogenic variant below. This result is consistent with this individual's clinical diagnosis.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification
Hypertrophic cardiomyopathy, Autosomal dominant	Left ventricular hypertrophy	MYBPC3 NM_000256.3	Heterozygous c.2827C>T p.Arg943X	Pathogenic

II. OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

A. MONOGENIC DISEASE RISK: 0 VARIANTS IDENTIFIED

This test did not identify any genetic variants that may be responsible for existing disease or the development of disease in this individual's lifetime.

B. CARRIER RISK: 2 VARIANTS IDENTIFIED

This test identified carrier status for 2 autosomal recessive disorders.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification	Phenotype in carriers*
Cystic fibrosis, Autosomal recessive	Chronic lung and digestive disease	CFTR NM_000492.3	Heterozygous c.3846G>A p.Trp1282X	Pathogenic	None reported
Glycogen storage disease 7, Autosomal recessive	Severe exercise intolerance	PFKM NM_000295.5	Heterozygous c.237+1G>A	Pathogenic	None reported

As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's future children to be affected, the partner of this individual would also need to be tested for variants in these genes. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain phenotypes. Please see variant descriptions for more information.

C. PHARMACOGENOMIC ASSOCIATIONS

This test identified the following pharmacogenomic associations. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
Warfarin	Standard dose requirement
Clopidogrel	Typical response to clopidogrel
Digoxin	Intermediate metabolism and serum concentration of digoxin
Metformin	Typical glycemic response to metformin
Simvastatin	Typical risk of simvastatin-related myopathy

D. RED BLOOD CELL AND PLATELET ANTIGENS

This test identified the ABO Rh blood type as A Negative. This person showed a rare absence of the HPA-1a antigen indicating they are at risk of transfusion-related alloantibody formation and a very desirable platelet donor. Additional blood group information is available at the end of the report.

GENOME REPORT (CONTINUED)

It should be noted that the disease risk section of this report is limited only to variants with strong evidence for causing highly penetrant disease, or contributing to highly penetrant disease in a recessive manner. Not all variants identified have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information becomes available. For questions about this report, please contact the Genome Resource Center at GRC@partners.org

DETAILED VARIANT INFORMATION

I. RESULTS RELEVANT TO CLINICAL INDICATION

Disease Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence	References
Hypertrophic cardiomyopathy Autosomal dominant	MYBPC3 NM_000256.3	Heterozygous c.2827C>T p.Arg943X Pathogenic	Not previously reported	1/500	Van Driest 2004, Lekanne Deprez 2006, Tajsharghi 2010
VARIANT INTERPRETATION: The Arg943X variant in MYBPC3 has been reported in 8 individuals with HCM (Van Driest 2004, Lekanne Deprez 2006, Tajsharghi 2010, LMM unpublished data). Several of these individuals carried an additional clinically significant variant and presented with early onset disease (Lekanne Deprez 2006, Tajsharghi 2010, LMM unpublished data). This variant leads to a premature stop at codon 943, which was shown to result in a stable mRNA, and is therefore predicted to generate a truncated protein (Lekanne Deprez 2006). Pathogenic nonsense variants in MYBPC3 are prevalent among individuals with HCM. In summary, this variant meets our criteria to be classified as pathogenic (http://pcpgm.partners.org/LMM).					
DISEASE INFORMATION: Hypertrophic cardiomyopathy (HCM), caused by mutations in genes encoding components of the sarcomere, is characterized by left ventricular hypertrophy (LVH) in the absence of predisposing or existing cardiac conditions (e.g., aortic stenosis or long-standing hypertension). The clinical manifestations of HCM range from asymptomatic to progressive heart failure to sudden cardiac death. Common symptoms include shortness of breath, chest pain, palpitations, orthostasis, presyncope, and syncope. Adapted from GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1768/					
FAMILIAL RISK: HCM due to pathogenic variants in the MYBPC3 gene is typically inherited in an autosomal dominant pattern. Each first-degree relative has a 50% chance of inheriting the variant and its risk for disease.					

II. OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

A. MONOGENIC DISEASE RISK

This test did not identify any genetic variants that may be responsible for existing disease or the development of disease in this individual's lifetime.

B. CARRIER RISK

Disease Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in carriers
Cystic fibrosis Autosomal recessive	CFTR NM_000492.3	Heterozygous c.3846G>A p.Trp1282X Pathogenic	6/8600 (0.07%) European American	1/3200 European American (1/25)	Hamosh 1991 Kerem 1990 Shoshani 1992 Vidaud 1990	None reported
VARIANT INTERPRETATION: The Trp1282X variant in CFTR has been identified in numerous patients with cystic fibrosis (Viduad 1990, Kerem1990, Hamosh 1991, Shoshani 1992). This variant is present on the American Board of Medical Genetics CFTR mutation panel (http://www.acmg.net/Pages/ACMG_Activities/stds-2002/cf.htm). This nonsense variant leads to a premature termination codon at position 1282, which is predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria for pathogenicity.						
DISEASE INFORMATION: Cystic fibrosis effects the epithelia of the respiratory tract, exocrine pancreas, intestine, male genital tract, hepatobiliary system, and exocrine sweat glands, resulting in a complex multisystem disease. Pulmonary disease is the major cause of morbidity and mortality in CF. Affected individuals have lower airway inflammation and chronic endobronchial infection, progressing to end-stage lung disease characterized by extensive airway damage (bronchiectasis, cysts, and abscesses) and fibrosis of lung parenchyma. Meconium ileus occurs at birth in 15%-20% of newborns with CF. Pancreatic insufficiency with malabsorption occurs in the great majority of individuals with CF. More than 95%of males with CF are infertile as a result of azoospermia caused by absent, atrophic, or fibrotic Wolffian duct structures. Adapted from GeneReviews abstract: http://www.ncbi.nlm.nih.gov/books/NBK1250/						
FAMILIAL RISK: Cystic fibrosis (CF) due to pathogenic variants in the CFTR gene is inherited in an autosomal recessive manner. The risk of this patient's child having CF is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with CF. Other biologically related family members may also be carriers of this variant.						

GENOME REPORT (CONTINUED)

Disease Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in carriers
Glycogen storage disease 7 Autosomal recessive	PFKM NM_000289.5	Heterozygous c.237+1G>A Pathogenic	Not previously reported	Unknown (Unknown)	Raben 1993	None reported

VARIANT INTERPRETATION: The 237+1G>A variant in PFKM has been previously identified in one homozygous patient with glycogen storage disease 7 and was found to segregate with disease in an affected homozygous relative (Raben 1993). This variant is located in the 5' splice region and computational tools do suggest an impact to splicing. In summary, this variant meets our criteria for pathogenicity.

DISEASE INFORMATION: Glycogen storage disease 7 is caused by a deficiency of muscle phosphofructokinase activity. Symptoms usually appear in adulthood and are characterized by exercise intolerance with muscle cramps that can be accompanied by attacks of myoglobinuria. Some patients also experience compensated hemolytic anemia and early onset myogenic hyperuricemia. In addition to the accumulation of normal glycogen in muscle, an abnormal glycogen, resembling amylopectin, can be found in some muscle fibers. Adapted from Online Metabolic and Molecular Basis of Inherited Disease abstract: http://www.ommbid.com//OMMBID/the_online_metabolic_and_molecular_bases_of_inherited_disease/b/abstract/part7/ch71

FAMILIAL RISK: Glycogen storage disease 7 (GSD7) due to pathogenic variants in PFKM is inherited in an autosomal recessive manner. The risk of this patient's child having GSD is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with GSD7. Other biologically related family members may also be carriers of this variant.

PHARMACOGENOMIC ASSOCIATIONS AND BLOOD GROUPS

C. PHARMACOGENOMIC ASSOCIATIONS

Drug (Indication)	Summary	Variants Evaluated and Genotypes Identified	Interpretation	References (PMID)
Warfarin (Anti-coagulation)	**Standard dose requirement**	*CYP2C9* rs1799853 rs1057910 Genotype: *1/*2 c.[430C;1075A]; c.[430C>T;1075A] *VKORC1* rs9923231 Genotype: AA	Patients with the CYP2C9*1/*2 genotype may require a lower dose of warfarin as compared to patients with theCYP2C9*1/*1 genotype. Patients with theVKORC1 AA genotype may require a lower dose of warfarin as compared to patients with the VKORC1 GG or GA genotypes. However, patients with the combination of the CYP2C9*1/*2 genotype and VKORC1 AA genotype are predicted to require standard doses of warfarin compared to other patients. Refer to warfarindosing.org for dosing based on genotype and other clinical factors.	Johnson 2011

VKORC1/CYP2C9 genotype combination frequencies

Dosing Group	VKORC1 rs9923231	CYP2C9 Genotypes	Approximate Frequency
Lower	AA	*1/*3, *2/*2, *2/*3, *3/*3	6%
	GA	*2/*3, *3/*3	3%
Standard	AA	*1/*1, *1/*2	37%
	GA	*1/*2, *1/*3, *2/*2	14%
	GG	*1/*3, *2/*2, *2/*3	<1%
Higher	GA	*1/*1	28%
	GG	*1/*1, *1/*2	13%

<table>
<tr><th>Drug (Indication)</th><th>Summary</th><th>Variants Evaluated and Genotypes Identified</th><th>Interpretation</th><th>References (PMID)</th></tr>
<tr><td>Clopidogrel (Anti-coagulation)</td><td rowspan="2">Typical response to clopidogrel</td><td>CYP2C19
rs4244285
rs4986893
rs12248560

Genotype: *1/*1
c.[806C(;)681G(;)636G];
[-806C(;)681G(;)636G]</td><td>Patients with the CYP2C19 *1/*1 genotype may have extensive (typical) metabolism of clopidogrel as well as well as typical response to clopidogrel as compared to ultrarapid or poor clopidogrel metabolizers. Additional information and dosing recommendations for this result can be found at: http://www.pharmgkb.org/drug/PA449053.</td><td>Scott 2013</td></tr>
<tr><td colspan="3">CYP2C19 genotype frequencies
<table>
<tr><th>Metabolism</th><th>Genotypes</th><th>Frequency</th></tr>
<tr><td>Ultrarapid</td><td>*1/*17, *17/*17</td><td>5-30%</td></tr>
<tr><td>Extensive (typical)</td><td>*1/*1</td><td>35-50%</td></tr>
<tr><td>Intermediate</td><td>*1/*2, *1/*3, *2/17, *3/*17</td><td>18-35%</td></tr>
<tr><td>Poor</td><td>*2/*2, *2/*3, *3/*3</td><td>2-15%</td></tr>
</table></td></tr>
<tr><td>Digoxin (Dysrhythmias, heart failure)</td><td>Intermediate metabolism and serum concentration of digoxin</td><td>ABCB1
rs1045642
Genotype: CT

Genotype frequencies:
CC: 22% CT: 51% TT:27%</td><td>Patients with the CT genotype who take oral digoxin may have intermediate metabolism and serum concentrations of digoxin as compared to patients with the CC and TT genotypes.</td><td>Aarnoudse 2008, Kurata 2002, Hoffmeyer 2000</td></tr>
<tr><td>Metformin (Type 2 diabetes mellitus)</td><td>Typical glycemic response to metformin</td><td>C11orf65
rs11212617
Genotype: TT

Genotype frequencies:
TT:37% TG:48% GG:15%</td><td>Patients with the TT genotype who have Type 2 Diabetes Mellitus and are treated with metformin may have a decreased glycemic response as compared to patients with the GG genotype. An association with increased or decreased glycemic response to metformin was not seen in people diagnosed with impaired glucose tolerance in the absence of Type 2Diabetes Mellitus.</td><td>Florez 2012, GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group 2011</td></tr>
<tr><td>Simvastatin (Hyperlipidemia)</td><td>Typical risk of simvastatin-related myopathy</td><td>SLCO1B1
rs4149056
Genotype: TT

Genotype frequencies:
TT:68% TC:30% CC:2%</td><td>Patients with the TT genotype may have a lower risk of simvastatin-related myopathy as compared to patients with the CT or CC genotype.</td><td>Wilke 2012</td></tr>
</table>

D. RED BLOOD CELL AND PLATELET ANTIGENS

SUMMARY

ABO Rh Blood type: A Negative

Rare RBC Antigens

No rare presence or absence of RBC antigens was identified.

Rare Platelet Antigens

Antigen	Frequency	Comments
HPA-1(a-)	2%	Increased risk of alloantibody formation in individual. Very desirable antigen negative donor.

DISCUSSION

These red blood cell (RBC) and human platelet antigen (HPA) predictions are based on published genotype to phenotype correlations for the alleles present. Some antigens have also been serologically determined using traditional blood typing methods.
During pregnancy or transfusion alloantibodies to blood group antigens and platelet antigens can form against foreign RBCs that contain immunogenic blood group and platelet antigens that the recipient is missing. These alloantibodies can cause clinically important complications during future transfusions and pregnancy.

Blood Production Transfusion

This test revealed an absence of the high frequency platelet HPA-1a antigen, which is present in 98% of the population. Therefore this individual has an increased risk of forming an unusual and clinically significant anti-HPA-1a alloantibody that is associated with immune-mediated platelet transfusion refractoriness/clearance. In pregnant women, this may can cause destruction of mismatched fetal and neonatal platelets.

Blood Production Donation

This individual would be a rare and very desirable platelet donor given that only 2% of the population is HPA-1(a-). Anti-HPA-1a alloantibodies are the most common anti-HPA alloantibody cause of a life threatening destruction of fetal/neonatal platelets, known as Fetal/Neonatal alloimmune thrombocytopenia (FNAIT).

RED BLOOD CELL ANTIGENS

A	B	H	D	C	c	E	e	K	k	Jk(a)	Jk(b)	Fy(a)	Fy(b)
+	-	+	-	-	+	-	+	-	+	+	+	+	-

M	N	S	S	Lu(a)	Lu(b)	Au(a)	Au(b)	Kp(a)	Kp(b)	Kp(c)	Di(a)	Di(b)
+	-	+	-	[+]	[+]	[+]	[+]	[-]	[+]	[-]	[-]	[+]

Wr(a)	Wr(b)	Yt(a)	Yt(b)	Sc1	Sc2	Do(a)	Do(b)	Jo(a)	Hy	Co(a)	Co(b)	LW(a)	LW(b)
[-]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[+]	[-]

Cr(a)	Kn(a)	Kn(b)	Sl(a)	Vil	Yk(a)	KCAM	McC(a)	McC(b)	In(a)	In(b)
[+]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[-]	[+]

Ok(a)	MER2	JMHK	JMHL	FORS
[+]	[+]	[+]	[+]	[-]

PLATELET ANTIGENS

1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6bw	7bw	8bw	9bw
[-]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[-]	[-]	[-]	[-]	[-]

10bw	11bw	12bw	13bw	14bw	15a	15b	16bw	17bw	18bw	19bw	20bw	21bw	22bw
[-]	[-]	[-]	[-]	[-]	[+]	[+]	[-]	[-]	[-]	[-]	[-]	[-]	[-]

23bw	24bw	25bw	26bw	27bw
[-]	[-]	[-]	[-]	[-]

Key: [+] presence of antigen predicted by genotyping; + presence of antigen predicted by genotyping and confirmed by serology; +* presence of antigen detected by serology, genotype prediction not available; [+w] weak presence of antigen predicted by genotyping; +w weak presence of antigen predicted by genotyping and confirmed by serology; +w* weak presence of antigen detected by serology, genotype prediction not available; [−] absence of antigen predicted by genotyping; - absence of antigen predicted by genotyping and confirmed by serology, -* absence of antigen detected by serology, genotype prediction not available; NC indicates no sequencing coverage, Dis indicates discordant. Rare (less than 5% population frequency) presence or absence of antigen is indicated in **red**.

METHODOLOGY

Genomic sequencing is performed using next generation sequencing on the Illumina HiSeq platform. Genomes are sequenced to at least 30X mean coverage and a minimum of 95% of bases are sequenced to at least 8X coverage. Paired-end 100bp reads are aligned to the NCBI reference sequence (GRCh37) using the Burrows-Wheeler Aligner (BWA), and variant calls are made using the Genomic Analysis Tool Kit (GATK). Variants are subsequently filtered to identify: (1) variants classified as disease causing in public databases; (2) nonsense, frameshift, and +/-1,2 splice-site variants that are novel or have a minor allele frequency <1% in European American or African American chromosomes from the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); and (3) rs11212617 (C11orf65; metformin), rs12248560 (CYP2C19; clopidogrel), rs4244285 (CYP2C19; clopidogrel), rs4986893 (CYP2C19; clopidogrel), rs28399504 (CYP2C19; clopidogrel), rs41291556 (CYP2C19; clopidogrel), rs72552267 (CYP2C19; clopidogrel), rs72558186 (CYP2C19; clopidogrel), rs56337013 (CYP2C19; clopidogrel), rs1057910 (CYP2C9; warfarin), rs1799853 (CYP2C9; warfarin), rs7900194 (CYP2C9; warfarin), rs9332131 (CYP2C9; warfarin), rs28371685 (CYP2C9; warfarin), rs28371686 (CYP2C9; warfarin), rs9923231 (VKORC1; warfarin), rs4149056 (SLCO1B1; simvastatin), and rs1045642 (ABCB1; digoxin). The evidence for phenotype-

causality is then evaluated for each variant resulting from the filtering strategies above and variants are classified according to LMM criteria (http://pcpgm.partners.org/LMM). Only those variants with evidence for causing highly penetrant disease or contributing to disease in a recessive manner are reported. Before reporting, all variants are confirmed via Sanger sequencing or another orthogonal technology. The initial sequencing component of this test was performed by the Illumina Clinical Services Laboratory (San Diego, CA CLIA# 05D1092911) and the alignment, variant calling, data filtering, Sanger confirmation and interpretation were performed by the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine (Cambridge, MA CLIA#22D1005307). This test has not been cleared or approved U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

LIMITATIONS

It should be noted that this test does not sequence all bases in a human genome and not all variants have been identified or interpreted. Triplet repeat expansions, translocations and large copy number events are currently not reliably detected by genome sequencing. Furthermore, not all disease-associated genes have been identified and the clinical significance of variation in many genes is not well understood. It is recommended that genomic sequencing data is periodically reinterpreted, especially when new symptoms arise.

COVERAGE OF ANALYZED GENES RELEVANT TO CARDIOVASCULAR DISEASE

The table below provides a list of genes relevant to cardiovascular disease that were evaluated during this individual's genome sequencing analysis. The proportion of the gene covered at ≥8X, *e.g.* the proportion of the gene with at least 8 mapped reads, is also provided. Please note that the presence of pathogenic variation in genes not analyzed or with incomplete coverage cannot be fully excluded.

Gene	Test Coverage >8X (%)[a]	HCM	DCM	ARVC	CPVT	LVNC	RCM	Relative contribution to HCM (%)[b]
MYBPC3	100	**X**	X			X		50%
MYH7	100	**X**	X			X	(X)	33%
TNNI3	100	**X**	X				(X)	5%
TNNT2	100	**X**	X			X	(X)	4%
TPM1	100	**X**	X					3%
MYL2	100	**X**						2%
PRKAG2	100	**X†**						1%
GLA	100	**X†**						1%
MYL3	100	**X**						1%
LAMP2	99.9	**X**	X					1%
ACTC1	100	**X**	X			X	(X)	0.4%
PLN	100	**X**	X					0.1%
ACTN2	100	**X**	X					unknown
CSRP3	100	**X**	X					unknown
MYOZ2	100	**X**						unknown
NEXN	100	**X**	X					unknown
PTPN11	99.9	**X†**						unknown
RAF1	100	**X†**						unknown
TNNC1	100	**X**	X					unknown
TTR	100	**X†**						unknown
ANKRD1	100	(X)	(X)					unknown
BAG3	100	(X)†	X				(X)†	unknown
CAV3	100	(X)	(X)					unknown
JPH2	99.8	(X)						unknown
LDB3	99.2	(X)	X			X		unknown
MYH6	100	(X)	(X)					unknown
MYLK2	100	(X)						unknown
MYOM1	100	(X)						unknown
MYPN	100	(X)						unknown
PDLIM3	100	(X)	(X)					unknown
RYR2	100	(X)		(X)	X			unknown

Gene	Test Coverage >8X (%)[a]	HCM	DCM	ARVC	CPVT	LVNC	RCM	Relative contribution to HCM (%)[b]
TCAP	100	(X)	X†					unknown
TTN	99.6	(X)	X	(X)				unknown
VCL	99.7	(X)	X			X		unknown
ABCC9	100		X					unknown
CASQ2	100				X	X		unknown
CHRM2	100		(X)					unknown
CRYAB	100		(X)					unknown
DES	100		X	(X)			(X)	unknown
DMD	99.2		X					unknown
DOLK	99.9		(X)					unknown
DSC2	100		(X)	X				unknown
DSG2	100		(X)	X				unknown
DSP	100		X	X				unknown
DTNA	100					X		unknown
EMD	96.8		X†					unknown
FHL2	100		(X)					unknown
GATAD1	99.9		X					unknown
ILK	100		(X)					unknown
JUP	100			X				unknown
LAMA4	100		(X)					unknown
LMNA	100		X			X		unknown
MURC	100		(X)					unknown
NEBL	100		(X)					unknown
PKP2	100		(X)	X				unknown
PRDM16	99.7		(X)					unknown
RBM20	100		X					unknown
SCN5A	100		X					unknown
SGCD	100		X†					unknown
TAZ	99.7		X†			X†		unknown
TMEM43	100			X				unknown
TRDN	100				X			unknown

X = genes with an established or likely role in the noted cardiomyopathy; X† = cardiomyopathy seen as part of larger disease spectrum; (X) = genes with limited evidence for disease association

[a]Indicates % coverage of gene at ≥8X in this patient's WGS analysis

[b]Based on LMM unpublished data

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Name: Doe, Jane
DOB: 12/34/5678
Sex: Female
Race: Caucasian
MRN: 123456789
Specimen: Blood, Peripheral
Received: 12/34/5678
Accession ID: PMXX-12345
Family #: F12345
Referring physician: MedSeq
Referring facility: MedSeq
Indication for testing: Clinical diagnosis of dilated cardiomyopathy, MedSeq
Test: WGS-pnlA, SeqConV2, WGS-GGR

GENOME REPORT

RESULT SUMMARY

Sequencing of this individual's genome was performed and covered 95.3% of all positions at 8X coverage or higher, resulting in over 5.3 million variants compared to a reference genome. These data were analyzed to identify previously reported variants of potential clinical relevance as well as novel variants that could reasonably be assumed to cause disease (see methodology below). All results are summarized on page 1 with further details on subsequent pages.

I. RESULTS RELEVANT TO INDICATION FOR TESTING

For this patient with a diagnosis of cardiomyopathy, we reviewed all variants found in 62 genes with known association with hereditary cardiovascular disease and identified one variant of uncertain significance. More information is needed to determine if this variant contributes to disease.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification
Dilated cardiomyopathy, Autosomal dominant	Ventricular chamber enlargement	RBM20 NM_001134363.1	Heterozygous c.2662G>A p.Asp888Asn	Uncertain significance

II. OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

A. MONOGENIC DISEASE RISK: 0 VARIANTS IDENTIFIED

This test did not identify any genetic variants that may be responsible for existing disease or the development of disease in this individual's lifetime.

B. CARRIER RISK: 2 VARIANTS IDENTIFIED

This test identified carrier status for 2 autosomal recessive disorders.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification	Phenotype in carriers*
Alpha-1 Antitrypsin Deficiency Disorder, Autosomal recessive	Emphysema +/- liver disease	SERPINA1 NM_000295.4	Heterozygous c.1096G>A p.Glu366Lys	Pathogenic	None reported
Hepatic lipase deficiency, Autosomal recessive	Elevated plasma cholesterol and triglyceride levels	LIPC NM_000236.2	Heterozygous c.866C>T p.Ser289Phe	Uncertain significance: Favor pathogenic	Elevated HDL

As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's future children to be affected, the partner of this individual would also need to be tested for variants in these genes. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain phenotypes. Please see variant descriptions for more information.

C. PHARMACOGENOMIC ASSOCIATIONS

This test identified the following pharmacogenomic associations. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
Warfarin	Standard dose requirement
Clopidogrel	Typical response to clopidogrel
Digoxin	Intermediate metabolism and serum concentration of digoxin
Metformin	Typical glycemic response to metformin
Simvastatin	Typical risk of simvastatin-related myopathy

D. RED BLOOD CELL AND PLATELET ANTIGENS

This test identified the ABO Rh blood type as B Negative. Additional blood group information is available at the end of the report.

GENOME REPORT (CONTINUED)

It should be noted that the disease risk section of this report is limited only to variants with strong evidence for causing highly penetrant disease, or contributing to highly penetrant disease in a recessive manner. Not all variants identified have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information becomes available. For questions about this report, please contact the Genome Resource Center at GRC@partners.org

DETAILED VARIANT INFORMATION

I. RESULTS RELEVANT TO CLINICAL INDICATION

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence	References
Dilated cardiomyopathy, Autosomal dominant	RBM20 NM_001134363.1	Heterozygous c.2662G>A p.Asp888Asn Uncertain significance	Not in large population studies	~1/2,500	Refaat 2012
VARIANT INTERPRETATION: The Asp888Asn variant in RBM20 has been reported in 1 individual with DCM and was absent from 1200 control chromosomes (1000 Caucasian and 200 Black; Refaat 2012). Our laboratory has detected this variant in >5 individuals with clinical features of or a clinical diagnosis of DCM (LMM unpublished data). This variant has also been identified in 0.5% (3/570) of European chromosomes by the ClinSeq Project (dbSNP rs201370621). It was initially reported as being present in European American chromosomes from the NHLBI Exome Sequencing Project, but was then removed due to insufficient data quality (http://evs.gs.washington.edu/EVS/). Computational prediction tools and conservation analysis do not provide strong support for or against an impact to the protein. In summary, the clinical significance of the Asp888Asn variant is uncertain.					
DISEASE INFORMATION: Dilated cardiomyopathy (DCM) is characterized by left ventricular enlargement and systolic dysfunction. DCM usually presents with any one of the following: Heart failure with symptoms of congestion and/or reduced cardiac output, arrhythmias and/or conduction system disease and thromboembolic disease including stroke. The incidence of DCM is currently underestimated. Familial dilated cardiomyopathy is principally caused by genetic mutations in genes that encode for cytoskeletal and sarcomeric proteins in the cardiac myocyte. Adapted from GeneReviews abstract: http://www.ncbi.nlm.nih.gov/books/NBK1309/.					
FAMILIAL RISK: Dilated Cardiomyopathy due to pathogenic variants in the RBM20 gene is typically inherited in an autosomal dominant pattern. Each first-degree relative has a 50% chance of inheriting the variant and its risk for disease.					

II. OTHER VARIANTS OF MEDICAL SIGNIFICANCE (INCIDENTAL FINDINGS)

A. MONOGENIC DISEASE RISK

This test did not identify any genetic variants that may be responsible for existing disease or the development of disease in this individual's lifetime.

B. CARRIER RISK

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in Carriers
Alpha-1 Antitrypsin Deficiency Disorder, Autosomal recessive	SERPINA1 NM_000295.4	Heterozygous c.1096G>A p.Glu366Lys Pathogenic	1.6% (140/8,600) European American	1/5,000-1/7,000 European American (1/60)	Stoller 2012	None Reported
VARIANT INTERPRETATION: The p.Glu366Lys variant in SERPINA1 (also known as p.Glu342Lys or PI*Z) is the most common alpha-1 antitrypsin deficiency allele, leading to a high risk of emphysema (and to a lesser extent liver disease) when homozygous. In summary, even with the high population frequency of this variant, it meets our criteria to be classified as pathogenic.						
DISEASE INFORMATION: Alpha-1 Antitrypsin Deficiency Disorder (AATD) is one of the most common metabolic disorders in persons of northern European heritage, occurring in approximately one in 5,000-7,000 individuals in North America and one in 1,500-3,000 in Scandinavians. COPD, specifically emphysema, is the most common manifestation of AATD and smoking is the major factor influencing age of onset and course of disease. Some individuals also present with liver disease. AATD is caused by homozygosity for the common deficiency allele, PI*Z, of SERPINA1. Clinical manifestations are infrequent in heterozygotes, except in some smokers. Adapted from GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1519/						
FAMILIAL RISK: AATD is inherited in an autosomal recessive manner. The risk of this patient's child having AATD is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with AATD. Other biologically related family members may also be carriers of this variant.						

GENOME REPORT (CONTINUED)

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in Carriers
Hepatic lipase deficiency, Autosomal recessive	LIPC NM_000236.2	Heterozygous c.866C>T p.Ser289Phe Uncertain significance: Favor pathogenic	0.13% (11/8,584) European American	Unknown (Unknown)	Hegele 1991 Durstenfeld 1994	Elevated HDL

VARIANT INTERPRETATION: The p.Ser289Phe variant in LIPC has been reported in 1 compound heterozygous individual with hepatic lipase deficiency and segregated with disease in 3 affected compound heterozygous relatives from 1 family (Hegele 1991). This variant has been identified in 0.13% (11/8584) of European American chromosomes and 0.05% (4/4384) of African American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/; dbSNP rs121912502). Although this variant has been seen in the general population, its frequency is low enough to be consistent with a recessive carrier frequency. In vitro assays indicate the p.Ser289Phe variant leads to reduced LIPC activity (Durstenfeld 1994). However, these types of assays may not accurately represent biological function. Computational prediction tools and conservation analysis also suggest that the p.Ser289Phe variant may impact the protein, though this information is not predictive enough to determine pathogenicity. In summary, while there is some suspicion for a pathogenic role, the clinical significance of the p.Ser289Phe variant is uncertain.

DISEASE INFORMATION: Hepatic lipase deficiency (HLD) is characterized by elevated plasma cholesterol and triglyceride levels. Premature atherosclerosis has been reported in some individuals with HLD. Carriers for HLD may have elevated HDL cholesterol levels.

FAMILIAL RISK: HLD due to mutations in the LIPC gene is typically inherited in an autosomal recessive manner. The risk of this patient's child having hepatic lipase deficiency is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with the disease. Other biologically related family members may also be carriers of this variant.

PHARMACOGENOMIC ASSOCIATIONS AND BLOOD GROUPS

C. PHARMACOGENOMIC ASSOCIATIONS

Drug (Indication)	Summary	Variants Evaluated and Genotypes Identified	Interpretation	References (PMID)
Warfarin (Anti-coagulation)	**Standard dose requirement**	*CYP2C9* rs1799853 rs1057910 Genotype: *1/*2 c.[430C;1075A]; c.[430C>T;1075A] *VKORC1* rs9923231 Genotype: AA	Patients with the CYP2C9*1/*2 genotype may require a lower dose of warfarin as compared to patients with theCYP2C9*1/*1 genotype. Patients with theVKORC1 AA genotype may require a lower dose of warfarin as compared to patients with the VKORC1 GG or GA genotypes. However, patients with the combination of the CYP2C9*1/*2 genotype and VKORC1 AA genotype are predicted to require standard doses of warfarin compared to other patients. Refer to warfarindosing.org for dosing based on genotype and other clinical factors.	Johnson 2011

VKORC1/CYP2C9 genotype combination frequencies

Dosing Group	VKORC1 rs9923231	CYP2C9 Genotypes	Approximate Frequency
Lower	AA	*1/*3, *2/*2, *2/*3, *3/*3	6%
	GA	*2/*3, *3/*3	3%
Standard	AA	*1/*1, *1/*2	37%
	GA	*1/*2, *1/*3, *2/*2	14%
	GG	*1/*3, *2/*2, *2/*3	<1%
Higher	GA	*1/*1	28%
	GG	*1/*1, *1/*2	13%

<table>
<tr><th>Drug (Indication)</th><th>Summary</th><th>Variants Evaluated and Genotypes Identified</th><th>Interpretation</th><th>References (PMID)</th></tr>
<tr><td>Clopidogrel (Anti-coagulation)</td><td rowspan="2">Typical response to clopidogrel</td><td>CYP2C19
rs4244285
rs4986893
rs12248560

Genotype: *1/*1
c.[806C(;)681G(;)636G];
[-806C(;)681G(;)636G]</td><td>Patients with the CYP2C19 *1/*1 genotype may have extensive (typical) metabolism of clopidogrel as well as well as typical response to clopidogrel as compared to ultrarapid or poor clopidogrel metabolizers. Additional information and dosing recommendations for this result can be found at: http://www.pharmgkb.org/drug/PA449053.</td><td>Scott 2013</td></tr>
<tr><td colspan="3">CYP2C19 genotype frequencies
<table>
<tr><th>Metabolism</th><th>Genotypes</th><th>Frequency</th></tr>
<tr><td>Ultrarapid</td><td>*1/*17, *17/*17</td><td>5-30%</td></tr>
<tr><td>Extensive (typical)</td><td>*1/*1</td><td>35-50%</td></tr>
<tr><td>Intermediate</td><td>*1/*2, *1/*3, *2/17, *3/*17</td><td>18-35%</td></tr>
<tr><td>Poor</td><td>*2/*2, *2/*3, *3/*3</td><td>2-15%</td></tr>
</table></td></tr>
<tr><td>Digoxin (Dysrhythmias, heart failure)</td><td>Intermediate metabolism and serum concentration of digoxin</td><td>ABCB1
rs1045642
Genotype: CT

Genotype frequencies:
CC: 22% CT: 51% TT:27%</td><td>Patients with the CT genotype who take oral digoxin may have intermediate metabolism and serum concentrations of digoxin as compared to patients with the CC and TT genotypes.</td><td>Aarnoudse 2008, Kurata 2002, Hoffmeyer 2000</td></tr>
<tr><td>Metformin (Type 2 diabetes mellitus)</td><td>Typical glycemic response to metformin</td><td>C11orf65
rs11212617
Genotype: TT

Genotype frequencies:
TT:37% TG:48% GG:15%</td><td>Patients with the TT genotype who have Type 2 Diabetes Mellitus and are treated with metformin may have a decreased glycemic response as compared to patients with the GG genotype. An association with increased or decreased glycemic response to metformin was not seen in people diagnosed with impaired glucose tolerance in the absence of Type 2Diabetes Mellitus.</td><td>Florez 2012, GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group 2011</td></tr>
<tr><td>Simvastatin (Hyperlipidemia)</td><td>Typical risk of simvastatin-related myopathy</td><td>SLCO1B1
rs4149056
Genotype: TT

Genotype frequencies:
TT:68% TC:30% CC:2%</td><td>Patients with the TT genotype may have a lower risk of simvastatin-related myopathy as compared to patients with the CT or CC genotype.</td><td>Wilke 2012</td></tr>
</table>

D. RED BLOOD CELL AND PLATELET ANTIGENS

SUMMARY

ABO Rh Blood type: B Negative

Rare RBC Antigens

No rare presence or absence of RBC antigens was identified.

Rare Platelet Antigens

No rare presence or absence of platelet antigens was identified.

DISCUSSION

These red blood cell (RBC) and human platelet antigen (HPA) predictions are based on published genotype to phenotype correlations for the alleles present. Some antigens have also been serologically determined using traditional blood typing methods.
During pregnancy or transfusion alloantibodies to blood group antigens and platelet antigens can form against foreign RBCs that contain immunogenic blood group and platelet antigens that the recipient is missing. These alloantibodies can cause clinically important complications during future transfusions and pregnancy.

Blood Production Transfusion

This individual does NOT have an increased risk of forming unusual RBC or platelet alloantibodies, since this test revealed a normal presence of high frequency antigens and no antigen gene rearrangements.

GENOME REPORT (CONTINUED)

Blood Production Donation

This individual does NOT pose an increased risk to blood product recipients since this test revealed a normal presence of high frequency antigens and no antigen gene rearrangements.

RED BLOOD CELL ANTIGENS

A	B	H	D	C	c	E	e	K	k	Jk(a)	Jk(b)	Fy(a)	Fy(b)
-	+	+	-	-	+	-	+	-	+	+	+	+	-

M	N	S	S	Lu(a)	Lu(b)	Au(a)	Au(b)	Kp(a)	Kp(b)	Kp(c)	Di(a)	Di(b)
+	-	+	-	[+]	[+]	[+]	[+]	[-]	[+]	[-]	[-]	[+]

Wr(a)	Wr(b)	Yt(a)	Yt(b)	Sc1	Sc2	Do(a)	Do(b)	Jo(a)	Hy	Co(a)	Co(b)	LW(a)	LW(b)
[-]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[+]	[-]

Cr(a)	Kn(a)	Kn(b)	Sl(a)	Vil	Yk(a)	KCAM	McC(a)	McC(b)	In(a)	In(b)
[+]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[-]	[+]

Ok(a)	MER2	JMHK	JMHL	FORS
[+]	[+]	[+]	[+]	[-]

PLATELET ANTIGENS

1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6bw	7bw	8bw	9bw
[+]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[-]	[-]	[-]	[-]	[-]

10bw	11bw	12bw	13bw	14bw	15a	15b	16bw	17bw	18bw	19bw	20bw	21bw	22bw
[-]	[-]	[-]	[-]	[-]	[+]	[+]	[-]	[-]	[-]	[-]	[-]	[-]	[-]

23bw	24bw	25bw	26bw	27bw
[-]	[-]	[-]	[-]	[-]

Key: [+] presence of antigen predicted by genotyping; + presence of antigen predicted by genotyping and confirmed by serology; +* presence of antigen detected by serology, genotype prediction not available; [+w] weak presence of antigen predicted by genotyping; +w weak presence of antigen predicted by genotyping and confirmed by serology; +w* weak presence of antigen detected by serology, genotype prediction not available; [−] absence of antigen predicted by genotyping; - absence of antigen predicted by genotyping and confirmed by serology, -* absence of antigen detected by serology, genotype prediction not available; NC indicates no sequencing coverage, Dis indicates discordant. Rare (less than 5% population frequency) presence or absence of antigen is indicated in **red**.

METHODOLOGY

Genomic sequencing is performed using next generation sequencing on the Illumina HiSeq platform. Genomes are sequenced to at least 30X mean coverage and a minimum of 95% of bases are sequenced to at least 8X coverage. Paired-end 100bp reads are aligned to the NCBI reference sequence (GRCh37) using the Burrows-Wheeler Aligner (BWA), and variant calls are made using the Genomic Analysis Tool Kit (GATK). Variants are subsequently filtered to identify: (1) variants classified as disease causing in public databases; (2) nonsense, frameshift, and +/-1,2 splice-site variants that are novel or have a minor allele frequency <1% in European American or African American chromosomes from the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); and (3) rs11212617 (C11orf65; metformin), rs12248560 (CYP2C19; clopidogrel), rs4244285 (CYP2C19; clopidogrel), rs4986893 (CYP2C19; clopidogrel), rs28399504 (CYP2C19; clopidogrel), rs41291556 (CYP2C19; clopidogrel), rs72552267 (CYP2C19; clopidogrel), rs72558186 (CYP2C19; clopidogrel), rs56337013 (CYP2C19; clopidogrel), rs1057910 (CYP2C9; warfarin), rs1799853 (CYP2C9; warfarin), rs7900194 (CYP2C9; warfarin), rs9332131 (CYP2C9; warfarin), rs28371685 (CYP2C9; warfarin), rs28371686 (CYP2C9; warfarin), rs9923231 (VKORC1; warfarin), rs4149056 (SLCO1B1; simvastatin), and rs1045642 (ABCB1; digoxin). The evidence for phenotype-causality is then evaluated for each variant resulting from the filtering strategies above and variants are classified according to LMM criteria (http://pcpgm.partners.org/LMM). Only those variants with evidence for causing highly penetrant disease or contributing to disease in a recessive manner are reported. Before reporting, all variants are confirmed via Sanger sequencing or another orthogonal technology. The initial sequencing component of this test was performed by the Illumina Clinical Services Laboratory (San Diego, CA CLIA# 05D1092911) and the alignment, variant calling, data filtering, Sanger confirmation and interpretation were performed by the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine (Cambridge, MA

GENOME REPORT (CONTINUED)

CLIA#22D1005307). This test has not been cleared or approved U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

LIMITATIONS

It should be noted that this test does not sequence all bases in a human genome and not all variants have been identified or interpreted. Triplet repeat expansions, translocations and large copy number events are currently not reliably detected by genome sequencing. Furthermore, not all disease-associated genes have been identified and the clinical significance of variation in many genes is not well understood. It is recommended that genomic sequencing data is periodically reinterpreted, especially when new symptoms arise.

COVERAGE OF ANALYZED GENES RELEVANT TO CARDIOVASCULAR DISEASE

The table below provides a list of genes relevant to cardiovascular disease that were evaluated during this individual's genome sequencing analysis. The proportion of the gene covered at ≥8X, *e.g.* the proportion of the gene with at least 8 mapped reads, is also provided. Please note that the presence of pathogenic variation in genes not analyzed or with incomplete coverage cannot be fully excluded.

Gene	Test Coverage >8X (%)[a]	DCM	HCM	ARVC	CPVT	LVNC	RCM	Relative contribution to DCM (%)[b]
TTN	100	**X**	(X)	(X)				12%
LMNA	99.9	**X**				X		4%
MYH7	100	**X**	X			X	(X)	3%
TNNT2	100	**X**	X			X	(X)	3%
DSP	100	**X**		X				2%
TPM1	100	**X**	X					2%
RBM20	99.4	**X**						1%
VCL	100	**X**	(X)			X		0.70%
DES	99.6	**X**		(X)			(X)	0.50%
TAZ	100	**X†**				X†		0.30%
TNNI3	100	**X**	X				(X)	0.30%
ABCC9	100	**X**						0.20%
CSRP3	100	**X**	X					0.20%
PLN	100	**X**	X					0.10%
ACTC1	100	**X**	X			X	(X)	unknown
ACTN2	100	**X**	X					unknown
BAG3	100	**X**	(X)†				(X)†	unknown
MYBPC3	100	**X**	X			X		unknown
NEXN	100	**X**	X					unknown
DMD	100	**X**						unknown
EMD	99.9	**X†**						unknown
GATAD1	99.4	**X**						unknown
LAMP2	100	**X**	X					unknown
LDB3	97.5	**X**	(X)			X		unknown
SCN5A	100	**X**						unknown
SGCD	100	**X†**						unknown
TCAP	100	**X†**	(X)					unknown
TNNC1	100	**X**	X					unknown
ANKRD1	99.9	(X)	(X)					unknown
CAV3	100	(X)	(X)					unknown
CHRM2	100	(X)						unknown

Gene	Test Coverage >8X (%)[a]	DCM	HCM	ARVC	CPVT	LVNC	RCM	Relative contribution to DCM (%)[b]
CRYAB	100	(X)						unknown
DOLK	100	(X)						unknown
DSC2	100	(X)		X				unknown
DSG2	100	(X)		X				unknown
FHL2	100	(X)						unknown
ILK	100	(X)						unknown
LAMA4	100	(X)						unknown
MURC	100	(X)						unknown
MYH6	100	(X)	(X)					unknown
NEBL	100	(X)						unknown
PDLIM3	100	(X)	(X)					unknown
PKP2	100	(X)		X				unknown
PRDM16	100	(X)						unknown
CASQ2	100				X	X		unknown
DTNA	100					X		unknown
GLA	100		X†					unknown
JPH2	99.6		(X)					unknown
JUP	100			X				unknown
MYL2	100		X					unknown
MYL3	100		X					unknown
MYLK2	100		(X)					unknown
MYOM1	100		(X)					unknown
MYOZ2	100		X					unknown
MYPN	100		(X)					unknown
PRKAG2	100		X†					unknown
PTPN11	100		X†					unknown
RAF1	100		X†					unknown
RYR2	100		(X)	(X)	X			unknown
TMEM43	100			X				unknown
TRDN	100				X			unknown
TTR	100		X†					unknown

X = genes with an established or likely role in the noted cardiomyopathy; X† = cardiomyopathy seen as part of larger disease spectrum; (X) = genes with limited evidence for disease association

[a]Indicates % coverage of gene at ≥8X in this patient's WGS analysis

[b]Based on LMM unpublished data

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PARTNERS HEALTHCARE | PERSONALIZED MEDICINE | MASSACHUSETTS GENERAL HOSPITAL | BRIGHAM AND WOMEN'S HOSPITAL

Name: Doe, Jeffrey
DOB: 12/34/5678 **MRN: 123456789**
Sex: Male **Specimen: Blood, Peripheral**
Race: Caucasian **Received: 12/34/5678**
Indication for testing: MedSeq
Test: WGS-pnlA, SeqConV2, WGS-GGR

Accession ID: PMXX-12345
Family #: F12345
Referring physician: MedSeq
Referring facility: MedSeq

GENOME REPORT

RESULT SUMMARY

Sequencing of this individual's genome was performed and covered 95.7% of all positions at 8X coverage or higher, resulting in over 5.4 million variants compared to a reference genome. These data were analyzed to identify previously reported variants of potential clinical relevance as well as novel variants that could reasonably be assumed to cause disease (see methodology below). All results are summarized on page 1 with further details on subsequent pages.

A. MONOGENIC DISEASE RISK: 1 VARIANT IDENTIFIED

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification
Factor V Leiden thrombophilia, Multi-factorial	Venous thromboembolism	F5 NM_000130.4	Heterozygous c.1601G>A p.Arg534Gln	Risk allele

B. CARRIER RISK: 2 VARIANTS IDENTIFIED

This test identified carrier status for 2 autosomal recessive disorders.

Disease, Inheritance	Phenotype	Gene Transcript	Zygosity Variant	Classification	Phenotype in carriers *
Achromatopsia, Autosomal recessive	Color blindness	CNGA3 NM_001298.2	Heterozygous c.101+1G>A	Likely pathogenic	None reported
Hereditary hemochromatosis, Autosomal recessive	Excessive iron storage	HFE NM_000410.3	Heterozygous c.187C>G p.His63Asp	Pathogenic	None reported

As a carrier for recessive genetic variants, this individual is at higher risk for having a child with one or more of these highly penetrant disorders. To determine the risk for this individual's future children to be affected, the partner of this individual would also need to be tested for variants in these genes. Other biologically related family members may also be carriers of these variants. *Carriers for some recessive disorders may be at risk for certain phenotypes. Please see variant descriptions for more information.

C. PHARMACOGENOMIC ASSOCIATIONS

This test identified the following pharmacogenomic associations. Additional pharmacogenomic results may be requested, but will require additional molecular confirmation prior to disclosure.

Drug	Risk and Dosing Information
Warfarin	Standard dose requirement
Clopidogrel	Typical response to clopidogrel
Digoxin	Intermediate metabolism and serum concentration of digoxin
Metformin	Typical glycemic response to metformin
Simvastatin	Typical risk of simvastatin-related myopathy

D. RED BLOOD CELL AND PLATELET ANTIGENS

This test identified the ABO Rh blood type as B Negative. Additional blood group information is available at the end of the report.

It should be noted that the disease risk section of this report is limited only to variants with strong evidence for causing highly penetrant disease, or contributing to highly penetrant disease in a recessive manner. Not all variants identified have been analyzed, and not all regions of the genome have been adequately sequenced. These results should be interpreted in the context of the patient's medical evaluation, family history, and racial/ethnic background. Please note that variant classification and/or interpretation may change over time if more information becomes available. For questions about this report, please contact the Genome Resource Center at GRC@partners.org

GENOME REPORT (CONTINUED)

DETAILED VARIANT INFORMATION

A. MONOGENIC DISEASE RISK

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence	References
Factor V Leiden Thrombophilia, Multi-factorial	F5 NM_000130.4	Heterozygous c.1601G>A p.Arg534Gln Risk allele	3-8% European American	Unknown	Kujovich 2011 Simone 2013
VARIANT INTERPRETATION: The Arg534Gln (legacy name Arg506Gln) in F5 is commonly referred to as "factor V Leiden" and has been associated with increased risk for venous thromboembolism (VTE) with an overall odds ratio (OR) of 4.3 (Simone 2013). The frequency of this variant varies by population, with the highest heterozygosity rate found in Europe. It has been identified in 3% (259/8600) of European American chromosomes and 0.4% (19/4406) of African American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/; dbSNP rs6025). The factor V Leiden mutation is present in approximately 15-20% of individuals with a first deep vein thrombosis (DVT) and in up to 50% of individual with recurrent VTE or an estrogen-related thrombosis. Many individuals with the factor V Leiden allele never develop thrombosis, however, evidence suggests that the relative risk for VTE is increased 3- to 8-fold in factor V Leiden heterozygotes and 10- to 80-fold in homozygotes. Lastly, heterozygosity for factor V Leiden is associated with a 2- to 3-fold increase in relative risk for pregnancy loss, and possibly other pregnancy complications such as preeclampsia, fetal growth retardation, and placental abruption.					
DISEASE INFORMATION: Factor V Leiden thrombophilia is the most common inherited form of thrombophilia. It is characterized by a poor anticoagulant response to activated protein C (APC) and an increased risk for venous thromboembolism (VTE), which is a common complex (multifactorial) disease. Deep venous thrombosis (DVT) is the most common VTE, with the legs being the most common site. Thrombosis in unusual locations is less common. The clinical expression of factor V Leiden thrombophilia is influenced by the number of factor V Leiden alleles, coexisting genetic or acquired thrombophilic disorders and circumstantial risk factors (e.g., travel, pregnancy, oral contraceptives, central venous catheters, advancing age and surgery). Adapted from GeneReviews: http://www.ncbi.nlm.nih.gov/books/NBK1368/.					
FAMILIAL RISK: This Factor V Leiden risk allele increases the chance of developing thrombophilia, likely in conjunction with other genetic and/or environmental risk factors. For a heterozygous individual, there is a 50% chance of passing on the variant and its risk of thrombophilia to a first degree relative. For homozygous individuals, there is a 100% chance of passing on the variant and its risk of thrombophilia to a first degree relative.					

B. CARRIER RISK

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in carriers
Achromatopsia, Autosomal recessive	CNGA3 NM_001298.2	Heterozygous c.101+1G>A Likely pathogenic	0.08% (7/8600) European American	<1/30,000 (Unknown)	Wissinger 2001	None reported
VARIANT INTERPRETATION: The c.101+1G>A variant in CNGA3 has not been previously reported in individuals with achromatopsia, but has been identified in 0.08% (7/8600) of European American chromosomes and 0.02% (1/4406) of African American chromosomes by the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/; dbSNP rs147118493). Although this variant has been seen in the general population, its frequency is low enough to be consistent with a recessive carrier frequency. This variant occurs in the invariant region (+/- 1,2) of the splice consensus sequence and is predicted to cause altered splicing leading to an abnormal or absent protein. Complete loss of CNGA3 function is an established disease mechanism in individuals with achromatopsia (Wissinger 2001). In summary, although additional studies are required to fully establish its clinical significance, the c.101+1G>A variant is likely pathogenic.						
DISEASE INFORMATION: Achromatopsia is characterized by reduced visual acuity, pendular nystagmus, increased sensitivity to light (photophobia), a small central scotoma, eccentric fixation, and reduced or complete loss of color discrimination. Individuals with achromatopsia have impaired color discrimination along all three axes of color vision (red, green, and blue). Adapted from GeneReviews abstract: http://www.ncbi.nlm.nih.gov/books/NBK1418/						
FAMILIAL RISK: Achromatopsia is inherited in an autosomal recessive manner. The risk of this patient's child having the disease is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with achromatopsia. Other biologically related family members may also be carriers of this variant.						

GENOME REPORT (CONTINUED)

Disease, Inheritance	Gene Transcript	Zygosity Variant Classification	Variant Frequency	Disease Prevalence (Carrier Freq.)	References	Phenotype in Carriers
Hereditary hemochromatosis, Autosomal recessive	HFE NM_000410.3	Heterozygous c.187C>G p.His63Asp Pathogenic	15% (1301/8600) European American	3-5/1000 (9%)	Gochee 2002 Beutler 2002 Gurrin 2009 Pederson 2009	None reported

VARIANT INTERPRETATION: The His63Asp variant in HFE is a well-studied variant for hereditary hemochromatosis (HH) (Gochee 2002). Although it is considered pathogenic, the penetrance is much reduced. Although 13% of His63Asp homozygous individuals and 17% of Cys282Tyr/His63Asp compound heterozygous individuals exhibit elevated transferrin saturation (Pederson 2009), the clinical penetrance is even lower with ≤5% in individuals who are homozygous or compound heterozygous for pathogenic HFE variants exhibiting symptoms (Beutler 2002, Gurrin 2009). In summary, this variant meets our criteria for pathogenicity but with much reduced penetrance.

DISEASE INFORMATION: Hereditary hemochromatosis (HH) is characterized by excessive storage of iron in the liver, skin, pancreas, heart, joints, and testes. In untreated individuals, symptoms may include abdominal pain, weakness, lethargy, weight loss, increased skin pigmentation, diabetes mellitus, congestive heart failure and/or arrhythmias, arthritis, and hypogonadism. The risk of cirrhosis is significantly increased when the serum ferritin is > 1,000 ng/mL. The biochemical and clinical penetrance of HH varies (Beutler 2002, Gurrin 2009, Pederson 2009) and HH is more common in men than women. Adapted from GeneReviews abstract: http://www.ncbi.nlm.nih.gov/books/NBK1440/

FAMILIAL RISK: Hereditary hemochromatosis is inherited in an autosomal recessive manner. The risk of this patient's child having the disease is dependent on the carrier status of the patient's partner. Two carriers have a 25% risk for having a child with Hereditary hemochromatosis. Other biologically related family members may also be carriers of this variant.

PHARMACOGENOMIC ASSOCIATIONS AND BLOOD GROUPS

C. PHARMACOGENOMIC ASSOCIATIONS

Drug (Indication)	Summary	Variants Evaluated and Genotypes Identified	Interpretation	References (PMID)
Warfarin (Anti-coagulation)	**Standard dose requirement**	*CYP2C9* rs1799853 rs1057910 Genotype: *1/*2 c.[430C;1075A]; c.[430C>T;1075A] *VKORC1* rs9923231 Genotype: AA	Patients with the CYP2C9*1/*2 genotype may require a lower dose of warfarin as compared to patients with theCYP2C9*1/*1 genotype. Patients with theVKORC1 AA genotype may require a lower dose of warfarin as compared to patients with the VKORC1 GG or GA genotypes. However, patients with the combination of the CYP2C9*1/*2 genotype and VKORC1 AA genotype are predicted to require standard doses of warfarin compared to other patients. Refer to warfarindosing.org for dosing based on genotype and other clinical factors.	Johnson 2011

VKORC1/CYP2C9 genotype combination frequencies

Dosing Group	VKORC1 rs9923231	CYP2C9 Genotypes	Approximate Frequency
Lower	AA	*1/*3, *2/*2, *2/*3, *3/*3	6%
	GA	*2/*3, *3/*3	3%
Standard	AA	*1/*1, *1/*2	37%
	GA	*1/*2, *1/*3, *2/*2	14%
	GG	*1/*3, *2/*2, *2/*3	<1%
Higher	GA	*1/*1	28%
	GG	*1/*1, *1/*2	13%

<table>
<tr><th>Drug (Indication)</th><th>Summary</th><th>Variants Evaluated and Genotypes Identified</th><th>Interpretation</th><th>References (PMID)</th></tr>
<tr><td>Clopidogrel (Anti-coagulation)</td><td rowspan="2">Typical response to clopidogrel</td><td>CYP2C19
rs4244285
rs4986893
rs12248560

Genotype: *1/*1
c.[806C(;)681G(;)636G];
[-806C(;)681G(;)636G]</td><td>Patients with the CYP2C19 *1/*1 genotype may have extensive (typical) metabolism of clopidogrel as well as well as typical response to clopidogrel as compared to ultrarapid or poor clopidogrel metabolizers. Additional information and dosing recommendations for this result can be found at: http://www.pharmgkb.org/drug/PA449053.</td><td>Scott 2013</td></tr>
<tr><td colspan="3">CYP2C19 genotype frequencies
<table>
<tr><th>Metabolism</th><th>Genotypes</th><th>Frequency</th></tr>
<tr><td>Ultrarapid</td><td>*1/*17, *17/*17</td><td>5-30%</td></tr>
<tr><td>Extensive (typical)</td><td>*1/*1</td><td>35-50%</td></tr>
<tr><td>Intermediate</td><td>*1/*2, *1/*3, *2/17, *3/*17</td><td>18-35%</td></tr>
<tr><td>Poor</td><td>*2/*2, *2/*3, *3/*3</td><td>2-15%</td></tr>
</table></td></tr>
<tr><td>Digoxin (Dysrhythmias, heart failure)</td><td>Intermediate metabolism and serum concentration of digoxin</td><td>ABCB1
rs1045642
Genotype: CT

Genotype frequencies:
CC: 22% CT: 51% TT:27%</td><td>Patients with the CT genotype who take oral digoxin may have intermediate metabolism and serum concentrations of digoxin as compared to patients with the CC and TT genotypes.</td><td>Aarnoudse 2008, Kurata 2002, Hoffmeyer 2000</td></tr>
<tr><td>Metformin (Type 2 diabetes mellitus)</td><td>Typical glycemic response to metformin</td><td>C11orf65
rs11212617
Genotype: TT

Genotype frequencies:
TT:37% TG:48% GG:15%</td><td>Patients with the TT genotype who have Type 2 Diabetes Mellitus and are treated with metformin may have a decreased glycemic response as compared to patients with the GG genotype. An association with increased or decreased glycemic response to metformin was not seen in people diagnosed with impaired glucose tolerance in the absence of Type 2Diabetes Mellitus.</td><td>Florez 2012, GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group 2011</td></tr>
<tr><td>Simvastatin (Hyperlipidemia)</td><td>Typical risk of simvastatin-related myopathy</td><td>SLCO1B1
rs4149056
Genotype: TT

Genotype frequencies:
TT:68% TC:30% CC:2%</td><td>Patients with the TT genotype may have a lower risk of simvastatin-related myopathy as compared to patients with the CT or CC genotype.</td><td>Wilke 2012</td></tr>
</table>

D. RED BLOOD CELL AND PLATELET ANTIGENS

SUMMARY

ABO Rh Blood type: B Negative

Rare RBC Antigens

No rare presence or absence of RBC antigens was identified.

Rare Platelet Antigens

No rare presence or absence of platelet antigens was identified.

DISCUSSION

These red blood cell (RBC) and human platelet antigen (HPA) predictions are based on published genotype to phenotype correlations for the alleles present. Some antigens have also been serologically determined using traditional blood typing methods.
During pregnancy or transfusion alloantibodies to blood group antigens and platelet antigens can form against foreign RBCs that contain immunogenic blood group and platelet antigens that the recipient is missing. These alloantibodies can cause clinically important complications during future transfusions and pregnancy.

Blood Production Transfusion

This individual does NOT have an increased risk of forming unusual RBC or platelet alloantibodies, since this test revealed a normal presence of high frequency antigens and no antigen gene rearrangements.

Blood Production Donation

This individual does NOT pose an increased risk to blood product recipients since this test revealed a normal presence of high frequency antigens and no antigen gene rearrangements.

RED BLOOD CELL ANTIGENS

A	B	H	D	C	c	E	e	K	k	Jk(a)	Jk(b)	Fy(a)	Fy(b)
-	+	+	-	-	+	-	+	-	+	+	+	+	-

M	N	S	S	Lu(a)	Lu(b)	Au(a)	Au(b)	Kp(a)	Kp(b)	Kp(c)	Di(a)	Di(b)
+	-	+	-	[+]	[+]	[+]	[+]	[-]	[+]	[-]	[-]	[+]

Wr(a)	Wr(b)	Yt(a)	Yt(b)	Sc1	Sc2	Do(a)	Do(b)	Jo(a)	Hy	Co(a)	Co(b)	LW(a)	LW(b)
[-]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[+]	[-]

Cr(a)	Kn(a)	Kn(b)	Sl(a)	Vil	Yk(a)	KCAM	McC(a)	McC(b)	In(a)	In(b)
[+]	[+]	[-]	[+]	[-]	[+]	[+]	[+]	[-]	[-]	[+]

Ok(a)	MER2	JMHK	JMHL	FORS
[+]	[+]	[+]	[+]	[-]

PLATELET ANTIGENS

1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6bw	7bw	8bw	9bw
[+]	[+]	[+]	[-]	[+]	[-]	[+]	[-]	[+]	[-]	[-]	[-]	[-]	[-]

10bw	11bw	12bw	13bw	14bw	15a	15b	16bw	17bw	18bw	19bw	20bw	21bw	22bw
[-]	[-]	[-]	[-]	[-]	[+]	[+]	[-]	[-]	[-]	[-]	[-]	[-]	[-]

23bw	24bw	25bw	26bw	27bw
[-]	[-]	[-]	[-]	[-]

Key: [+] presence of antigen predicted by genotyping; + presence of antigen predicted by genotyping and confirmed by serology; +* presence of antigen detected by serology, genotype prediction not available; [+w] weak presence of antigen predicted by genotyping; +w weak presence of antigen predicted by genotyping and confirmed by serology; +w* weak presence of antigen detected by serology, genotype prediction not available; [−] absence of antigen predicted by genotyping; - absence of antigen predicted by genotyping and confirmed by serology, -* absence of antigen detected by serology, genotype prediction not available; NC indicates no sequencing coverage, Dis indicates discordant. Rare (less than 5% population frequency) presence or absence of antigen is indicated in **red**.

METHODOLOGY

Genomic sequencing is performed using next generation sequencing on the Illumina HiSeq platform. Genomes are sequenced to at least 30X mean coverage and a minimum of 95% of bases are sequenced to at least 8X coverage. Paired-end 100bp reads are aligned to the NCBI reference sequence (GRCh37) using the Burrows-Wheeler Aligner (BWA), and variant calls are made using the Genomic Analysis Tool Kit (GATK). Variants are subsequently filtered to identify: (1) variants classified as disease causing in public databases; (2) nonsense, frameshift, and +/-1,2 splice-site variants that are novel or have a minor allele frequency <1% in European American or African American chromosomes from the NHLBI Exome Sequencing Project (http://evs.gs.washington.edu/EVS/); and (3) rs11212617 (C11orf65; metformin), rs12248560 (CYP2C19; clopidogrel), rs4244285 (CYP2C19; clopidogrel), rs4986893 (CYP2C19; clopidogrel), rs28399504 (CYP2C19; clopidogrel), rs41291556 (CYP2C19; clopidogrel), rs72552267 (CYP2C19; clopidogrel), rs72558186 (CYP2C19; clopidogrel), rs56337013 (CYP2C19; clopidogrel), rs1057910 (CYP2C9; warfarin), rs1799853 (CYP2C9; warfarin), rs7900194 (CYP2C9; warfarin), rs9332131 (CYP2C9; warfarin), rs28371685 (CYP2C9; warfarin), rs28371686 (CYP2C9; warfarin), rs9923231 (VKORC1; warfarin), rs4149056 (SLCO1B1; simvastatin), and rs1045642 (ABCB1; digoxin). The evidence for phenotype-causality is then evaluated for each variant resulting from the filtering strategies above and variants are classified according to LMM criteria (http://pcpgm.partners.org/LMM). Only those variants with evidence for causing highly penetrant disease or contributing to disease in a recessive manner are reported. Before reporting, all variants are confirmed via Sanger sequencing or another orthogonal technology. The initial sequencing component of this test was performed by the Illumina Clinical Services Laboratory (San Diego, CA CLIA# 05D1092911) and the alignment, variant calling, data filtering, Sanger confirmation and interpretation were performed by the Laboratory for Molecular Medicine at the Partners Healthcare Center for Personalized Genetic Medicine (Cambridge, MA

CLIA#22D1005307). This test has not been cleared or approved U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

LIMITATIONS

It should be noted that this test does not sequence all bases in a human genome and not all variants have been identified or interpreted. Triplet repeat expansions, translocations and large copy number events are currently not reliably detected by genome sequencing. Furthermore, not all disease-associated genes have been identified and the clinical significance of variation in many genes is not well understood. It is recommended that genomic sequencing data is periodically reinterpreted, especially when new symptoms arise.

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