Published in final edited form as:

Mayo Clin Proc. 2018 November ; 93(11): 1600–1610. doi:10.1016/j.mayocp.2018.06.026.

The Return of Actionable Variants Empirical (RAVE) Study, a Mayo Clinic Genomic Medicine Implementation Study: Design and Initial Results

Iftikhar J. Kullo, MD, **Janet Olson, PhD**, **Xiao Fan, PhD**, **Merin Jose, MBBS**, **Maya Safarova, MD, PhD**, **Carmen Radecki Breitkopf, PhD**, **Erin Winkler, CGC**, **David C. Kochan, BS**, **Sara Snipes, BS**, **Joel E. Pacyna, MA**, **Meaghan Carney, BA**, **Christopher G. Chute, MD, DrPH**, **Jyoti Gupta, MS**, **Sheethal Jose, BS**, **Eric Venner, PhD**, **Mullai Murugan, PhD**, **Yunyun Jiang, PhD**, **Magdi Zordok, MBBS**, **Medhat Farwati, MD**, **Maraisha Philogene, BA**, **Erica Smith, BS**, **Gabriel Q. Shaibi, PhD**, **Pedro Caraballo, MD**, **Robert Freimuth, PhD**, **Noralane M. Lindor, MD**, **Richard Sharp, PhD**, **Stephen N. Thibodeau, PhD**

Department of Cardiovascular Medicine (I.J.K., X.F., M.J., M.S., S.S., D.C.K., M.C., M.Z., M.F., M.P., E.S.), Department of Health Sciences Research, Biomedical Ethics Program (J.O., C.R.B., J.E.P., R.F., R.S.), Center for Individualized Medicine-Genomics (E.W.), Department of Internal Medicine (P.C.), and Department of Laboratory Medicine and Pathology (S.N.T.), Mayo Clinic, Rochester, MN; Johns Hopkins University, Schools of Medicine, Public Health, and Nursing, Baltimore, MD (C.G.C.); National Human Genome Research Institute, Bethesda, MD (J.G., S.J.); Baylor College of Medicine Human Genome Sequencing Center, Houston, TX (E.V., M.M., Y.J.); Center for Health Promotion and Disease Prevention, Arizona State University, Phoenix, AZ (G.Q.S.); and Department of Health Sciences Research, Mayo Clinic, Scottsdale, AZ (N.M.L.)

Abstract

Objectives: To identify clinically actionable genetic variants from targeted sequencing of 68 disease-related genes, estimate their penetrance, and assess the impact of disclosing results to participants and providers.

Patients and Methods: The Return of Actionable Variants Empirical (RAVE) Study investigates outcomes following the return of pathogenic/likely pathogenic (P/LP) variants in 68 disease-related genes. The study was initiated in December 2016 and is ongoing. Targeted sequencing was performed in 2533 individuals with hyperlipidemia or colon polyps. The electronic health records (EHRs) of participants carrying P/LP variants in 36 cardiovascular disease (CVD) genes were manually reviewed to ascertain the presence of relevant traits. Clinical outcomes, health care utilization, family communication, and ethical and psychosocial

Correspondence: Address to Iftikhar J. Kullo, MD, Department of Cardiovascular Medicine and the Gonda Vascular Center, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Kullo.Iftikhar@mayo.edu).

SUPPLEMENTAL ONLINE MATERIAL

Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Potential Competing Interests: The authors report no competing interests.

implications of disclosure of genomic results are being assessed by surveys, telephone interviews, and EHR review.

Results: Of 29,208 variants in the 68 genes, 1915 were rare (frequency <1%) and putatively functional, and 102 of these (60 in 36 CVD genes) were labeled P/LP based on the American College of Medical Genetics and Genomics framework. Manual review of the EHRs of participants (n=73 with P/LP variants in CVD genes) revealed that 33 had the expected trait(s); however, only 6 of 45 participants with non–familial hypercholesterolemia (FH) P/LP variants had the expected traits.

Conclusion: Expected traits were present in 13% of participants with P/LP variants in non-FH CVD genes, suggesting low penetrance; this estimate may change with additional testing performed as part of the clinical evaluation. Ongoing analyses of the RAVE Study will inform best practices for genomic medicine.

Although genome sequencing holds great promise for preventive and precision medicine, there are several barriers to its use in clinical practice, including difficulty in reliably identifying pathogenic/likely pathogenic (P/LP) variants, lack of estimates of penetrance of such variants, and the unclear consequences of disclosing these variants to patients and providers. To address these challenges we designed the Return of Actionable Variants Empirical (RAVE) Study as part of phase 3 of the electronic MEdical Records and GEnomics (eMERGE) network.[1] We aimed to (1) identify P/LP variants in 68 disease-related genes, (2) assess penetrance of P/LP variants, (3) develop methods for placing genomic results in the electronic health record (EHR) with linkage to clinical decision support (CDS),[2] and (4) investigate whether disclosing clinically actionable findings from targeted sequencing of 68 disease-related genes leads to changes in medical decisions, health care utilization, psychosocial responses, and family communication about health and disease.

Participants were ascertained based on the presence of hypercholesterolemia or colon polyps to enrich for familial hypercholesterolemia (FH) and hereditary colorectal cancer (CRC; Lynch syndrome), 2 diseases labeled by the Office of Public Health Genomics, Centers for Disease Control and Prevention[3] as tier 1 genomic applications due to the potential for positive effect on public health based on available evidence-based guidelines and recommendations.[4] Familial hypercholesterolemia is the most commonly inherited cause of premature coronary heart disease, with an estimated 15 to 20 million prevalent cases around the world.[5] Approximately 1 in 250 people in the United States, or an estimated 1.3 million, have FH due to a mutation in *LDLR, APOB*, or *PCSK9*.[6,7] Unfortunately, FH remains significantly under-diagnosed even though effective treatment is available.[5,8] Colorectal cancer is the third most common invasive cancer diagnosed in both men and women and the second leading cause of cancer-related deaths in the United States. The lifetime risk of CRC for Americans is approximately 5%, and the American Cancer Society projected 135,430 new cases of hereditary CRC in the United States in 2017.[9]

Herein we describe the design and initial results of the RAVE Study. Participants were recruited from Mayo Clinic biobanks in Rochester, Minnesota, and Phoenix, Arizona. This report focuses on the protocol for the Rochester participants. The Phoenix participants

included Mexican Americans attending a Federally Qualified Health Center and study processes and timelines were tailored differently for that population.[10]

PARTICIPANTS AND METHODS

Overall Study Design

We recruited 3030 participants from Mayo Clinic biobanks in Rochester who had hypercholesterolemia, colon polyps, or both, thereby enriching for FH and monogenic causes of CRC (Figure 1). The DNA from 2538 samples was sent to Baylor College of Medicine Human Genome Sequencing Center (BCM-HGSC), a Central Laboratory Improvement Amendment–certified facility, for targeted sequencing using the eMERGEseq panel of 109 genes and 1706 single nucleotide variants (SNVs).[11] The disease-related genes (n=68) included those on the American College of Medical Genetics and Genomics (ACMG)[12] list as well as 14 additional genes deemed actionable by eMERGE investigators. Three genes associated with FH (*LDLR, APOB*, and *PCSK9*) and 10 with CRC (*APC, BMPR1A, SMAD4, PMS2, MLH1, MSH2, MSH6, POLD1, POLE*, and biallelic *MUTYH* variants) were on the eMERGEseq panel. Once sequencing data were available, actionable variants in cardiovascular disease (CVD) genes were identified as described later herein. In parallel, the BCM-HGSC laboratory independently identified actionable variants and issued clinical reports that were reviewed by a variant curation group at Mayo Clinic before disclosure to participants. We developed EHR-based CDS for actionable variants in FH/CRC genes to facilitate decision making at the point of care. Estimates of penetrance of the P/LP variants in CVD genes were based on information available in the EHR at the time of return of results. Subsequent participant outcomes, costs and utilization of health care resources, and behavioral and psychosocial outcomes are being assessed at several time points after disclosure of genetic results.

Participant Recruitment

Participants were selected from 2 biobanks at Mayo Clinic—the Mayo Clinic Biobank[13] and the Vascular Diseases Biorepository[14]—based on the presence of hyperlipidemia or colon polyps in the EHR. We screened 51,525 Mayo Clinic Biobank and 10,000 Vascular Diseases Biorepository participants to identify individuals who met the following eligibility criteria: (1) residents of southeast Minnesota who were alive and aged 18 to 70 years; (2) low-density lipoprotein cholesterol (LDL-C) level greater than 155 mg/dL (to convert to mmol/L, multiply by 0.0259) (>116 mg/dL on lipid-lowering therapy) in the absence of a known cause of secondary hyperlipidemia, or the presence of colon polyps on colonoscopy; and (3) no cognitive impairment or dementia that would compromise ability to provide written informed consent. Recruitment was conducted in waves and used mailed recruitment packets consisting of a study brochure, a written informed consent form, a baseline psychosocial questionnaire, and a return postage-paid envelope.

Human Subjects Protection

This study was approved by the Mayo Clinic Institutional Review Board. The consent process incorporated input from the Community Advisory Board for the Mayo Clinic Biobank and was consistent with national standards and best practices. The membership of

the Community Advisory Board reflects the sociocultural characteristics of the Mayo Clinic Biobank population and provided early and regular feedback regarding Mayo Clinic Biobank procedures. Although consent was obtained via postal mail, counseling and telephone support was available to participants who had additional questions about the RAVE Study.[15] Those who elected to participate returned the informed consent document with their signature and an accompanying questionnaire. To participate, individuals had to agree to receive primary findings (ie, those related to hypercholesterolemia or colon cancer risk) but could opt out of receiving secondary findings. Participants could withdraw from the study at any time before the disclosure of results. Once disclosed, results were also placed in the EHR. Additional details of informed consent are described elsewhere.[1]

Central Laboratory Improvement Amendment Sequencing and Genotyping

DNA was sent to the BCM-HGSC laboratory for targeted sequencing using the eMERGEseq panel. The 68 actionable genes and 14 actionable SNVs tested are listed in Supplemental Tables 1 and 2 (available online at http://www.mayoclinicproceedings.org) (Figure 2). The postcapture library DNA was subjected to sequence analysis using the HiSeq platform (Illumina Inc) for 100–base pair paired-end reads. The following quality control metrics of the sequencing data were generally achieved: greater than 70% of reads aligned to target, greater than 99% target bases covered at greater than 20×, greater than 98% target bases covered at greater than 40×, average coverage of target bases greater than 200×; single nucleotide polymorphism (SNP) concordance to SNPtrace genotype array greater than 99%. As a quality control measure, each individual's DNA was also analyzed by an SNP array (SNPtrace panel; Fluidigm Corp).[16] The SNP data were compared with the sequencing panel data to ensure correct sample identification and to assess sequencing quality. All genes had 100% of targeted bases sequenced to redundant coverage of 20 or greater with the following exceptions: *APOB* (99.39%), *CAC-NA1B* (97.55%), *COL5A1* (98.03%), *KCNQ1* (97.02%), *RYR1* (98.79%), and *TGFBR1* (93.56%).

Variant Calling and Annotation—Data were analyzed by the Mercury 3.4 pipeline (Supplemental Material, available online at http://www.mayoclinicproceedings.org).[17] The output data from HiSeq were converted from BCL files to FASTQ files by Illumina bcl2fastq software, version 1.8.3, and mapped to the hg19 human genome reference by the BWA program.[18] The variant calls were performed using Atlas-SNP and Atlas-indel. Variants passing quality control (n=42,054) were mapped to gene loci using SeattleSeqAnnotation138. Using the latest guidelines from the ACMG, we binned variants into 5 categories: P, LP, variant of uncertain significance, likely benign, or benign.[19,20] We used InterVar,[21] a tool that assesses genetic variants based on 18 of 28 rules recommended by ACMG guidelines, and collected additional evidence from ClinVar,[22] the Human Gene Mutation Database,[23] the Genome Aggregation Database,[24] and recent literature. We identified P/LP variants and variants of uncertain significance in the 68 actionable genes that had a frequency less than 0.01 and at least 2 pieces of evidence suggesting pathogenicity.

Return of Results

The BCM-HGSC laboratory independently reported P/LP variants in the 68 medically actionable genes and genotypes at 14 medically actionable SNVs (Supplemental Tables 1

and 2). For autosomal recessive disorders, only homozygous or biallelic variants were returned. Actionable variants were confirmed by Sanger sequencing by the laboratory. Each clinical report from the BCM-HGSC laboratory was reviewed by a multidisciplinary variant curation team at Mayo Clinic that included clinical molecular geneticists, a cardiovascular genomics expert, a genetic counselor, and a medical geneticist. The variant curation process included manual review of the EHR of participants with P/LP variants in CVD genes and review of the literature and relevant databases and data sources.

Participants with actionable variants in any of the 68 genes were sent a letter stating that results were available and that the participant should contact the RAVE Study team to schedule an appointment with the RAVE Study genetic counselor to discuss his or her sequencing results. A genetic counselor disclosed the results, assisted participants with interpreting and understanding their results, discussed potential implications for medical management and risks to family members, and arranged for referrals to an appropriate specialist or to medical genetics to facilitate further clinical management. If the participant was geographically distant, he/she was offered the opportunity to receive results from the genetic counselor over the telephone. With participant permission, genetic counselor encounters were videotaped for future qualitative analysis of the interaction. Before and after the genetic counseling appointment, participants were asked to complete surveys addressing intentions to share genetic information with family members. If a participant with an actionable variant did not respond after 3 contacts, the information was placed in the EHR 6 months after the initial contact.

Participants without actionable variants were notified by postal mail and offered the opportunity to speak with a genetic counselor by telephone if they had questions. Results were placed in the EHR, and an electronic alert was sent to the primary care provider. In addition to the genetic counselor support line specifically set up for this study, investigators were available to answer any questions that primary care providers may have about sequencing results.

Penetrance

We assessed the penetrance of P/LP variants by EHR review. For this report we limited the estimates of prevalence to P/LP variants in genes associated with cardiovascular phenotypes. In addition, we reviewed the EHRs of participants with actionable genotypes at SNV sites. Trained abstractors reviewed EHRs of carriers of P/LP variants/actionable genotypes for the presence of expected traits.

EHR Integration and CDS

Results from the BCM-HGSC laboratory were transferred to DNAnexus, a secure cloud-based platform (DNAnexus Inc). Two different formats of each report were provided: first, a PDF file that was scanned into the EHR, and second, structured data necessary to integrate CDS into the clinical workflow. The structured format included discrete results and associated variant interpretations and used an Extensible Markup Language (XML) schema developed by the eMERGE network and based on the HL7 version 2 (Health Level Seven International) genetic test results standard.[25] After disclosure, PDF reports were scanned

into the EHR and a results letter, generated from the structured data, was sent to primary care providers via the EHR inbox. Summary results were placed in patient portals, and participants were encouraged to share results with at-risk family members. To increase awareness of the availability of genomic test results in the EHR and to assist primary care providers in interpreting the results, we developed CDS for actionable variants in FH and CRC genes. The CDS included links to relevant educational content about actionable variants, including evidence-based guidelines for additional diagnostic interventions and treatment (Ask-MayoExpert) recommendations.

Outcomes

The RAVE Study is examining 3 types of outcomes consequent to return of genomic results (Tables 1 and 2): (1) clinical outcomes, health care utilization, and cascade screening in family members; (2) psychosocial outcomes, and (3) sharing of genetic results with family members.

Clinical Outcomes and Health Care Utilization.—The EHR is periodically reviewed after disclosure of results to assess whether the disclosure led to initiation of medical therapy or ordering of additional tests. Process outcomes associated with the placement of actionable variants in the EHR (Table 1), including new diagnoses, initiation of statins, stress testing, colonoscopy, etc, and potential costs associated with tests and therapies are assessed. Outcomes of family communication, including the number of family members informed, their pursuit of genetic testing, and the broader impact of results sharing on family relationships, are examined. We initiated familial case finding for individuals with actionable variants in FH genes and assessed effectiveness using defined outcomes (eg, number of relatives contacted, number of relatives tested, number of cases identified).

Psychosocial Outcomes.—Participants with clinically actionable variants completed questionnaires immediately before and after receiving genetic results and 6 months after the genetic counseling session. Questionnaires include psychosocial measures of perceived benefits and harms as they relate to sharing genetic results with family members, perceived social support, and emotional experiences related to learning genetic results (Table 2). Participants without any actionable variants were asked to complete a post-results questionnaire assessing their understanding of the results they received and their overall experiences with genetic testing. In-depth interviews were conducted in a subgroup of 100 such participants.

Familial Sharing.—We assessed whether, how, when, and why individuals shared the results of genomic testing with family members, as well as the impact of their sharing genomic risk information.[34] We also assessed participants' intentions to share genomic results with family or others both before and after receiving actionable genomic results.[32] As part of the post-results survey, variables such as perceived vulnerability and receptivity of family members were measured because these will likely inform choices regarding the sharing of results and with whom. We evaluated motivations for sharing or not sharing results,[35] the content of what was shared, and the method of communication used.

Data Management and Statistical Methods

All study data were placed in REDCap (Research Electronic Data Capture)[36] and exported to an SAS database (SAS Institute Inc) for analyses. Questionnaire data were processed by Mayo Clinic's Survey Research Center using standardized tracking protocols and double data entry procedures. Usual initial evaluations of the data included general inspection of the raw data, plots of summary scores, examination of outliers and group distributions, and evaluation of missing data. The frequencies (percentages) of categorical factors were compared using either the χ^2 test or the Fisher exact test.

RESULTS

Of 51,525 individuals enrolled in the Mayo Clinic BioBank and 10,000 in the Vascular Diseases Biorepository, 5270 (8.6%) met the eligibility criteria for the study and were invited to participate (Figure 1). The mean age of the source population was 57 years, and 2898 (55%) were women. We received signed informed consent from 3030 participants (57.5%), of whom 198 (6.5%) were from the Mayo Vascular Diseases Biorepository. However, only 2538 of these individuals (83.8%) underwent sequencing due to prespecified limitations on the number of participants who could undergo sequencing. Participants were prioritized for sequencing based on their LDL-C levels and presence of colon polyps. Three samples could not be sequenced because of low DNA concentration, and 2 participants withdrew from the study after their DNA was sequenced. The characteristics of the 2533 RAVE Study participants with sequencing data are presented in Table 3. Of participants with hypercholesterolemia, 640 (25.3%) had LDL-C levels greater than or equal to 190 mg/dL (or >142 mg/dL on a statin).

Variant Annotation

Of 29,208 variants in the 68 genes, 1915 were rare (frequency <1%) and putatively functional, and of these, 102 (60 in 36 CVD genes) were labeled P/LP based on the ACMG framework. We identified P/LP variants in 4.0% of participants using the framework described in the "Participants and Methods" section. In total there were 60 P/LP variants in CVD genes, 32 in cancer genes, and 10 related to additional traits (Supplemental Table 1). Three actionable genotypes at the 14 SNVs were identified in 22 patients (Supplemental Table 2).

Penetrance of P/LP Variants/Actionable Genotypes in CVD Genes

Nearly half of the participants (45.2%) with P/LP variants or actionable genotypes related to CVD had the expected traits in the EHR (Tables 4 and 5). This number was inflated by the high penetrance of P/LP variants in FH genes. Half of the P/LP variant carriers in FH genes had a Dutch Lipid Clinic Network score of at least 6 (commonly used threshold to define the FH phenotype),[37] but nearly all had an LDL-C level greater than 190 mg/dL. The penetrance of the non-FH CVD gene variants was low at 13%. Only 18% of the participants who carried P/LP variants for diabetes (*HNFIA, HNFIB*) and 25% of those with long QT syndrome variants (*SCN5A, KCNQ1, KCNH2*) expressed related traits; 1 in 8 participants with P/LP variants for arterial aneurysmal disease and 1 in 9 participants with P/LP variants for hypertrophic cardiomyopathy had the expected traits. However, an electrocardiography

report was not available in the EHR for 1 patient with a P/LP variant in arrhythmia genes, and an echocardiogram report was not available in 44% of the participants who had P/LP variants in cardiomyopathy genes. History of venous thromboembolism was noted in 1 of 5 participants homozygous for the factor V Leiden variant. Relevant traits were present in nearly one-third of participants homozygous for the c.845G>A variant in *HFE* that is associated with hemochromatosis (Table 5).

DISCUSSION

As institutions prepare to implement genomic medicine, persistent challenges relate to a lack of knowledge about (1) how to assign pathogenicity to rare putatively functional variants identified in individuals not preselected for a phenotype, ie, the interpretive gap; (2) penetrance of P/LP variants in disease-related genes; (3) how best to integrate genomic sequencing results into the EHR with linkage to CDS; (4) the consequences of disclosing actionable results from genome sequencing on health care utilization and cost; (5) the psychosocial impact of returning genome sequencing results; and (6) best practices for promoting the sharing of results with at-risk family members. The goal of the RAVE Study is to attempt to address these gaps in knowledge and thereby facilitate implementation of genomic medicine.

We used a framework based on ACMG guidelines to identify P/LP variants. The prevalence (4.0%) of P/LP variants in the RAVE Study cohort was somewhat higher than the 1% to 3.4% prevalence reported in other genome sequencing studies.[38–40] This finding is likely due to ascertainment of participants based on hyperlipidemia and colon polyps. When we used “expected” prevalence of FH and Lynch syndromes, the proportion with P/LP variants dropped to 3.2%. Because the sequencing laboratory independently identified P/LP variants, we will be able to compare interpretations and provide insights into any discordance in variant stratification. We will deposit variant/phenotype associations relevant to the eMERGEseq panel in ClinVar,[41] an important public repository of variant annotations. Such data from the other genomic medicine projects being conducted across the eMERGE network, most of which included participants who did not have an indication for testing, will contribute to efforts aimed at closing the interpretive gap.[42,43]

Variants predicted to disrupt the function of critical genes seem to be more common than the diseases with which they are associated. In the RAVE Study cohort, we detected 102 P/LP variants that could have phenotypic consequences and assessed penetrance of 60 P/LP variants in CVD genes. Reliable information on the penetrance of P/LP variants is necessary to guide patients, their families, and their clinicians on the actions to be taken once these are detected. Linking genome sequence data to the broad range of phenotypes in the EHR is one approach to obtain insights into the penetrance and expression of presumed P/LP genetic variants, with the caveat that absence of clinical features that are associated with a particular P/LP variant may be due to truly reduced penetrance or absence of complete phenotyping, eg, lack of electrocardiograms or echocardiograms. Although expected traits were present in 45% of participants with P/LP variants, this proportion was inflated by the high penetrance of P/LP variants in FH genes. The penetrance of P/LP variants in non-FH genes was low at 13%.

An important goal of the RAVE Study is to integrate genomic sequence results into the EHR with linkage to CDS, thereby facilitating delivery of relevant information to clinicians at the point of care. Availability of actionable genomic results in the EHR triggers an inbox message with specific recommendations for use of the results in further evaluation, diagnosis, and treatment. Such CDS also helps clinicians answer questions patients may have and facilitates ongoing care of patients with actionable genomic data. These CDS interventions are being developed, implemented, and monitored by a multidisciplinary group of experts that includes clinicians, informaticians, and information technology experts with extensive knowledge of CDS integration.

After actionable RAVE Study results are returned to participants, additional testing is likely to be performed in the clinical setting for further evaluation and management. Analyzing these outcomes will provide insights into health care utilization and potential costs after disclosure of genome sequence results to individuals and their care providers. Such information is important for the eventual incorporation of genome sequencing into clinical practice, particularly to inform payers. In addition to medical outcomes, the potential positive and negative impact of disclosure of genetic risk on participants is being assessed. We included measures to better understand ways in which disclosure of genetic testing results affects the individual participants. We assessed participants' understanding of genomic results and their views about placing genomic data in the EHR. In addition, we assessed perception of genetic risk and perceived value of genetic risk information. By examining these psychosocial outcomes over an extended timeframe, we will obtain critical perspectives on the delivery and receipt of genetic risk information in real-world settings that anticipate greater application of genomic testing in the future.

It is important to know whether clinically actionable findings relevant to FH and other monogenic disorders are shared with others, particularly family members. A unique aspect of the RAVE Study is that family members of probands with FH are directly contacted with permission from the study participant who provides the study team with contact information of first-degree biological relatives. For other actionable variants, probands are encouraged by the genetic counselor to inform at-risk family members.

CONCLUSION

We describe the design and initial results for the RAVE Study, which identified actionable genomic variants from targeted sequencing of 68 disease-related genes and 14 SNVs in 4.0% of the study cohort. There were 60 P/LP variants in CVD-related genes carried by 73 participants, and review of the EHR found expected traits in 45.2% of P/LP variant carriers. However, this proportion was inflated by the high penetrance of P/LP variants in FH genes as the penetrance of P/LP variants in non-FH genes was low at 13%. Additional work is needed to determine how much of this is due to incomplete phenotype data vs truly reduced penetrance. This ongoing study will inform best practices for implementing genomic sequencing in the clinical setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank participants of the Mayo Clinic Biobank and Vascular Diseases Biorepository who enrolled in the study. We acknowledge Benjamin Welkie, Yijing Chen, Kelly Lyke, and Matthew Hathcock for Mayo Clinic Biobank and Vascular Diseases Biorepository database searches; Melody Powers for sample accession; Donna Muzny, Yaping Yang, and Richard Gibbs from BCM-HGSC for facilitating targeted sequencing of the eMERGEseq panel in the study participants; and Luanne Wussow for help with manuscript preparation.

Grant Support: This study was funded as part of the National Human Genome Research Institute–supported electronic MEdical Records and GEnomics (eMERGE) network (grant U0IHG006379) and the Mayo Center for Individualized Medicine. The Mayo Clinic Biobank is funded by the Mayo Clinic Center for Individualized Medicine, and the Vascular Diseases Biorepository is funded by grant HL-75794 from the National Heart, Lung, and Blood Institute and a Marriot Award for Individualized Medicine. Dr Kullo is additionally funded by grants HLI35879 and HLI370I0 from the NHLBI.

Abbreviations and Acronyms:

ACMG	American College of Medical Genetics and Genomics
BCM-HGSC	Baylor College of Medicine Human Genome Sequencing Center
CDS	clinical decision support
CRC	colorectal cancer
CVD	cardiovascular disease
EHR	electronic health record
eMERGE	electronic MEdical Records and GEnomics
FH	familial hypercholesterolemia
LDL-C	low-density lipoprotein cholesterol
P/LP	pathogenic/likely pathogenic
RAVE	Return of Actionable Variants Empirical
SNP	single nucleotide polymorphism
SNV	single nucleotide variant

REFERENCES

1. Fossey R, Kochan D, Winkler E, et al. Ethical considerations related to return of results from genomic medicine projects: the eMERGE Network (Phase III) experience. J Pers Med. 2018;81.
2. Kullo IJ, Jarvik GP, Manolio TA, Williams MS, Roden DM. Leveraging the electronic health record to implement genomic medicine. Genet Med. 2013;15(4):270–271. [PubMed: 23018749]
3. Bowen MS, Kolor K, Dotson WD, Ned RM, Khoury MJ. Public health action in genomics is now needed beyond newborn screening. Public Health Genomics. 2012;15(6):327–334. [PubMed: 22986915]

4. Khoury MJ, Reyes M, Gwinn M, Feero WG. A genetic test registry: bringing credible and actionable data together. Public Health Genomics. 2010;13(6):360–361. [PubMed: 19940456]
5. Knowles JW, O'Brien EC, Greendale K, et al. Reducing the burden of disease and death from familial hypercholesterolemia: a call to action. Am Heart J. 2014;168(6):807–811. [PubMed: 25458642]
6. Safarova MS, Kullo IJ. My approach to the patient with familial hypercholesterolemia. Mayo Clin Proc. 2016;91(6):770–786. [PubMed: 27261867]
7. Safarova M, Liu H, Kullo IJ. Rapid identification of familial hypercholesterolemia from electronic health records: the SEARCH Study. J Clin Lipidol. 2016;10(5):1230–1239. [PubMed: 27678441]
8. Benn M, Watts GF, Tybjaerg-Hansen A, Nordestgaard BG. Familial hypercholesterolemia in the Danish general population: prevalence, coronary artery disease, and cholesterol-lowering medication. J Clin Endocrinol Metab. 2012;97(11):3956–3964. [PubMed: 22893714]
9. Siegel R, Miller KD, Fedewa SA, et al. Colorectal cancer statistics, 2017. CA Cancer J Clin. 2017;67(3):177–193. [PubMed: 28248415]
10. Shaibi G, Singh D, De Filippis E, et al. The Sangre PorSalud Biobank: facilitating genetic research in an underrepresented Latino community. Public Health Genomics. 2016;19(4):229–238. [PubMed: 27376364]
11. National Human Genome Resource Institute. Electronic Medical Records and Genomics (eMERGE) Network. https://www.genome.gov/27540473/electronic-medical-records-and-genomics-emerge-network/ Accessed October 5, 2018.
12. Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017;19(2):249–255. [PubMed: 27854360]
13. Olson JE, Ryu E, Johnson KJ, et al. The Mayo Clinic Biobank: a building block for individualized medicine. Mayo Clin Proc. 2013;88(9):952–962. [PubMed: 24001487]
14. Ye Z, Kalloo FS, Dalenberg AK, Kullo IJ. An electronic medical record-linked biorepository to identify novel biomarkers for atherosclerotic cardiovascular disease. Glob Cardiol Sci Pract. 2013;2013(1):82–90. [PubMed: 24689004]
15. Sutton EJ, Kullo IJ, Sharp RR. Making pretest genomic counseling optional: lessons from the RAVE study [published online February 1, 2018]. Genet Med. In press.
16. Liang-Chu MM, Yu M, Haverty PM, et al. Human biosample authentication using the high-throughput, cost-effective SNPtrace(TM) system. PLoS One 2015;10(2):e0116218. [PubMed: 25714623]
17. Reid JG, Carroll A, Veeraraghavan N, et al. Launching genomics into the cloud: deployment of Mercury, a next generation sequence analysis pipeline. BMC Bioinformatics. 2014;15:30. [PubMed: 24475911]
18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14): 1754–1760. [PubMed: 19451168]
19. Berg JS, Adams M, Nassar N, et al. An informatics approach to analyzing the incidentalome. Genet Med. 2013;15(1):36–44. [PubMed: 22995991]
20. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–424. [PubMed: 25741868]
21. Li Q, Wang K. InterVar Clinical interpretation of genetic variants by the 2015 ACMG-AMP Guidelines. Am J Hum Gen. 2017;100(2):267–280.
22. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42(database issue):D980–D985. [PubMed: 24234437]
23. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database: 2008 update. Genome Med. 2009;1(1):13. [PubMed: 19348700]

24. Perez de Isla L, Alonso R, Watts GF, et al. Attainment of LDL-cholesterol treatment goals in patients with familial hypercholesterolemia: 5-Year SAFEHEART Registry follow-up. J Am Coll Cardiol. 2016;67(11):1278–1285. [PubMed: 26988947]
25. Health Level Seven® International. HL7 Implementation Guide for CDA® Release 2: Genetic Testing Reports, Release 1. http://www.hl7.org/implement/standards/product_brief.cfm?product_id=292. Accessed October 5, 2018.
26. DeSalvo KB, Fan VS, McDonell MB, Fihn SD. Predicting mortality and healthcare utilization with a single question. Health Serv Res. 2005;40(4):1234–1246. [PubMed: 16033502]
27. O'Connor AM. Validation of a decisional conflict scale. Med Decis Making. 1995;15(1):25–30. [PubMed: 7898294]
28. Wallston KA, Wallston BS, DeVellis R. Development of the Multidimensional Health Locus of Control (MHLC) Scales. Health Educ Monogr. 1978;6(2):160–170. [PubMed: 689890]
29. Kaphingst KA, Facio FM, Cheng MR, et al. Effects of informed consent for individual genome sequencing on relevant knowledge. Clin Genet. 2012;82(5):408–415. [PubMed: 22694298]
30. Moos RH. Conceptual and empirical approaches to developing family-based assessment procedures: resolving the case of the Family Environment Scale. Fam Process. 1990;29(2):199–208; discussion 209–111. [PubMed: 2373215]
31. Mesters I, van den Borne H, McCormick L, Pruyn J, de Boer M, Imbos T. Openness to discuss cancer in the nuclear family: scale, development, and validation. Psychosom Med. 1997; 59(3): 269–279. [PubMed: 9178338]
32. Procidano ME, Heller K. Measures of perceived social support from friends and from family: three validation studies. Am J Community Psychol. 1983;11(1):1–24. [PubMed: 6837532]
33. Brehaut JC, O'Connor AM, Wood TJ, et al. Validation of a decision regret scale. Med Decis Making. 2003;23(4):281–292. [PubMed: 12926578]
34. Gaff CL, Clarke AJ, Atkinson P, et al. Process and outcome in communication of genetic information within families: a systematic review. Eur J Hum Genet. 2007;15(10):999–1011. [PubMed: 17609674]
35. Hughes C, Lerman C, Schwartz M, et al. All in the family: evaluation of the process and content of sisters' communication about BRCA1 and BRCA2 genetic test results. Am J Med Genet. 2002;107(2):143–150. [PubMed: 11807889]
36. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap): a metadata-driven methodology and workflow process for providing translational research informatics support. J Biomed Inform. 2009;42(2):377–381. [PubMed: 18929686]
37. Fouchier SW, Defesche JC, Umans-Eckenhausen MW, Kastelein JP. The molecular basis of familial hypercholesterolemia in The Netherlands. Hum Genet. 2001;109(6): 602–615. [PubMed: 11810272]
38. Dorschner MO, Amendola LM, Turner EH, et al. Actionable, pathogenic incidental findings in 1,000 participants' exomes. Am J Hum Genet. 2013;93(4):631–640. [PubMed: 24055113]
39. Amendola LM, Dorschner MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. Genome Res. 2015;25(3):305–315. [PubMed: 25637381]
40. Olfson E, Cottrell CE, Davidson NO, et al. Identification of medically actionable secondary findings in the 1000 Genomes. PLoS One. 2015;10(9):e0135193. [PubMed: 26332594]
41. Landrum MJ, Lee JM, Benson M, et al. ClinVar: public archive of interpretations of clinically relevant variants. Nucleic Acids Res. 2016;44(D1):D862–D868. [PubMed: 26582918]
42. Rehm HL, Berg JS, Brooks LD, et al. ClinGen: the Clinical Genome Resource. N Engl J Med. 2015;372(23):2235–2242. [PubMed: 26014595]
43. Cutting GR. Annotating DNA variants is the next major goal for human genetics. Am J Hum Genet. 2014;94(1):5–10. [PubMed: 24387988]

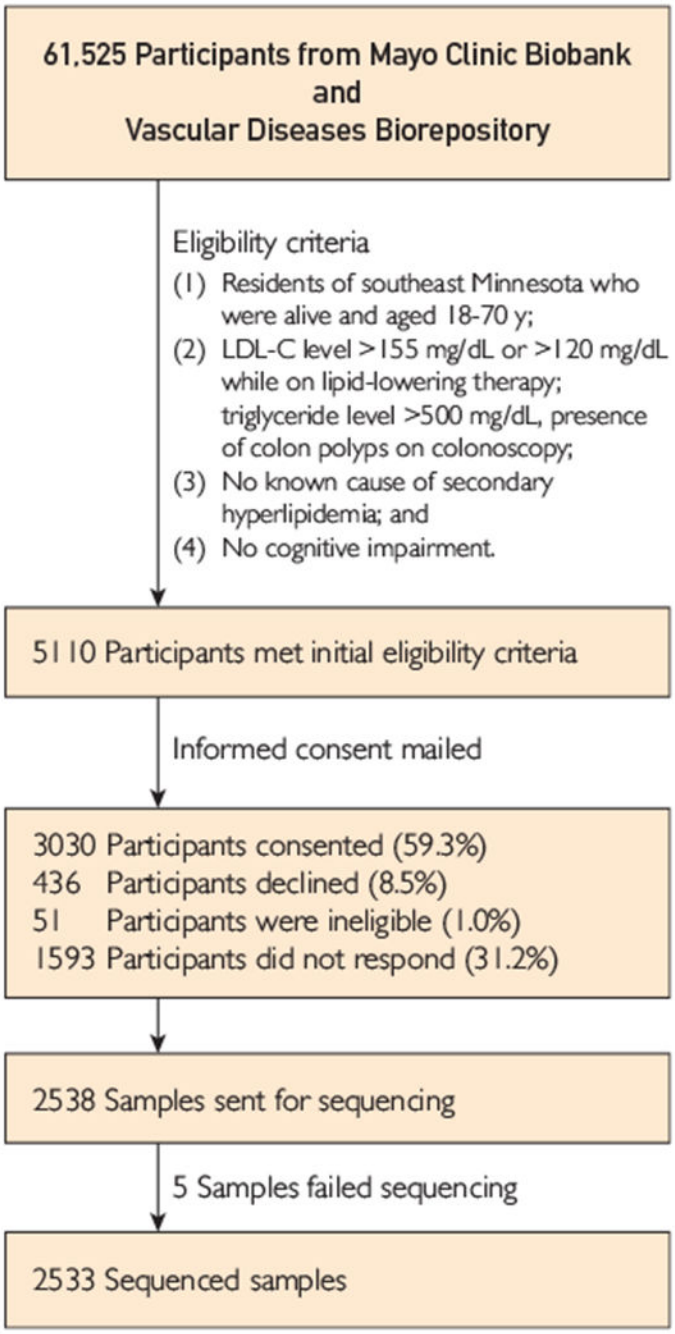

FIGURE 1.

Participant recruitment for the Return of Actionable Variants Empirical Study in Rochester, Minnesota. LDL-C = low-density lipoprotein cholesterol. To convert LDL-C values to mmol/L, multiply by 0.0259; triglyceride values to mmol/L, multiply by 0.0113.

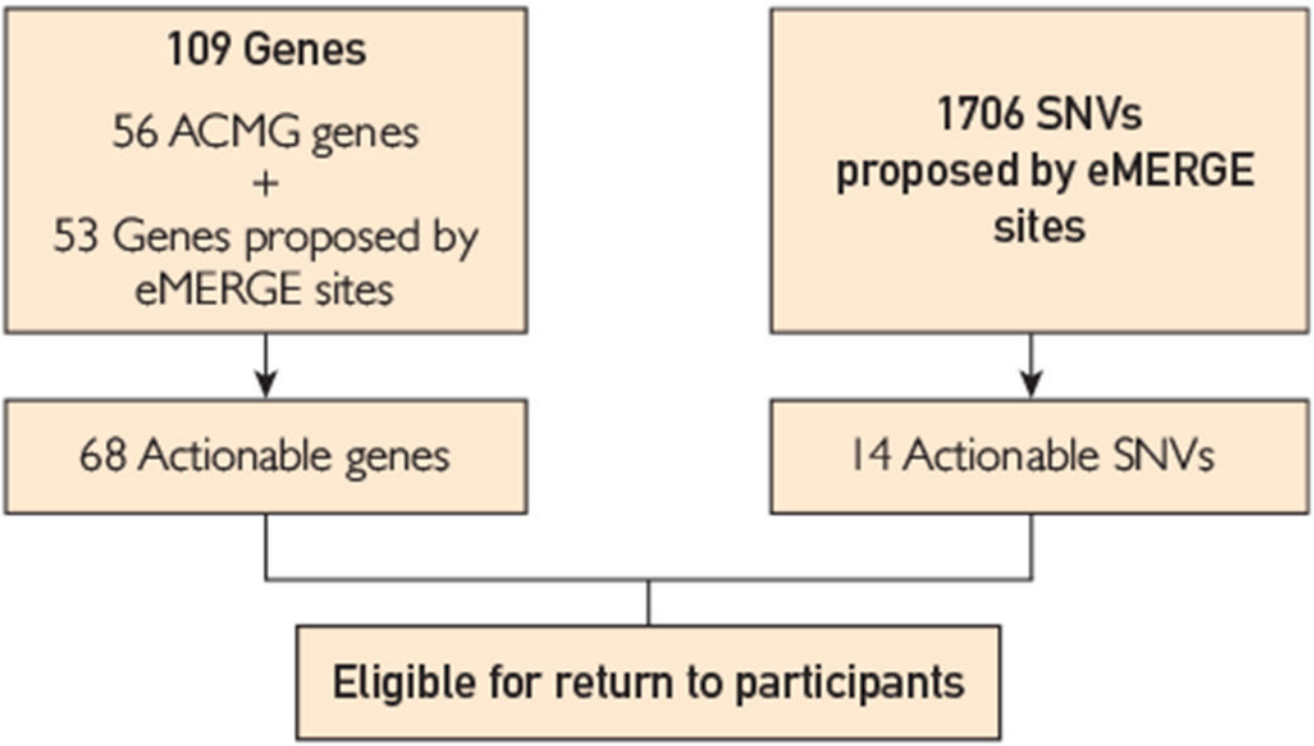

FIGURE 2.
Selection of actionable genes and single nucleotide variants (SNVs). ACMG = American College of Medical Genetics and Genomics; eMERGE = electronic MEdical Records and GEnomics.

TABLE 1.

Examples of Outcomes That Were Assessed After Disclosure of Results

Patient outcomes	New tests ordered: imaging studies to assess for subclinical coronary heart disease, stress testing, coronary angiography; colonoscopy; initiation of lipid-lowering therapy, polypectomy Specialty referrals
Impact on family	Number of family members who (1) are contacted, (2) undergo lipid screening/colonoscopy, (3) undergo genetic testing for familial hypercholesterolemia/colorectal cancer New cases detected
Electronic health record integration	Number of alerts fired Use of AskMayoExpert Patient portal accessed

TABLE 2.

Psychosocial Domains and Measures Assessed

Domain	All pretest (n=2895)	Participants receiving results in person	
		At ROR appointment (n ≈ 150)	6 mo post-ROR (n ≈ 150)
Demographic characteristics	●		
Self-reported health status[26]	●		
Previous experience with genetics	●		
Decisional conflict[27]	●		
Health locus of control[28]	●		
Expectations about results	●		
Self-efficacy in coping with results	●		
Concerns about results	●		
Knowledge about genetic sequencing[29]	●		
Orientation toward genomics	●		
Intent to share	●	●	●
Sharing behavior		●	●
Health expressiveness in families[30,31]		●	
Perceived social support[32]		●	
Consequences of sharing			●
Sharing content			●
Sharing barriers, facilitators			●
Decisional regret[33]			●

ROR = return of results.

TABLE 3.

Demographic and Clinical Characteristics of the 2533 Study Participants[a,b]

Characteristic	Value
Age (y), mean ± SD	62.2±7.7
Female sex (No. [%])	1453 (57.4)
White race (No. [%])	2465 (97.3)
Body mass index, mean ± SD	30.3±5.9
Electrocardiogram (No. [%])	2304 (91.0)
Echocardiogram (No. [%])	1092 (43.1)
Lipid profiles	
Median LDL-C (mg/dL), mean ± SD	166.8±39.3
Maximum LDL-C (mg/dL) , mean ± SD	170.3±41.0
Triglycerides (mg/dL), mean ± SD	263.9±265.7
Statin use (No. [%])	125 (4.9)
Maximum LDL-C ≥155 mg/dL (No. [%])	1752 (69.4)
Maximum LDL-C ≥190 mg/dL (No. [%])	640 (25.2)
Maximum triglyceride level > 150 mg/dL (No. [%])	1856 (73.3)
Maximum triglyceride level >500 mg/dL (No. [%])	198 (7.8)
Primary indication for testing	
Hyperlipidemia (No. [%])	1351 (53.3)
Colon polyp (No. [%])	599 (23.6)
Colon polyp + hyperlipidemia (No. [%])	583 (23.0)

[a] LDL-C = low-density lipoprotein cholesterol (in participants who were taking statins, the LDL-C level was imputed).

[b] SI conversion factors: To convert LDL-C values to mmol/L, multiply by 0.0259; to convert triglyceride values to mmol/L, multiply by 0.0113.

TABLE 4.

Pathogenic/Likely Pathogenic Variants Identified in the 36 Cardiovascular Disease–Related Genes and Prevalence of Expected Traits in Participants With These Variants

Disease	Genes	Variants (No.)	P/LP variants	P/LP carriers	Expected traits (%)		
					Yes	No	Uncertain[a]
Aortopathy/aneurysmal disease							
Ehlers-Danlos syndrome	*COL5A1, COL3A1*	1796, 523	1	1	0	0	1
Familial thoracic aortic aneurysm	*MYH11, ACTA2, MYLK*	1178, 133, 700	0	0	0	0	0
Loeys-Dietz syndrome	*TGFBR1, TGFBR2, SMAD3*	153, 228, 319	2	2	0	0	2
Marfan syndrome	*FBN1*	1024	3	5	1	0	4
Arrhythmia							
Catecholaminergic polymorphic ventricular tachycardia	*RYR2, TMEM43*	2591, 179	2	2	0	2	0
Long QT syndrome/Brugada syndrome	*SCN5A, KCNQ1, KCNH2, KCNE1, KCNJ2*	663, 760, 359, 125, 38	8	8	2	3	3
Cardiomyopathy							
Arrhythmogenic cardiomyopathy	*DSG2, DSC2, DSP, PKP2*	269, 280, 462, 311	6	6	0	1	5
Dilated cardiomyopathy	*LMNA*	208	1	1	0	1	0
Hypertrophic cardiomyopathy	*MYBPC3, MYH7, MYL2, ACTC1, TPM1, PRKAG2, TNN13, MYL3, TNNT2, GLA*	401, 407, 125, 81, 288, 617, 123, 69, 261, 132	7	9	1	3	5
Metabolic							
Familial hypercholesterolemia	*LDLR, APOB, PCSK9*	440, 574, 285	19	28	27	1	0
Diabetes	*HNF1A, HNF1B*	198, 209	11	11	2	9	0

[a]Uncertain includes those with a borderline abnormal trait or absence of key phenotype data (eg, electrocardiogram/echocardiogram).

TABLE 5.

Actionable Genotypes at the 14 SNVs

SNP	Gene	Molecular consequence	Associated disease	Disease category	Homozygous carriers (No.)	Participants with expected traits (%)
rs77931234	*ACADM*	c.985A>C (p.Lys329Gln)	Medium-chain acyl-CoA dehydrogenase deficiency	Inborn error of metabolism	1	0
rs6025	*F5*	c.1601G>A (p.Arg534Gln)	Factor V Leiden thrombophilia	Clotting disorder	5	20
rs1800562	*HFE*	c.845G>A (p.Cys282Tyr)	Hereditary hemochromatosis	Iron storage	16	31

SNP = single nucleotide polymorphism; SNV = single nucleotide variant.