HUMAN GENETICS

Aggregate penetrance of genomic variants for actionable disorders in European and African Americans

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In populations that have not been selected for family history of disease, it is unclear how commonly pathogenic variants (PVs) in disease-associated genes for rare Mendelian conditions are found and how often they are associated with clinical features of these conditions. We conducted independent, prospective analyses of participants in two community-based epidemiological studies to test the hypothesis that persons carrying PVs in any of 56 genes that lead to 24 dominantly inherited, actionable conditions are more likely to exhibit the clinical features of the corresponding diseases than those without PVs. Among 462 European American Framingham Heart Study (FHS) and 3223 African-American Jackson Heart Study (JHS) participants who were exome-sequenced, we identified and classified 642 and 4429 unique variants, respectively, in these 56 genes while blinded to clinical data. In the same participants, we ascertained related clinical features from the participants' clinical history of cancer and most recent echocardiograms, electrocardiograms, and lipid measurements, without knowledge of variant classification. PVs were found in 5 FHS (1.1%) and 31 JHS (1.0%) participants. Carriers of PVs were more likely than expected, on the basis of incidence in noncarriers, to have related clinical features in both FHS (80.0% versus 12.4%) and JHS (26.9% versus 5.4%), yielding standardized incidence ratios of 6.4 [95% confidence interval (CI), 1.7 to 16.5; $P = 7 \times 10^{-4}$) in FHS and 4.7 (95% CI, 1.9 to 9.7; $P = 3 \times 10^{-4}$) in JHS. Individuals unselected for family history who carry PVs in 56 genes for actionable conditions have an increased aggregated risk of developing clinical features associated with the corresponding diseases.

INTRODUCTION

Clinical exome and genome sequencing is increasingly applied in the practice of medicine, but many challenges remain (*1*–*5*). There has been extensive discussion of the merits of selection, ascertainment, and reporting of incidental or secondary findings that come to light during sequencing, especially when they may be of medical value to patients and their families (*6*–*8*). In 2013, the American College of Medical Genetics and Genomics (ACMG) recommended that laboratories providing clinical sequencing for any medical indication should search for and report pathogenic variants (PVs) in 56 genes (the ACMG56) that represent 24 rare Mendelian conditions for which there are recommended treatments (*7*, *9*). The ACMG recommendations have generated debate (*10*, *11*), in part because the risk associated with PVs in families with many affected relatives is not always the same for persons whose families are not enriched with affected relatives (*12*–*15*), and thus it has been unclear whether in the absence of a family history these variants truly represent an increase in risk.

In addition, large-scale biobanks are being sequenced for research purposes, and investigators are struggling with recent recommendations about whether and how to return genomic findings of potential medical importance to participants and their family members (*8*, *16*). Although the genes and variants to be returned are not specified in these recommendations, the ACMG56 have become a convenient starting point for these discussions and for the generation of lists of genes that are actually being reported to the participants. For example, information about the ACMG56, with some modifications, is being returned to the participants by some sites within the eMERGE III (Electronic Medical Records and Genomics Phase III) network (*17*), as well as the Geisinger MyCode research project (*18*). These research initiatives presage the use of genome sequencing for population screening and raise the issue of whether this is appropriate (*19*–*23*).

Estimating the association between specific PVs in individual genes for rare Mendelian conditions and clinical phenotypes in an unselected population is challenging because PVs associated with Mendelian diseases are rare, variants are difficult to categorize with confidence, even among experts (*24*), and clinical phenotypes unrelated to known genetic changes are relatively common in the general population. Because most of what we know in genetics has been from patients presenting to specialized clinics, and there have been few population-based estimates of variant penetrance, it is surprisingly difficult to demonstrate the seemingly straightforward idea that unselected persons carrying PVs in a group of genes known to be associated with disease are actually at increased risk for those conditions or to estimate the effect size of this increased risk. To address this, we devised an unbiased method to prospectively examine the aggregate association between PVs in a set

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of genes and clinical features among research participants from the Framingham Heart Study (FHS) and, separately, in the Jackson Heart Study (JHS), all of whom had been exome-sequenced and systematically phenotyped. In these two independent populations, we tested the hypothesis that participants with PVs in any of the ACMG56 genes were more likely to exhibit related clinical features (RCFs) than would be expected in participants without a PV.

RESULTS

Participant description

The FHS participants were drawn from the FHS Offspring cohort [n = 290, 35.7% female; mean age at enrollment, 36.8 (SD, 9.3) years] and the FHS Generation 3 cohort (n = 172, 35.7% female; mean age at enrollment, 44.5 (SD, 8.9) years]. All FHS participants were European American, and comprehensive clinical phenotypes were gleaned from the most recent clinical examination. The participants in the FHS cohort were followed for an average of 20.4 (SD, 14.3) years. Among the 3223 JHS participants, all were African-American, 62.4% were female, and mean age at enrollment was 55.6 (SD, 12.8) years. The JHS participants are being followed longitudinally, but comprehensive clinical phenotypes were only available from the baseline examination.

Overview of study design and phenotype characterization

We designed a procedure for unbiased analysis of the association between exome sequencing and phenotype data from 462 participants in the FHS and 3223 participants in the JHS. Family history was not considered in selecting participants for enrollment in either cohort, nor was it considered in the selection of participants for sequencing. Without knowledge of the phenotypes, we classified all variants in the ACMG56 genes, using a previously described multistep algorithm (*25*, *26*) and following recently revised ACMG variant classification recommendations (*27*). We tabulated RCFs (Table 1) corresponding to the 24 disease conditions associated with the ACMG56 from clinical records of the FHS and JHS participants while blinded to the results of sequencing.

Variant classification

By analyzing exomes, we identified 642 unique variants within the ACMG56 genes in the 462 FHS participants and 4429 unique variants in the 3223 JHS participants, and then subsequently classified these while blinded to the phenotype information (see Materials and Methods). Among FHS participants, we identified five PVs in five individuals (1.1% of the FHS cohort) and two likely PVs (LPVs) in two individuals (0.4% of the FHS cohort). Among JHS participants, we identified 19 PVs in 31 individuals (1.0% of the JHS cohort) and 4 LPVs in 4 individuals (0.1% of the JHS cohort). A description of the variants classified as PVs and LPVs, along with the presence or absence of clinical features suggestive of the corresponding diseases, is shown in Table 2 for the FHS participants and Table 3 for the JHS participants. A listing of the specific transcripts that corresponded to the sequenced genes is shown in table S1, and the evidence from the literature that we used to classify variants into PVs and LPVs from FHS and JHS participants is described in table S2. Variants of uncertain significance (VUSs) in the ACMG56 were identified in 146 FHS participants (31.6%) and 917 JHS participants (28.5%). The ACMG guidelines do not recommend returning VUSs, so these were excluded from further analysis.

Comparison of observed and expected proportions of phenotypes

To examine our primary hypothesis, we tested whether carriers of PVs in any of the ACMG56 genes were more likely to exhibit corresponding RCFs than would be expected in participants without a PV. We compared the observed number of RCFs in individuals with any PV to the expected number, assuming that the fraction of carriers of particular PVs who exhibited an RCF was equal to the fraction of noncarriers exhibiting those RCFs (see Materials and Methods). Of five FHS participants with PVs, four displayed an RCF, and this proportion (80%) was higher than expected (12.4%; one-sided binomial mixture test, $P = 7 \times 10^{-4}$). The standardized incidence ratio (SIR), which is the ratio of observed RCFs among those with PVs to the number expected on the basis of incidence rates among those without PVs, was 6.4 in the FHS [95%

Table 1. Prespecified clinical features among sequenced participants. SW, septal width; HR, heart rate; bpm, beats per minute; LV, left ventricle; RV, right ventricle; RA, right atrium; ECG, electrocardiogram.

Genes recommended by ACMG for return of secondary findings	RCFs for conditions associated with each gene
APC, BRCA1, BRCA2, MEN1, MLH1, MSH2, MSH6, MUTYH, NF2, PMS2, PTEN, RB1, RET, SDHD, SDHAF2, SDHB, SDHC, STK11, TP53, TSC1, TSC2, VHL, WT1	Previous diagnosis of cancer
COL3A1, FBN1, TGFBR1, TGFBR2, SMAD3, ACTA2, MYLK, MYH11	Echocardiography with aortic aneurysm (aortic root diameter >3.7 cm)
MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA	Echocardiography with posterior LV, posterior wall thickness, or SW >12 mm, or echocardiography with LV diastolic diameter >6 cm and fractional shortening <20%
RYR2	HR >100 bpm
PKP2, DSP, DSC2, TMEM43, DSG2	Echocardiography with abnormal RV or RA appearance
KCNQ1, KCNH2, SCN5A	ECG with prolonged QT interval [QT >450 mm (in women) and QT >460 mm (in men)]
LDLR, APOB, PCSK9	Elevation of LDL >190 mg/dl on no cholesterol medications or elevation of LDL >130 mg/dl on cholesterol medications
RYR1, CACNA1S	No phenotype data available

Table 2. Individuals with PVs and LPVs in the FHS. LOF, loss of function; F, female; M, male; PW, posterior wall width; LVD, left ventricular diameter; FS, fractional shortening; BC, breast cancer; OC, ovarian cancer; HCM, hypertrophic cardiomyopathy; HCL, hypercholesterolemia; ARVD/C, arrhythmogenic right ventricular dysplasia/cardiomyopathy.

Gene	Variant and amino acid	Amino acid	Summary of classification evidence*	Associated condition	RCFs	Age†	Sex
PVs							
BRCA2	c.5213_5216del	p.Thr1738Ilefs*2	Previously reported, LOF is a known mechanism of disease	BC; OC	Breast cancer	27–60	F
BRCA2	c.4398_4402del	p.Leu1466Phefs*2	Previously reported, LOF is a known mechanism of disease	BC; OC	Prostate cancer	48–75	M
MYBPC3	c.1504C>T	p.Arg502Trp	Well-established PV	HCM	SW, 1.03 cm PW, 1.07 cm LVD, 6.25 cm FS, 17%	41–71	M
MYBPC3	c.26-2A>G	p.?	Previously reported, some segregation, affects canonical splice site	HCM	Normal appearance of heart on echocardiography	38–73	M
LDLR	c.429C>A	p.Cys143*	Not previously reported, LOF is a known mechanism of disease	HCL	LDL, 195 mg/dl, on no cholesterol medication	35–68	F
LPVs							
GLA	c.335G>A	p.Arg112His	Previously reported in cases, limited segregation and functional evidence	Fabry	Normal appearance of heart on echocardiography	49–83	F
DSP	c.4180C>T	p.Gln1394*	Not previously reported, LOF is a suspected mechanism of disease	ARVD/C	Normal appearance of heart on echocardiography	24–58	F

*Further details of classification evidence are provided in table S2. †Ages followed in the FHS.

confidence interval (CI), 1.7 to 16.5]. Of 26 JHS participants with PVs, 7 displayed an RCF, and this proportion (26.9%) was also higher than expected (5.4%; $P = 3 \times 10^{-4}$), corresponding to an SIR of 4.7 (95% CI, 1.9 to 9.7). The addition of LPV carriers to this analysis to estimate SIR for PVs and LPVs together yielded similar results [SIR, 4.9; 95% CI, 1.3 to 12.6 in FHS ($P = 0.004$) and SIR, 4.3; 95% CI, 1.8 to 8.4 in JHS ($P = 2 \times 10^{-4}$)]. Preplanned secondary analyses of individuals with cancer and cardiovascular diseases revealed that the incidence of RCFs was also significantly higher than expected for carriers of PVs associated with cancer and cardiovascular diseases (Table 4).

Description of individuals carrying PVs

In the FHS, a participant with an *LDLR* nonsense variant (p.Cys143*) had an untreated low-density lipoprotein (LDL) cholesterol level of 195 mg/dl (optimal <130 mg/dl). Notably, this individual was selected for sequencing in a hypertension study and thus was not selected for sequencing on the basis of her lipid status. A participant with a pathogenic missense variant in *MYBPC3* (p.Arg502Trp) had manifestations of dilated cardiomyopathy. Two participants with two different *BRCA2* frameshift variants (p.Leu1466Phefs*2 and p.Thr1738Ilefs*2) had (respectively) grade 3 (poorly differentiated), Gleason score 5 prostate cancer diagnosed at age 78 and ductal carcinoma in situ breast cancer at age 55. Neither of the individuals carrying LPVs had RCFs. No PV or LPV carrier was a first-degree relative of another carrier.

In the JHS, there were three individuals who had PVs in cancer predisposition genes who reported a history of cancer, although the type of cancer was not recorded. An individual with a *BRCA2* frameshift mutation (p.Val220Ilefs*2) was diagnosed with cancer at age 60. A carrier of *MLH1* p.Arg687Trp was diagnosed with cancer at age 36. A carrier of *TP53* p.Arg273His, who was enrolled at age 93, reported a diagnosis of cancer at age 89. A carrier of *MYH7* p.Ala797Thr had left ventricular hypertrophy with an interventricular septal thickness of 13.2 mm and posterior wall thickness of 12.8 mm (normal, <11 mm). Carriers of *KCNQ1* p.Arg518* and *KCNQ1* p.Val205Met had corrected QT (QTc) intervals of 477 and 494 ms [normal, <440 ms in men and <460 ms in women]. Furthermore, a carrier of the LPV *KCNQ1* p.Gly179Ser had a QTc interval of 506 ms. A participant with *LDLR* p.Pro685Leu had a markedly elevated untreated LDL cholesterol level (357.5 mg/dl; optimal, <130 mg/dl). One family of six, and two additional pairs of first-degree relatives each harbored the same PV, but none of these 10 individuals displayed an RCF, and thus familial presence of the same variant did not inflate the observed association.

DISCUSSION

In genetics, penetrance is the proportion of individuals harboring a particular variant who exhibit, or eventually exhibit, the associated disease (*28*). Estimating the penetrance of PVs in populations that are not enriched for family history is a challenge because specific PVs in any given gene are rare, and therefore an exceedingly large population would need to be systematically examined over many years to ascertain accurate phenotype information, which could emerge at any time in the lifetime of the individual. Our analyses do not address the penetrance of specific variants within individual genes. Instead, we tested whether pathogenic variants in a set of genes are collectively associated with RCFs for those conditions, and, if so, what is the effect size of this aggregated association. To answer this question, we conducted two separate, prospective, hypothesis-driven analyses of 462 European Americans and 3223 African-Americans for a group of 56 genes associated with disease conditions where early intervention could lead to prevention or better outcomes. We found that persons carrying PVs in a subset of these 56 genes demonstrate an increased aggregate risk of having clinical features associated with that gene

Table 3. Individuals with PVs and LPVs in the JHS. CRC, colorectal carcinoma; MH, malignant hyperthermia; HPP, hypokalemic periodic paralysis; FDB, familial dysbetalipoproteinemia; HNPCC, hereditary nonpolyposis CRC; LQTS, long QT syndrome; IVS, interventricular septum; PWT, left ventricular posterior wall thickness; LFS, Li-Fraumeni syndrome; LVIDd, left ventricular internal diastolic diameter; NA, not analyzed, that is, individual was not included in the analysis because the expected phenotype was either unavailable (see Materials and Methods) or missing for the given individual.

Gene	Variant and amino acid	Amino acid	Summary of classification evidence*	Associated condition	RCFs	Age†	Sex
PVs							
APOB	c.10580G>A	p.Arg3527Gln	Well-established PV	FDB	LDL-C, 165 mg/dl	69	F
MLH1	c.2059C>T	p.Arg687Trp	Well-established PV	HNPCC	Cancer, 36	65	M
SCN5A	c.3214G>T	p.Glu1072*	Not previously reported, LOF is a known mechanism of disease	Brugada	NA	36	F
					NA	43	F
					NA	61	F
MYL3	c.170C>G	p.Ala57Gly	Well-established PV	HCM	IVS, 8.7 mm PWT, 7.4 mm	81	F
KCNQ1	c.613G>A	p.Val205Met	Previously reported, homozygosity associated with a more severe phenotype	LQTS	QTc, 477 ms	46	F
KCNQ1	c.1552C>T	p.Arg518*	Well-established PV, LOF is a known mechanism of disease	LQTS	QTc, 494 ms	63	F
MYBPC3	c.1504C>T	p.Arg502Trp	Well-established PV	HCM	IVS, 10.9 mm PWT, 7.8 mm	79	F
PKP2	c.1689-1G>C	p.?	Previously reported, LOF is a known mechanism of disease	ARVD/C	Normal RA/RV	49	M
PKP2	c.1237C>T	p.Arg413*	Previously reported, LOF is a known mechanism of disease	ARVD/C	Normal RA/RV	46	F
					Normal RA/RV	47	F
					NA	50	F
					Normal RA/RV	51	F
					Normal RA/RV	72	F
					Normal RA/RV	74	F
					Normal RA/RV	74	F
BRCA2	c.658_659del	p.Val220Ilefs*4	Well-established PV, LOF is a known mechanism of disease	BC; OC	Cancer, 60	65	M
BRCA2	c.5611_5615del	p.Lys1872Asnfs*2	Previously reported, LOF is a known mechanism of disease	BC; OC	No cancer	39	F
BRCA2	c.5855T>A	p.Leu1952*	Not previously reported, LOF is a known mechanism of disease	BC; OC	No cancer	29	F
					No cancer	54	M
					No cancer	51	F
					No cancer	77	F
BRCA2	c.9382C>T	p.Arg3128*	Previously reported, LOF is a known mechanism of disease	BC; OC	No cancer	43	F
					No cancer	47	F
MYH7	c.2389G>A	p.Ala797Thr	Well-established PV	HCM	IVS, 13.2 mm PWT, 12.8 mm	66	M
TP53	c.818G>A	p.Arg273His	Well-established PV	LFS	Cancer, 89	93	M

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Gene	Variant and amino acid	Amino acid	Summary of classification evidence*	Associated condition	RCFs	Age†	Sex
BRCA1	c.5177_5180del	p.Arg1726fs*3	Previously reported, LOF is a known mechanism of disease	BC	No cancer	69	F
BRCA1	c.3607C>T	p.Arg1203*	Previously reported, Well-established PV, LOF is a known mechanism of disease	BC	No cancer	64	M
LDLR	c.2054C>T	p.Pro685Leu	Well-established PV	HCL	LDL-C, 358 mg/dl	36	M
RYR1	c.7300G>A	p.Gly2434Arg	Well-established PV	MH	NA	43	M
LPVs							
MLH1	c.1153C>T	p.Arg385Cys	Previously reported in cases, limited segregation and functional evidence	HNPCC	No cancer	36	M
DSP	c.3865C>T	p.Gln1289*	Not previously reported, heterogeneous expression of LOF mutations	ARVD/C	Normal RA/RV	66	M
KCNQ1	c.535G>A	p.Gly179Ser	Previously reported in cases, limited segregation but supportive functional evidence	LQTS	QTc, 506 ms	69	F
KCNQ1	c.1085A>G	p.Lys362Arg	Previously reported in cases, limited segregation but supportive functional evidence	LQTS	QTc, 432 ms	57	F

*Further details of classification evidence are provided in table S2. †Age at exam 1, cancer phenotype is reported with age of diagnosis.

Table 4. Observed proportion of participants with PV or LPV who had RCFs of the associated condition compared to the expected proportion.

	Observed*	Expected†	SIR‡	*P*§
Framingham Heart Study				
All ACMG genes	4/5 (80.0%)	0.62/5 (12.4%)	6.4 (1.7–16.5)	7×10^{-4}
Cancer	2/2 (100%)	0.15/2 (7.5%)	13.0 (1.5–47.0)	0.006
Cardiovascular	2/3 (66.7%)	0.46/3 (15.3%)	4.2 (0.5–15.4)	0.06
Jackson Heart Study				
All ACMG genes	7/26 (26.9%)	1.4/26 (5.4%)	4.7 (1.9–9.7)	3×10^{-4}
Cancer	3/12 (25.0%)	0.7/12 (5.8%)	4.3 (0.9–12.6)	0.03
Cardiovascular	4/14 (28.6%)	0.8/14 (5.7%)	5.1 (1.4–12.0)	4×10^{-3}

*Observed fraction of individuals carrying a PV who had the associated RCF. †Expected fraction of individuals based on the incidence of the RCF observed in individuals without PVs. ‡SIR and 95% CI. §*P* values comparing observed and expected fraction of PV carriers with RCFs, calculated with a binomial simulation.

in both the FHS (an entirely European American population) and JHS (an entirely African-American population). The difference in percentages of the cohort with the phenotypes of interest may be due to an enhanced healthy volunteer effect among the JHS cohort or to the fact that phenotypes were collected prospectively over several examinations spanning the course of decades in FHS but were based on a single examination thus far in JHS.

The frequencies we found for PVs and LPVs in the FHS and JHS populations are similar to recent assessments of PVs in medically actionable genes among large collections of individuals with exome sequences (*29*, *30*) and to others who have reported variants in the ACMG56 among collections of exomes or genomes (*31*, *32*). As in these reports, most of the PVs described here are predicted to encode null alleles and result in haploinsufficiency, a well-defined mechanism of pathogenicity for most genetic diseases. The range of frequencies for PVs in these other studies (from 0.8 to ~5%) could reflect differences in how the various populations were identified and recruited, but more likely reflect variability in variant classification. As we have demonstrated (*24*), even expert laboratories struggle to achieve complete concordance in variant classification. These issues reinforce the need to apply rigorous standards for variant classification and to share variant classification through mechanisms such as ClinGen (*33*), and also underscore the methodological importance of blinded variant classification in these analyses.

Understanding the association between PVs and RCFs in the general population is necessary for the informed use of genomics to evaluate patients for secondary findings (sometimes characterized as opportunistic screening) and for the use of sequencing in asymptomatic individuals (population screening) (*19*, *34*, *35*), but data to support or refute these practices are scarce. In a separate study of FHS participants, 21% of individuals with PVs in hypertrophic cardiomyopathy genes had clinical features suggestive of cardiomyopathy, a lower proportion than expected in multiplex families but a higher proportion than in persons without such variants (*36*). Specific founder mutations for long QT syndrome among the Finnish population are far from fully penetrant but are still highly associated with prolongation of QT interval in the relatively homogeneous Finnish population (*37*). However, analyses of medical records for evidence of cardiac arrhythmias did not demonstrate detectable penetrance of PVs in arrythmia-related genes, perhaps because variant classification was suboptimal (*38*, *39*). Screening for Lynch syndrome has been piloted among incident cases of colorectal cancer (*40*) but not among cancer-free individuals. A substantially increased risk for breast cancer associated with *BRCA* variants has recently been demonstrated (*41*), prompting a call for population-based screening of women around the age of 30 (*42*). For other genes and variants that are highly penetrant in multiplex families, an increased likelihood of clinical features among carriers cannot always be demonstrated in the general population: Individuals with well-established PVs for mature-onset diabetes of the young in the FHS and JHS do not exhibit an increased likelihood of having type 2 diabetes or impaired fasting glucose (*43*). Thus, the

literature contains mixed results as to whether PVs in some genes, even some of the genes included among the ACMG56, individually confer increased risk of disease in populations that are not selected for family history.

Estimations of gene-disease association are traditionally conceptualized as penetrance on a gene-by-gene and variant-by-variant basis, and predicting the likelihood of a phenotype from a particular variant in a particular gene is difficult when disease prevalence is low and carrier status prevalence is rare. However, there may be value in aggregating PVs across a number of genes to consider the prior probability as a compound hypothesis relating to numerous diseases. For example, among 951 individuals exome-sequenced as part of the ClinSeq cohort, 103 (10.8%) had putative loss-of-function variants in a large number of genes likely to cause a phenotype in heterozygotes (*44*). In ClinSeq, intensive targeted phenotyping of 79 of these individuals revealed 34 (43%) with personal or family histories that could be attributed to that gene. That analysis deliberately started with the PVs among a population recruited in part for cardiovascular risk and then searched for the corresponding phenotype in that participant or the participant's family, often uncovering evidence of a previously unrecognized but non–life-threatening genetic condition. In our analyses, we approached both variant classification and the tabulation of RCFs independently and blinded to each other and examined their association in a subset of genes that have been linked to life-threatening conditions in which early intervention or surveillance could potentially mitigate risk.

Our study has several important limitations. These analyses only examined the aggregate association of PVs with RCFs from the corresponding conditions but did not address the penetrance of individual variants or PVs within a specific gene, because this would have required vastly larger sample sizes. Although the FHS and JHS participants were neither enrolled nor sequenced on the basis of family history, the selection of participants for exome sequencing in FHS was based on their involvement in other studies and may therefore not be representative of the entire FHS population. This was not the case in the JHS where all consenting participants with available DNA were exome-sequenced. Our variant classification strategy may have missed some disease-associated variants by dismissing novel missense variants of unknown function from consideration (*45*). The a priori definition of both observed and expected RCFs in our analysis included any cancer, thus the cancers associated with PVs and the cancers counted in the comparison populations were appropriately included; however, had cancer cases been considered RCFs only when they had an onset early in life, the differences between the observed and expected penetrance of this group of variants might have been different. It is possible that some of the identified PVs occurred in multiplex families, although none of the participants were selected for sequencing based on family history. In JHS, a family of six individuals carried *PKP2* p.Arg413*, a PV expected to result in arrhythmogenic right ventricular dysplasia, but none of the family members displayed features of right ventricular abnormalities by echocardiography; therefore, the observed association was not inflated. The number of individuals with LPVs was too small to independently analyze this group, but adding PVs and LPVs together did not change the strength or significance of the association within each population.

These limitations are balanced by a number of strengths. The FHS and JHS cohorts are exceptionally well-studied populations where both sequence data and high-quality clinical data, including electrocardiograms, echocardiograms, and lipid levels, were available for all participants, not just those who had been recognized by the medical care system as patients. Aside from 25 FHS participants who were selected for sequencing on the basis of elevated LDL cholesterol, none of the participants were selected for sequencing on the basis of phenotypes examined in our analysis, and none of those identified in Table 2 with lipid abnormalities were from those 25 individuals. In addition, we prespecified our hypothesis and compared PVs and RCFs that were ascertained and classified independently of each other. Any misclassifications of variants, or censoring of phenotypes due to participant dropout or death, would be expected to bias the results toward the null. Performing these analyses in cohorts where all participants undergo phenotyping is advantageous, but even such systematic testing may incompletely capture some RCFs, such as right ventricular abnormalities on echocardiography for arrhythmogenic right ventricular dysplasia, limiting the ability to detect phenotypes and further biasing toward the null. The relatively few individuals with PVs in the ACMG56 is reflected in a wide CI for the analysis of each cohort; nevertheless, despite the small numbers and limited power, the associations range from a lower bound that is moderately strong to an upper bound that is extremely strong. Although aggregating that the exposure improves power, the combined carrier rate is low in a sample size of 3685, thereby limiting effect estimate precision. However, offsetting this issue is the fact that we independently demonstrated association in two ethnically distinct cohorts with similar relative effect estimates.

The ACMG recommendations for the return of secondary findings were expressly formulated for use in clinical sequencing (*7*). However, other groups have recommended the return of genomic variants that have medical actionability in research participants who request such information (*8*, *46*), and the ACMG recommendations for clinical sequencing have been suggested as a basis for selecting the appropriate list of genes and category of variant (*47*). As large-scale, hospital-based, national biobanks begin to generate genomic data, and research initiatives like the Precision Medicine Initiative affirm the right of research participants to have access to their research results (*48*), guidance regarding the management of such findings is urgently needed. It is important to note that it has not been demonstrated that detecting such variants actually results in improved health outcomes, and to many, the absence of this evidence remains a compelling objection to both opportunistic and population screening. Our results should be replicated in other populations that are followed for clinical outcomes and should be interpreted with caution, but may help inform the emerging debate about whether and how to offer the return of individual genomic results to participants in research cohorts and biobanks, as well as in clinical sequencing.

MATERIALS AND METHODS

Study design

We designed and carried out two independent analyses to estimate the association between PVs derived from exome sequencing in any of 56 genes and clinical features related to the actionable Mendelian conditions that have been linked to these genes. We examined all of the participants who had been sequenced at the time of the analysis in FHS and JHS, and used systematically collected phenotype information from each. Variants were classified as described below without knowledge of the clinical phenotypes, and phenotypes were assessed without knowledge of the variants. The association was estimated within each cohort independently, providing replication of the results.

Participants

The FHS is a multigenerational, longitudinal study of European Americans established in 1948 in Framingham, MA. Participants in this

analysis were from FHS Offspring (children and spouses of the Original cohort) and Generation 3 (children of the Offspring) cohorts (*49*, *50*). Offspring participants were examined every 4 to 8 years, for a total of eight exams. Generation 3 participants were examined twice. The JHS is a prospective, longitudinal study of African-Americans established in 1998 in Jackson, MS. The details of the cohort, including sampling, recruitment, and examinations, have been previously described (*51*–*53*).

For the FHS, as part of the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP), FHS Offspring and Generation 3 participants were selected for exome sequencing as follows: 41 cases and 135 controls for a study of myocardial infarction, 80 cases and 86 controls for a study of blood pressure, 13 cases and 12 controls for a study of LDL cholesterol, 30 cases with stroke, and 65 FHS participants who were randomly selected.

For the JHS, we analyzed genomic and phenotype data from participants who consented to DNA collection during the first examination (2000 to 2004). Exome sequencing was completed for all consenting JHS participants (3273 of the 5301 participants).

These studies were performed using protocols approved by ethics committees at FHS and JHS and by their institutional review boards, with informed consent from all participants.

Exome sequencing

Exome sequencing, variant detection, and quality control steps for the FHS samples have been previously described (*54*). Briefly, exome capture used either Agilent SureSelect Human All Exon v2 kit (*55*), or Roche/NimbleGen SeqCap EZ Human Exome Library v1.0 (~32 Mb; Roche NimbleGen EZ Cap v1) or EZ Cap v2 (~34 Mb). Enriched exome libraries were sequenced on an Illumina GAIIx or HiSeq 2000, aligned to human reference (GRCh37) using BWA (*56*), followed by duplicate removal, indel realignment, base quality score recalibration, and variant detection using Genome Analysis Toolkit (*57*).

Variant classification

Variants were adjudicated independently by two evaluators who made their classifications without any knowledge of the phenotype data and any differences resolved by consultation with a third evaluator. Variant classification was completed using a multistep algorithm as described (*25*, *26*, *45*) and was consistent with both the ACMG recommendation for secondary findings (*7*) and the more recently developed ACMG recommendations for variant classification (*27*).

Transcripts for analysis were those previously selected by the Partners HealthCare Laboratory for Molecular Medicine, a CLIA–certified molecular diagnostic laboratory, and were typically the longest (see table S1). Copy number variants were not evaluated because of the diversity of capture methods and sequencing platforms used for this data set. For variant classification, Alamut (Interactive Biosoftware) (*58*) and Variant Effect Predictor (*59*) were used to aggregate variant annotations from multiple sources, including transcript information and evolutionary conservation from the University of California Santa Cruz genome browser (*60*), and minor allele frequency from the ESP [Exome Variant Server, NHLBI ESP, Seattle, WA (http://evs.gs.washington.edu/EVS/), 15 December 2011] database, 1000 Genomes Project (http://browser.1000genomes.org/), and Exome Aggregation Consortium (http://exac.broadinstitute.org/) browsers. Previously published variants were identified by filtering against the Human Gene Mutation Database (HGMD) Professional (*61*), GeneInsight (*62*), and ClinVar (*63*) databases, the latter two databases were also used in variant classification to obtain additional unpublished data on HGMD-selected variants.

Only missense variants that had previously been reported in an index case, denoted as "disease mutations" in the HGMD nomenclature or classified as pathogenic by at least one clinical laboratory in ClinVar, as well as nonsense, frameshift, and splice variants, were considered. Variants previously reported only in the context of functional or in silico experiments, but not previously associated with a symptomatic individual, were not further considered.

Variants were classified as PV if, in addition to being absent or at a frequency in population databases not inconsistent with their disease penetrance: (i) They were protein-truncating variants (nonsense, frameshift, or ±1,2 splice) in a gene where loss of function is a well-established disease mechanism, and the variant was expected to result in nonsense-mediated decay; (ii) literature review identified significant segregation with disease (defined as ≥10 meioses); or (iii) literature review identified moderate segregation with disease (5 to 9 meioses), and the amino acid was conserved in at least mammals and birds, or the impact of the variant was supported by strong functional data. Variants were classified as an LPV if, in addition to being absent or at a frequency in population databases not inconsistent with their disease penetrance: (i) Literature review showed moderate segregation (5 to 9 meioses) with disease, the amino acid was conserved in all mammals and birds, but functional data were either limited or absent; (ii) literature review identified minimal familial segregation (<5 meioses), but the amino acid was both conserved in all mammals and supported by strong functional data; or (iii) they were protein-truncating variants (nonsense, frameshift, or ±1,2 splice) in a gene where loss-of-function variants have been observed but was not yet a well-established disease mechanism, and the variant was expected to result in nonsense-mediated decay. Variants were classified as benign if the frequency of the variant was above 0.3% for variants associated with dominantly inherited diseases. All other variants were classified as VUS.

For secondary analyses, we grouped the ACMG genes into 23 that are cancer-related (*APC*, *BRCA1*, *BRCA2*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF2*, *PMS2*, *PTEN*, *RB1*, *RET*, *SDHD*, *SDHAF2*, *SDHB*, *SDHC*, *STK11*, *TP53*, *TSC1*, *TSC2*, *VHL*, and *WT1*) and 31 that are cardiovascular-related (*ACTC1*, *GLA*, *LMNA*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *PRKAG2*, *TNNT2*, *TNNI3*, *TPM1*, *DSC2*, *DSG2*, *DSP*, *PKP2*, *TMEM43*, *KCNH2*, *KCNQ1*, *SCN5A*, *RYR2*, *ACTA2*, *COL3A1*, *FBN1*, *MYLK*, *MYH11*, *SMAD3*, *TGFBR1*, *TGFBR2*, *APOB*, *LDLR*, and *PCSK9*). Two genes conferring susceptibility to malignant hyperthermia (*CACNA1S* and *RYR1*) were not considered in the secondary analyses.

Phenotype data

FHS phenotypes were downloaded from the database of Genotypes and Phenotypes (dbGaP) and were available throughout the period of follow-up, whereas JHS phenotypes were only available from Exam 1 and were extracted from the JHS Vanguard Center package for Exam 1 (*53*). Sex, age, and date of examination for each subject were derived from data recorded during clinical examinations. RCFs for diseases corresponding with the ACMG genes were ascertained and tabulated without knowledge of the genetic data. For cancer, an aggregated FHS cancer database, with subject diagnoses confirmed from pathology reports and clinical notes, was queried (*64*, *65*), whereas cancer diagnoses in JHS were extracted from Exam 1 participant surveys. For both data sets, any history of cancer was recorded regardless of the age of onset of the cancer. For cardiovascular diseases, the most recent lipid levels, echocardiography, and electrocardiogram data were recorded and categorized according to prespecified criteria (Table 1). In both FHS and JHS, phenotypic data

sets were highly complete with less than 4% of participants having missing data for any phenotypic variable.

Statistical analyses

We calculated the expected number of RCFs among those with PVs as $\sum_i n_i \pi_i$, where n_i is the number of individuals with a PV in class *i* (cancer, hypertrophic and dilated cardiomyopathy, arrhythmogenic right ventricular dysplasia/cardiomyopathy, and dyslipidemia), and π_i is the fraction of individuals without PVs exhibiting an RCF in class *i*. In the FHS cohort, we selected individuals with breast, ovarian, prostate, and gastrointestinal cancer, whereas in the JHS, cancer subtypes were not available, so we used any history of cancer. We estimated statistical significance through simulation: We sampled a binomial random variable with size n_i and probability π_i for each class *i* and summed these five random variables (generating a mixture of binomials). We generated 100,000 replicates of this simulated RCF count and estimated the (one-sided) *P* value as the proportion of replicates where the simulated count was equal to, or exceeded, the observed count. Second, we repeated this procedure for cancer and cardiovascular PVs. We also calculated SIR as the ratio of the observed RCF count to the expected count (*66*). All statistical analyses were performed with R (version 3.0.2).

SUPPLEMENTARY MATERIALS

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Table S1. ACMG incidental findings genes and transcripts analyzed.
Table S2. Classification evidence for PVs and LPVs from FHS and JHS participants.
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Editor's Summary

The problem of penetrance

It seems obvious that people who have mutations in genes known to cause disease in well-studied families would be more likely to also have the clinical features of disease if they were selected from t he general population. But researchers have obtained mixed results on this point because of incomplete p enetrance, i.e., not everyone who has a certain disease-causing mutation (a pathogenic variant) has the disease, raising questions about the value of genetic screening of people who are not sick. Natarajan and colleagues bring some clarity to this issue by examining two large groups of subjects −−from the Framingham Heart Study and the African-American Jackson Heart Study−−for the presence of mutations in 56 disease-related genes and for clinical features of their corresponding diseases. Even though the authors examined the genetic results of almost 5000 people, the number of these mutations was small. Nevertheless, these data clearly show that carrying a pathogenic variant markedly increases the chances of having the related disease.

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Supplementary Materials for

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Table S2. Classification evidence for PVs and LPVs from FHS and JHS participants.
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Supplementary Materials

Table S1. ACMG incidental findings genes and transcripts analyzed.

Gene	Transcript
ACTA2	NM_001613.2
ACTC1	NM_005159.4
APC	NM_000038.4
APOB	NM_000384.2
BRCA1	NM_007294.3 NM_007300.3 NM_007297.3 NM_007298.3 NM_007299.3
BRCA2	NM_000059.3
CACNA1S	NM_000069.2
COL3A1	NM_000090.3
DSC2	NM_024422.3 NM_004949.3
DSG2	NM_001943.3
DSP	NM_004415.2
FBN1	NM_000138.4
GLA	NM_000169.2
KCNH2	NM_000238.3 NM_172057.2 NM_172056.2
KCNQ1	NM_000218.2 NM_181798.1
LDLR	NM_000527.4
LMNA	NM_170707.2
MEN1	NM_130799.2
MLH1	NM_000249.3
MSH2	NM_000251.1
MSH6	NM_000179.2
MUTYH	NM_012222.2
MYBPC3	NM_000256.3
MYH11	NM_001040113.1 NM_001040114.1
MYH7	NM_000257.2
MYL2	NM_000432.3
MYL3	NM_000258.2
MYLK	NM_053025.3
NF2	NM_181831.2 NM_181825.2 NM_181832.2 NM_181830.2 NM_181829.2 NM_181828.2 NM_016418.5 NM_000268.3 NM_181833.2
PCSK9	NM_174936.3

PKP2	NM_004572.3
PMS2	NM_000535.5
PRKAG2	NM_016203.3
PTEN	NM_000314.4
RB1	NM_000321.2
RET	NM_020975.4 NM_020630.4
RYR1	NM_001042723.1 NM_000540.2
RYR2	NM_001035.2
SCN5A	NM_198056.2 NM_001099404.1 NM_001160160.1
SDHAF2	NM_017841.2
SDHB	NM_003000.2
SDHC	NM_001035511.1 NM_003001.3
SDHD	NM_003002.2
SMAD3	NM_005902.3 NM_001145103.1
STK11	NM_000455.4
TGFBR1	NM_004612.2
TGFBR2	NM_003242.5 NM_001024847.2
TMEM43	NM_024334.2
TNNI3	NM_000363.4
TNNT2	NM_001001430.1 NM_000364.2
TP53	NM_000546.4
TPM1	NM_001018020.1 NM_001018008.1 NM_001018005.1 NM_000366.5
TSC1	NM_000368.4
TSC2	NM_000548.3
VHL	NM_000551.2
WT1	NM_001198552.1 NM_024426.4 NM_001198551.1 NM_000378.4 NM_024424.3

Table S2. Classification evidence for PVs and LPVs from FHS and JHS participants.

Gene	Variant and Transcript	Amino acid	Associated phenotype	Classification evidence
			PATHOGENIC VARIANTS	
APOB	c.10580G>A NM_000384.2	p.Arg3527Gln	HCL	The p.Arg3527Gln (also referred to as p.Arg3500Gln) variant is a well-established pathogenic variant in apolipoprotein B dysfunction and has been reported in more than 50 families (*67-73*). In vitro functional studies provide evidence that the p.Arg3527Gln variant may impact protein function (*68*). In summary, this variant meets our criteria to be classified as pathogenic for apolipoprotein B dysfunction in an autosomal dominant manner based upon segregation studies and functional evidence.
MLH1	c.2059C>T NM_000249.3	p.Arg687Trp	CC	The p.Arg687Trp variant in *MLH1* has been reported in several individuals with colorectal cancer, disrupts mismatch repair in yeast (*74*) and is considered "Pathogenic" by the ClinGen-approved expert panel InSiGHT variant interpretation committee (*75*). In summary, this variant meets our criteria to be classified as pathogenic for colorectal cancer in an autosomal dominant manner.
SCN5A	c.3214G>T NM_198056.2 NM_001099404.1 NM_001160160.1	p.Glu1072*	Brugada	The p.Glu1072* variant in *SCN5A* has not been previously reported in individuals with disease and was absent from large population studies. This nonsense variant leads to a premature termination codon at position 1072 which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *SCN5A* gene is an established disease mechanism in Brugada syndrome. In summary, this variant meets our criteria to be classified as pathogenic for Brugada syndrome in an autosomal dominant based upon absence from controls and predicted impact on the protein.
MYL3	c.170C>G NM_000258.2	p.Ala57Gly	HCM	The p.Ala57Gly variant in *MYL3* has been identified previously by the Partners LMM in 2 individuals with HCM, and in 2 individuals with family history of HCM. This variant was also reported in two Korean families and two Japanese individuals with HCM (*76-78*). The variant was shown to segregate with disease in 5 affected members of the two Korean families. *In vitro* and in vivo functional studies provide some evidence that the p.Ala57Gly variant impacts protein function resulting in a high level of fibrosis and hypertrophy in an animal model (*79, 80*). This variant has been identified in 6/67654 European chromosomes and in 4/8758 East Asian chromosomes by the Exome Aggregation Consortium (*81*). Although this variant has been seen in the general population, its frequency is not high enough to rule out a pathogenic role. In summary, this variant meets our criteria to be classified as pathogenic based upon case observations, segregation studies and functional evidence.
KCNQ1	c.613G>A NM_000218.2	p.Val205Met	LQTS	The p.Val205Met variant in *KCNQ1* has been reported in at least 2 presumably unrelated Gitxsan individuals with LQTS and segregated with the disease in 12 affected family members (*82*). Four individuals have been reported to be homozygous for this variant and present with a clinically more severe phenotype (*83*). This variant has been identified only in 0.023% (1/4402) of African American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs151344631). Computational prediction tools and conservation analysis suggest that the p.Val205Met variant may impact the protein, though this information is not predictive enough to determine pathogenicity. *In vitro* functional studies provide some evidence that the p.Val205Met variant may impact protein function (*82, 85*). In

				summary, this variant meets our criteria to be classified as pathogenic, with incomplete penetrance, for LQTS in an autosomal dominant manner based upon segregation studies, case studies and functional evidence.
KCNQ1	c.1552C>T NM_000218.2	p.Arg518*	LQTS	The p.Arg518* variant in *KCNQ1* is a well-established pathogenic variant associated with LQTS and Jervell and Lange-Nielsen Syndrome (JLNS) (*86*). This variant has been identified in 16/66,544 European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs17215500). This nonsense variant leads to a premature termination codon at position 518 which is predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria to be classified as pathogenic for LQTS in an autosomal dominant manner and for JLNS in an autosomal recessive manner.
MYBPC3	c.26-2A>G NM_000256.3	p.?	HCM	The c.26-2A>G variant in *MYBPC3* has been reported in at least 9 individuals with HCM and segregated with disease in 2 affected relatives from 1 family (*87-91*). In addition, this variant has been identified by the Partners LMM in 3 individuals with HCM and segregated with disease in 2 affected relatives from 2 families. This variant has also been identified in 4/45416 European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs376395543); however, for diseases with clinical variability and reduced penetrance, pathogenic variants may be present at a low frequency in the general population. Finally, this 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. Heterozygous splice variants in *MYBPC3* are prevalent in cases of HCM. In summary, this variant meets our criteria to be classified as pathogenic for HCM in an autosomal dominant manner based upon predicted variant impact, case observations and segregation studies.
MYBPC3	c.1504C>T NM_000256.3	p.Arg502Trp	HCM	The p.Arg502Trp variant in *MYBPC3* has been well reported in multiple individuals across multiple studies and is known to be pathogenic for HCM. This variant meets our criteria for pathogenicity based upon extensive segregation studies and functional evidence (*90, 92-98*). It is also the most common pathogenic HCM variant.
PKP2	c.1689-1G>C NM_004572.3	p.?	ARVD/ C	The c.1689-1G>C variant in *PKP2* has been reported in 2 individuals with arrhythmogenic right ventricular cardiomyopathy (*99, 100*) and was absent from large population studies. 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. In summary, this variant meets our criteria to be classified as pathogenic for arrhythmogenic right ventricular cardiomyopathy in an autosomal dominant manner based upon predicted impact on the protein, case observations and absence from controls.
PKP2	c.1237C>T NM_004572.3	p.Arg413*	ARVD/C	The p.Arg413* variant in *PKP2* has been identified in >10 individuals with ARVD/C and segregated with disease in at least 3 affected relatives from one family (*100-105*). Overexpression of this variant in mice increased right ventricular size and shortened ventricular action potential durations (*105*), though this assay may not accurately represent the biological disease state. This nonsense variant leads to a premature termination codon at position 413, which is predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria to be classified as pathogenic for ARVC in an autosomal dominant manner based upon segregation studies, case studies, absence from controls, and predicted gene impact.
BRCA2	c.658_659del NM_000059.3	p.Val220Ilefs*4	BC; OC	The p.Val220Ilefs*4 variant in *BRCA2* has been reported in the literature in numerous individuals with hereditary breast and ovarian cancer (*106-108*) and has been reported in 37 individuals

				with breast and/or ovarian cancer in the Breast Cancer Information Core (BIC) database. This p.Val220fs variant has also been identified in individuals with Fanconi anemia, Wilms tumor, glioblastoma, or medulloblastoma; however all these individuals also carry a second pathogenic *BRCA2* variant (*109-111*). This variant has been identified in 4/51064 European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs80359604). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 220 and leads to a premature termination codon 4 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon genetic studies and the predicted impact to the protein.
BRCA2	c.4398_4402del NM_000059.3	p.Leu1466Phefs*2	BC; OC	The p.Leu1466Phefs*2 variant in *BRCA2* has been reported in at least 1 individual with prostate cancer (*112*). This variant has been identified in 0.036% (3/8240) of European American chromosomes and 0.094% (4/4258) of African American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs80359444). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 1466 and leads to a premature termination codon 2 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *BRCA2* gene is an established disease mechanism in *BRCA2*-associated cancers. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon the predicted impact to the protein.
BRCA2	c.5213_5216del NM_000059.3	p.Thr1738Ilefs*2	BC; OC	The p.Thr1738Ilefs*2 variant in *BRCA2* has been reported in at least 2 individuals with breast and/or ovarian cancer (*113, 114*). This variant has been identified in 1/66156 of European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs80359493). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 1738 and leads to a premature termination codon 2 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *BRCA2* gene is an established disease mechanism in *BRCA2* associated cancers. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon the predicted impact to the protein.
BRCA2	c.5611_5615del NM_000059.3	p. Lys1872Asnfs*2	BC; OC	The p.Lys1872Asnfs*2 variant in *BRCA2* has been reported in literature in at least 1 individual with breast cancer (*115*). It has also been reported in 3 individuals with breast and/or ovarian cancer in the UMD database and is found at very low frequency (2/10144 African chromosomes) by the Exome Aggregation Consortium (*81*) (dbSNP rs80359525). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 1872 and leads to a premature termination codon 2 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *BRCA2* gene is an established disease mechanism in *BRCA2*-associated cancers. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon the predicted impact to the protein and case observations.

BRCA2	c.5855T>A NM_000059.3	p.Leu1952*	BC; OC	The p.Leu1952* variant in *BRCA2* has not been reported in the literature and is at very low frequency (1/4406 African American chromosomes) in the NHLBI Exome Sequencing Project (*84*) (dbSNP rs375064902). This nonsense variant leads to a premature termination codon at position 1952 which is predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon the predicted impact to the protein.
BRCA2	c.9382C>T NM_000059.3	p.Arg3128*	BC; OC	The p.Arg3128* variant in *BRCA2* has been reported in the literature in at least 2 individuals with prostate cancer and in one individual with breast cancer (*116-118*). This variant was observed at very low frequency (2/10406 African chromosomes) by the Exome Aggregation Consortium (*81*) (dbSNP rs80359212). This nonsense variant leads to a premature termination codon at position 3128 which is predicted to lead to a truncated or absent protein. In summary, this variant meets our criteria to be classified as pathogenic for *BRCA2*-associated cancers in an autosomal dominant manner based upon the predicted impact to the protein.
MYH7	c.2389G>A NM_000257.2	p.Ala797Thr	HCM	The p.Ala797Thr variant in *MYH7* has been identified in >30 individuals with HCM and segregated with disease in >10 affected family members (*119-124*). This variant has been identified in 4/121372 chromosomes from various ethnicities by the Exome Aggregation Consortium (*81*) (dbSNP rs3218716). Please note that, for diseases with clinical variability and reduced penetrance, pathogenic variants may be present at a low frequency in the general population. In summary, this variant meets our criteria to be classified as pathogenic for HCM in an autosomal dominant manner based upon case observations and segregation studies.
TP53	c.818G>A NM_000546.4	p.Arg273His	LFS	The p.Arg273His variant in *TP53* has been reported in numerous individuals with various types of Li Fraumeni-associated cancers, including sarcomas, gastric carcinoma, breast cancer, uterine serous cancer, rhabdomyosarcoma-associated renal cell carcinoma, and acute lymphoblastic leukemia (*125-132*). *In vitro* functional studies provide some evidence that the p.Arg273His variant may impact protein function (*131, 133-140*). However, these types of assays may not accurately represent biological function. In summary, this variant meets our criteria to be classified as pathogenic for Li-Fraumeni syndrome in an autosomal dominant manner based upon case studies, absence from controls, and functional evidence.
BRCA1	c.5177_5180del NM_007300.3	p.Arg1726Lysfs*3	BC; OC	The p.Arg1726Lysfs*3 variant in *BRCA1* has been reported in at least 5 individuals with breast and ovarian cancer syndrome (*114, 141-143*) and was absent from large population studies (has only been identified in 1/4264 African American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs80357975). This variant is predicted to cause a frameshift, which alters the protein's amino acid sequence beginning at position 1726 and leads to a premature termination codon 3 amino acids downstream. This alteration is then predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *BRCA1* gene is an established disease mechanism in breast and ovarian cancer syndrome. In summary, this variant meets our criteria to be classified as pathogenic for breast and ovarian cancer syndrome in an autosomal dominant manner (*144*).
BRCA1	c.3607C>T NM_007300.3 NM_007294.3	p.Arg1203*	BC; OC	The p.Arg1203* variant in *BRCA1* has been reported in several individuals with breast and ovarian cancer syndrome (*144, 145*) and was absent from large population studies (has only been identified in 1/4406 African American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs62625308).

				This nonsense variant leads to a premature termination codon at position 1203 which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *BRCA1* gene is an established disease mechanism in breast and ovarian cancer syndrome. In summary, this variant meets our criteria to be classified as pathogenic for breast and ovarian cancer syndrome in an autosomal dominant manner.
LDLR	c.429C>A NM_000527.4	p.Cys143*	HCL	The p.Cys143* variant in *LDLR* has not been previously reported in individuals with disease and has been identified in 3/66347 of European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs199774121). This nonsense variant leads to a premature termination codon at position 143 which is predicted to lead to a truncated or absent protein. Heterozygous loss of function of the *LDLR* gene is an established disease mechanism in hypercholesterolemia. In summary, this variant meets our criteria to be classified as pathogenic for hypercholesterolemia in an autosomal dominant manner based upon its predicted effect on the protein.
LDLR	c.2054C>T NM_000527.4	p.Pro685Leu	HCL	The p.Pro685Leu variant in *LDLR* has been reported in at least 29 individuals with clinical features of hypercholesterolemia and segregated with disease in their families (*67*, *146*). This variant has been identified in 3/8600 of European American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs28942084). *In vitro* functional studies provide evidence that the p.Pro685Leu variant may impact protein function (*147*). In summary, this variant meets our criteria to be classified as pathogenic for hypercholesterolemia in an autosomal dominant manner based upon segregation studies and functional evidence.
RYR1	c.7300G>A NM_001042723.1 NM_000540.2	p.Gly2434Arg	MHS	The p.Gly2434Arg variant in *RYR1* has been reported in more than 100 individuals with malignant hyperthermia and segregated with the disease in several families (*148-154*). *In vitro* functional studies provide some evidence that the p.Gly2434Arg variant may impact protein function (*152, 155*). This variant has only been identified in 2/66466 of European and 1/10366 African chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs121918593). In summary, this variant meets our criteria to be classified as pathogenic for malignant hyperthermia in an autosomal dominant based upon segregation studies, absence from controls, functional evidence.
		LIKELY PATHOGENIC VARIANTS		
MLH1	c.1153C>T NM_000249.3	p.Arg385Cys	CC	The p.Arg385Cys variant in *MLH1* has been reported in 3 individuals with colorectal cancer (*156-158*) and identified in 0.03% (3/8,648) of East Asian chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs63750760). The variant is considered "Likely Pathogenic" by the ClinGen-approved expert panel InSiGHT variant interpretation committee (*75*). In summary, although additional studies are required to fully establish its clinical significance, the p.Arg385Cys variant is likely pathogenic.
DSP	c.3865C>T NM_004415.2	p.Gln1289*	ARVD/C	The p.Gln1289* variant in *DSP* has not been previously reported in individuals with disease and had only been identified in 1/10188 of African chromosomes by the Exome Aggregation Consortium (*81*). This nonsense variant leads to a premature termination codon at position 1289 which is predicted to lead to a truncated or absent protein. Frameshift and nonsense variants in *DSP* have been well reported in patients with ARVD/C (*159*), but recent evidence supports that they can also cause DCM (*160*). In summary, although additional studies are required to fully establish its clinical significance, the p.Gln1289* variant is likely pathogenic.

DSP	c.4180C>T NM_004415.2	p.Gln1394*	ARVD/C	The p.Gln1394* variant in *DSP* has not been reported in individuals with disease and has only been identified in 1/66536 of European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs140474226). This nonsense variant leads to a premature termination codon at position 1394 which is predicted to lead to a truncated or absent protein. Frameshift and nonsense variants in DSP have been well reported in patients with ARVD/C (*159*), but recent evidence supports that they can also cause DCM (*160*). In summary, although additional studies are required to fully establish its clinical significance, the p.Gln1394* variant is likely pathogenic.
KCNQ1	c.535G>A NM_000218.2	p.Gly179Ser	LQTS	The p.Gly179Ser variant in *KCNQ1* has been reported in 4 probands (one homozygous, one compound heterozygous and 2 of unreported status) with LQTS as well as segregating in 3 additional family members with LQTS (*161-163*). An additional 3 family members had borderline QT intervals and 3 others were considered normal. This variant has only been identified in 0.023% (1/4,400) of African American chromosomes by the NHLBI Exome Sequencing Project (*84*) (dbSNP rs199473394). Computational prediction tools and conservation analysis suggest that the p.Gly179Ser variant may impact the protein, though this information is not predictive enough to determine pathogenicity. *In vitro* functional studies provide some evidence that the p.Gly179Ser variant impacts protein function in a homozygous state though no effect was seen when mixed with normal protein (*164*). In summary, the p.Gly179Ser variant is likely pathogenic though additional studies are required to confirm its clinical significance as well as evaluate penetrance which is likely incomplete in a heterozygous state.
KCNQ1	c.1085A>G NM_000218.2	p.Lys362Arg	LQTS	The p.Lys362Arg variant in *KCNQ1* has been reported in 3 heterozygous individuals with LQTS and in 2 compound heterozygous individuals with Lange-Nielsen syndrome without auditory phenotype (*165-168*). This variant has been identified in 1/10356 of African chromosomes and in 1/66474 European chromosomes by the Exome Aggregation Consortium (*81*) (dbSNP rs12720458). Although this variant has been seen in the general population, its frequency is not high enough to rule out a pathogenic role. Computational prediction tools and conservation analysis suggest that the p.Lys362Arg variant may impact the protein, though this information is not predictive enough to determine pathogenicity. In summary, the p.Lys362Arg variant is likely pathogenic though additional studies are required to confirm its clinical significance as well as evaluate penetrance which is likely incomplete in a heterozygous state.
GLA	c.335G>A NM_000169.2	p.Arg112His	Fabry	The p.Arg112His variant in *GLA* has been previously identified in 3 individuals with Fabry disease (*169-171*). This variant has been identified in 1/6728 European American chromosomes (female) by the NHLBI Exome Sequencing Project (*84*). Functional studies indicate this variant results in reduced *GLA* activity (*169, 171*). In summary, this variant is likely pathogenic, though additional studies are required to fully establish its clinical significance.

Abbreviations: P, pathogenic; LP, likely pathogenic; LOF, loss-of-function; HCL, hypercholesterolemia; CC, colorectal cancer; LQTS, long QT syndrome; HCM, hypertrophic cardiomyopathy; ARVD/C, arrhythmogenic right ventricular dysplasia / cardiomyopathy; BC, breast cancer; OC, ovarian cancer; LFS, Li-Fraumeni syndrome