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Actionable, Pathogenic Incidental Findings in 1,000 Participants' Exomes

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The incorporation of genomics into medicine is stimulating interest on the return of incidental findings (IFs) from exome and genome sequencing. However, no large-scale study has yet estimated the number of expected actionable findings per individual; therefore, we classified actionable pathogenic single-nucleotide variants in 500 European- and 500 African-descent participants randomly selected from the National Heart, Lung, and Blood Institute Exome Sequencing Project. The 1,000 individuals were screened for variants in 114 genes selected by an expert panel for their association with medically actionable genetic conditions possibly undiagnosed in adults. Among the 1,000 participants, 585 instances of 239 unique variants were identified as disease causing in the Human Gene Mutation Database (HGMD). The primary literature supporting the variants' pathogenicity was reviewed. Of the identified IFs, only 16 unique autosomal-dominant variants in 17 individuals were assessed to be pathogenic or likely pathogenic, and one participant had two pathogenic variants for an autosomal-recessive disease. Furthermore, one pathogenic and four likely pathogenic variants not listed as disease causing in HGMD were identified. These data can provide an estimate of the frequency (~3.4% for European descent and ~1.2% for African descent) of the high-penetrance actionable pathogenic or likely pathogenic variants in adults. The 23 participants with pathogenic or likely pathogenic variants were disproportionately of European (17) versus African (6) descent. The process of classifying these variants underscores the need for a more comprehensive and diverse centralized resource to provide curated information on pathogenicity for clinical use to minimize health disparities in genomic medicine.

Introduction

As whole-genome and -exome tests are incorporated into medicine, the resources required for the return of genomic incidental findings (IFs) must be explored. The American College of Medical Genetics and Genomics (ACMG) has recently recommended return of IFs from a minimum set of actionable genes.[1] However, no large-scale study to date has addressed the likelihood of identifying pathogenic mutations in actionable genes per individual or the time burden on health-care professionals to make these determinations. These data can inform the policy discussion. We reviewed the primary literature for possible actionable pathogenic single-nucleotide variants in 500 European- and 500 African-descent participants randomly selected from the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project (ESP). These 1,000 individuals were screened for variants in a list of 114 genes associated with medically actionable genetic conditions that might remain undiagnosed in adults; these include 52 genes associated with adult-onset conditions of the 56 genes recommended for return of IFs by the ACMG.

Material and Methods

Development of the Gene List

The gene list and criteria for data supporting the call of a known highly penetrant pathogenic variant for return were developed and agreed upon unanimously by the Clinical Sequencing and Exploratory Research (CSER) "NEXT Medicine" Return of Results Committee (RORC), funded by the University of Washington and National Human Genome Research Institute. The NEXT Medicine RORC comprises 24 experts with a combined 340 clinician years of medical genetics practice and includes 14 practicing clinical medical geneticists, two genetic counselors, and several other physician and nonphysician members with expertise in a variety of genetic specialties, including pediatric, adult, cancer, dermatologic, collagen, neurological, biochemical, cytogenetic, and molecular diagnostics. "Actionable" genes in adults were defined as having deleterious mutation(s) whose penetrance would result in specific, defined medical recommendation(s) both supported by evidence and, when implemented, expected to improve an outcome(s) in terms of mortality or the

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Table 1. Genes with Actionable Variants

Dominant	Dominant (cont.)	Dominant (cont.)	Dominant (cont.)	X-Linked	Recessive
ACTA2[a] (102620)	*FLCN* (607273)	***MYH11*** (160745)	***SCN5A*** (600163)	*DMD* (300377)	*ATP7B* (606882)
ACTC1 (102540)	*GCH1* (600225)	***MYH7*** (160760)	***SDHAF2*** (613019)	*EMD* (300384)	*BCHE* (177400)
ACVRL1 (601284)	*GPD1L* (611778)	***MYL2*** (160781)	***SDHB*** (185470)	***GLA*** (300644)	*BLM* (604610)
APC (611731)	*HCN4* (605206)	***MYL3*** (160790)	***SDHC*** (602413)	*OTC* (300461)	*CASQ2* (114251)
BMPR1A (601299)	*HMBS* (609806)	***MYLK*** (600922)	***SDHD*** (602690)	-	*COQ2* (609825)
BRCA1 (113705)	*KCNE1* (176261)	***NF2*** (607379)	*SERPINC1* (107300)	-	*COQ9* (612837)
BRCA2 (600185)	*KCNE2* (603796)	*PDGFRA* (173490)	*SGCD* (601411)	-	*CPT2* (600650)
CACNA1C (114205)	*KCNE3* (604433)	***PKP2*** (602861)	***SMAD3*** (603109)	-	*F5*[b] (612309)
CACNA1S (114208)	***KCNH2*** (152427)	*PLN* (172405)	*SMAD4* (600993)	-	*GAA* (606800)
CACNB2 (600003)	*KCNJ2* (600681)	***PMS2*** (600259)	*SMARCB1* (601607)	-	*HAMP* (606464)
CDC73 (607393)	***KCNQ1*** (607542)	***PRKAG2*** (602743)	***STK11*** (602216)	-	*HFE*[c] (613609)
CDH1 (192090)	*KIT* (164920)	*PRKAR1A* (188830)	*TGFB3* (190230)	-	*HFE2* (602390)
CNBP (116955)	***LDLR*** (606945)	*PROC* (176860)	***TGFBR1*** (190181)	-	*IDUA* (252800)
COL3A1 (120180)	***LMNA*** (150330)	*PROS1* (176880)	***TGFBR2*** (190182)	-	*LDLRAP1* (605747)
DMPK (605377)	***MEN1*** (613733)	*PTCH1* (601309)	***TMEM43*** (612048)	-	*PAH* (612349)
DSC2 (125645)	*MET* (164860)	***PTEN*** (601728)	***TNNI3*** (191044)	-	*PCBD1* (126090)
DSG2 (125671)	***MLH1*** (120436)	*RBM20* (613171)	***TNNT2*** (191045)	-	*PTS* (612719)
DSP (125647)	*MLH3* (604395)	***RET*** (164761)	***TP53*** (191170)	-	*QDPR* (612676)
ENG (131195)	***MSH2*** (609309)	***RYR1*** (180901)	***TPM1*** (191010)	-	*SERPINA1* (107400)
EPCAM (185535)	***MSH6*** (600678)	***RYR2*** (180902)	***TSC1*** (605284)	-	*SLC25A13* (603859)
FBN1 (134797)	***MUTYH*** (604933)	*SCN1B* (600235)	***TSC2*** (191092)	-	*SLC37A4* (604194)
FH (136850)	***MYBPC3*** (600958)	*SCN3B* (608214)	***VHL*** (608537)	-	*SLC7A9* (604144)

MIM numbers are shown in parentheses next to each gene.
[a]Bolded genes are recommended for return by the ACMG guidelines.[1]
[b]Only homozygotes for the *F5* c.1601 G>A (pArg534Gln) Factor V Leiden mutation are deemed actionable.[3]
[c]Only homozygotes for the *HFE* c.845G>A (p.Cys282Tyr) mutation are deemed actionable.[3]

avoidance of significant morbidity. The benefit of intervention had to be sufficient to counter any concerns raised by an unexpected predisposition to disease. Identifying actionable genes to be included on the IF gene list began with consideration of a list of "bin 1" genes,[2] available clinical tests for genetic disorders, and genes nominated by group members on the basis of their clinical expertise. Genes that were unanimously agreed upon to warrant return upon discovery of a known pathogenic mutation were determined by the committee. Genes that had unclear associations with disease, for which associated disease treatment and/or screening had debatable benefit, or for which more information was required for making a determination were assigned to group members for evaluation on the basis of interest or expertise. The list of genes determined to date to have actionable variants is given in Table 1. The committee continues to develop and refine this list as new evidence regarding the association between genes and disease or actionability becomes available. Notably, we are developing an actionable-variant database for an adult population; therefore, only disorders that might remain undiagnosed in adulthood were included. The impact on reproductive decision making (e.g., carrier-status reporting) was not included.

Criteria for Classification of Variants

The criteria for the classification of highly penetrant pathogenic variants (Table 2) were meant to be stringent. In the evaluation process, each variant was classified as (1) a pathogenic variant, (2) a likely pathogenic variant of uncertain significance (VUS), (3) a VUS, or (4) a likely benign VUS. It was decided not to categorize any variants as definitely benign, given that all variants listed as disease-causing mutations in HGMD have been reported in the literature at least once.

Criteria for each category were discussed and developed during several meetings of the NEXT Medicine RORC. The criteria included the allele frequency of the variant, segregation evidence, the number of affected individuals found with the variant, and status as a de novo mutation. For a variant to be assigned to categories other than VUS or likely benign VUS, its allele frequency was required to be less than that expected for the disease, considering the pattern of inheritance and penetrance. If a variant is present in the general population more commonly than would be expected given the frequency of the associated disease, it is unlikely that the variant causes a high-penetrance phenotype. Cosegregation of a variant was also considered a criterion in determining pathogenicity. A probability of the observed cosegregation

Table 2. Variant Classification Criteria

Variant Type	Classification Criteria
Pathogenic	allele frequency of variant below cutoff[a] AND segregation in at least two unrelated families[b] OR segregation in one family and identified in at least three unrelated affected individuals[c] OR segregation in one family and at least one de novo event in trio[d] OR protein truncation where this event is known to cause disease
Likely pathogenic VUS	allele frequency of variant below cutoff AND identified in at least three unrelated individuals OR segregation in one family OR at least one de novo event in trio
VUS	allele frequency of variant below cutoff AND identified in fewer than three unrelated affected individuals OR no segregation studies OR no de novo events in a trio
Likely benign VUS	allele frequency of variant well above cutoff AND/OR seen in combination with a known pathogenic mutation

[a]Based on disease frequency and inheritance pattern (see "Criteria for Classification of Variants").
[b]Defined as probability of consistent sharing in the family of $\leq 1/16$.
[c]Dependent on allele frequency.
[d]Mutation identified as de novo dominant in an affected offspring of unaffected individuals.

by random chance of 1/16 or less (equivalent to a probability of 0.0625) was decided upon by the NEXT Medicine RORC as a conservative cutoff. The group decided that the presence of only one affected individual with the rare variant was not sufficient to assert causality; however, more than one unrelated individual reported in the literature would considerably strengthen the evidence of a variant's pathogenicity. Thus, the criterion of three or more unrelated affected individuals with the rare variant of interest was decided upon. Finally, if a variant was described in the literature as de novo, this increased suspicion that the variant was pathogenic.

Additionally, in the NEXT Medicine project, we chose not to return VUSs to participants for IFs in genes unrelated to the presenting condition. Both the need for stringent evidence of pathogenicity and the decision not to return an IF VUS derive from the low prior probability that a participant has a pathogenic variant when incidental findings are considered. This is in contrast to an individual who presents clinically with a relevant disorder. For example, a *BRCA1* VUS is more likely to be pathogenic in a woman with breast or ovarian cancer and no known pathogenic variant than in a woman without a personal or family history of related cancers.

Data from multiple sources were evaluated for determining the potential pathogenicity of each variant. Primary literature was compiled from articles cited by the Human Genome Mutation Database (HGMD Professional 2012.3[4]) curators, PubMed, and Google. Variant classifications from databases including the Leiden Open Variant Database, InSiGHT, dbSNP, and the Breast Cancer Information Core (BIC) were also examined. The primary data from these sources and the allele frequency supplied by the NHLBI Exome Variant Server (EVS) were used for classifying variants according to the scheme detailed in Table 2.

Participants and Variant Selection

One thousand participants, 500 of European ancestry and 500 of African ancestry, were randomly selected from the 6,503 individuals in the NHLBI ESP. The variant data are derived from participants from 18 well-phenotyped populations. Details regarding these populations can be found on the ESP website. Ancestry was inferred from analysis of principal components.[5,6] These participants' exome variants were reviewed for the 114 genes of interest (Table 1) for each variant listed as disease causing in HGMD and any disruptive mutations expected to cause disease (truncating variants) but not identified by HGMD as disease-causing mutations. However, these "disease-causing" variants were assumed to be benign for rare autosomal-dominant (AD) disorders when the minor allele frequency (MAF) was >0.005 because they were too common to be highly penetrant pathogenic variants given the disease frequency.

Expert Variant Review

Each reported potential pathogenic variant listed as disease causing in HGMD was assigned to one of 19 expert reviewers. These reviewers were all geneticists: 14 were clinical geneticists, genetic counselors, or certified molecular geneticists, and the remainder had significant relevant genomic expertise. Each reviewer was charged with (1) determining whether the allele frequency was less than a disease-specific maximum frequency and (2) reviewing the primary literature, including all papers cited by HGMD, in order to document these data and determine whether the evidence met the specific pathogenicity criteria (Table 2). The maximum allowable allele frequencies for each disease were calculated under a very conservative model, including the assumption that the given disorder was wholly due to that variant. When disease frequencies were unknown, they were conservatively overestimated. Reviewers were provided with total MAF and ancestry-specific allele frequencies. Ten percent (24 of 239) of the variants were independently double reviewed for pathogenicity, including both the allele frequency and the primary literature review, for quality control. In all cases, the double reviewers were one of two senior scientist clinical geneticists, each with over 10 years of clinical expertise. Each reviewer filled out a spreadsheet that summarized the findings relevant to the pathogenicity categorization. The spreadsheet of all 239 unique variant classifications was also reviewed by a genetic counselor for ensuring that the classification matched the evidence summarized by the reviewer. Any reviewer could nominate a difficult-to-categorize variant for committee review. Thirteen available reviewers met as a group to review the difficult-to-categorize variants and decide on the final pathogenicity. This work was accomplished on data without identifiers or phenotypes, so genotype-phenotype correlations were not possible.

Each reviewer was also asked to record the number of minutes it took to review each variant and make a conclusion. The number of minutes of review time was reduced when electronic links were available for the primary literature cited by HGMD. A genetic counselor was tasked with locating and sending to reviewers any

reference that was not available through local sources. The time to locate a reference, time to train reviewers, and time for the committee to meet were not captured in the variant-review time-taking measures.

Variants that were disruptive, predicted to cause protein truncation, and not listed as disease causing in HGMD were also identified. These variants were further considered if the gained stop codon was in the first 90% of the amino acid sequence. The literature was reviewed for investigating whether truncating mutations are causative of the disease phenotypes for such genes. Literature and ClinVar were both reviewed in the search for prior reports of these variants and data regarding pathogenicity.

Results

One hundred and fourteen actionable genes were identified for return of results and are listed in Table 1. This list includes 88 AD, 4 X-linked, and 22 autosomal-recessive (AR) diseases. In the 1,000 exomes, after exclusion of variants with MAF > 0.005, 239 unique variants occurring a total of 585 times were identified as potentially pathogenic in these loci on the basis of their classification as "disease causing" in HGMD. Among the 239 unique putatively disease-causing variants, 230 were in AD loci, three were in X-linked loci, and six were in AR loci. The AR variants were three pairs of variants within single individuals, given that carrier status was not included for reporting. Five disruptive mutations not listed as disease causing in HGMD were also identified. These disruptive mutations were all in AD loci.

Of the 239 unique variants, 72 (30.1%) had an allele frequency above the disease-specific frequency cutoff in the NHLBI ESP 6,500-exome data set. Thus, these occurred too frequently in the sample to be considered potentially pathogenic for the relevant disorder; however, the literature supporting the disease-causing HGMD status of these 72 variants was still reviewed. None of these 72 were classified as pathogenic or likely pathogenic. Each of the 239 variants was observed between one and ten times in the 1,000 subjects; 123 of these 239 were seen only once. Fifteen of the 16 pathogenic or likely pathogenic variants for dominant diseases were observed once in these 1,000 subjects, and one variant was seen in two subjects. Given that 51.4% (123/239) of these variants were seen only once, these 15 out of 16 represent a significant excess of pathogenic variants of low MAF (binomial p value < 0.0004).

Notably, some variants identified in the literature as being found in multiple individuals with a disease were too common to be highly penetrant pathogenic alleles. Examples include the *PKP2* missense mutations c.1759G>A (p.Val587Ile) (rs146102241) and c.419C>T (p.Ser140Phe) (rs150821281), each of which was identified in five European-descent participants in our cohort and whose frequencies in 4,200 European-descent participants in the ESP were 0.005 and 0.003, respectively. These variants have been associated with arrhythmogenic right ventricular cardiomyopathy (MIM 609040).[7,8] This disorder is expected to affect fewer than 1 in 1,000 individuals, indicating an expected MAF of ~0.001. Similarly, two European-descent participants had an identical putative *TP53* mutation (causing Li-Fraumeni syndrome [MIM 151623]).[9] However, the frequency of this variant (c.847C>T [p.Arg283Cys]) (rs149633775) in the ESP was 0.0004, and 2 of our 500 European-descent participants were carriers (for a frequency of 0.001). The prevalence of Li-Fraumeni syndrome is estimated to be 1 in 20,000.[10] Although these could be low-penetrance alleles for overlapping phenotypes, they could also be benign alleles found in affected individuals by chance. The availability of allele frequencies from sources such as the EVS, with large sample sizes, greatly improves our ability to classify such alleles that are too common to be highly penetrant pathogenic mutations.

Literature and stringent criteria (Table 2) found that of the 239 unique HGMD disease-causing variants identified, only 16 unique AD variants (in 17 participants) and one AR variant pair were pathogenic or likely pathogenic. Thus, 1.8% (18 of 1,000) of the participants analyzed were found to have pathogenic or likely pathogenic actionable variants also listed as disease-causing mutations in HGMD. These variants are listed in Tables 3 and 4. The classifications of all 239 variants are included in Table S1, available online. The eight participants with confirmed pathogenic (versus likely pathogenic) mutations included three with increased risk of breast and ovarian cancer (MIM 604370, caused by *BRCA1* mutations, or MIM 612555, caused by *BRCA2* mutations), one with a mutation in *LDLR*, associated with familial hypercholesterolemia (MIM 614337), one with a mutation in *PMS2*, associated with Lynch syndrome (MIM 614337), and two with mutations in *MYBPC3*, associated with hypertrophic cardiomyopathy (MIM 115197), as well as one person with two *SERPINA1* mutations, associated with the autosomal-recessive disorder alpha-1-antitrypsin deficiency (MIM 613490). The phase of the *SERPINA1* mutations could not be addressed with these data, but compound heterozygosity is most likely given that the variants are unlikely to have occurred on the same ancestral haplotype.

The nominated yield of variants listed as disease causing in HGMD varied by ancestry group. Approximately 71.5% (419/585) of the total variants were identified in individuals of European descent, 25.0% (146/585) were identified in individuals of African descent, and 3.4% (20/585) were identified in the 16 individuals of Ashkenazi Jewish descent (Table 5). Of the 239 unique variants, only 94 (39.3%) were found in those of African descent. This significantly differs from the expected 50%, given that 500 of the 1,000 subjects were of African descent (binomial test p = 0.0006). Eighteen participants had likely pathogenic or pathogenic mutations, and of these, only three (16.7%) were of African descent; again, this is significantly less than the expected 50% (binomial test p = 0.0038).

All 239 unique variants listed as disease causing in HGMD had their spreadsheet of criteria reviewed by a

Table 3. Pathogenic Actionable Variants Listed as Disease Causing in HGMD

Gene	Variant	Reference SNP ID	Primary Associated Condition(s)	Inheritance	Ancestry (n)
BRCA1 (MIM 113705)	NP_009225.1: p.Arg1699Trp NM_007294.3: c.5095C>T NC_000017.10: g.41215948G>A	rs55770810	hereditary breast and ovarian cancer (MIM 604370)	AD	E (1)
BRCA1[a] (MIM 113705)	NP_009225.1: p.Glu908* NM_007294.3: c.2722G>T NC_000017.10: g.41244826C>A	rs80356978	hereditary breast and ovarian cancer (MIM 604370)	AD	E (1)
BRCA2[a] (MIM 600185)	NP_000050.2: p.Tyr1894* NM_000059.3: c.5682C>G NC_000013.10: g.32914174C>G	rs41293497	hereditary breast and ovarian cancer (MIM 612555)	AD	E (1)
LDLR (MIM 143890)	NP_000518.1: p.Ser99* NM_000527.4: c.296C>G NC_000019.9: g.11213445C>G	-	familial hypercholesterolemia (MIM 143890)	AD	E (1)
MYBPC3 (MIM 600958)	NP_000247.2: p.Ala833Thr NM_000256.3: c.2497G>A NC_000011.9: g.47359047C>T	rs199865688	hypertrophic cardiomyopathy (MIM 115197)	AD	E (1)
MYBPC3 (MIM 600958)	NP_000247.2: p.Arg502Trp NM_000256.3: c.1504C>T NC_000011.9: g.47364249G>A	-	hypertrophic cardiomyopathy (MIM 115197)	AD	E (1)
PMS2 (MIM 600259)	NP_000526.1: p.Ser46Ile NM_000535.5: c.137G>T NC_000007.13: g.6045549C>A	rs121434629	Lynch syndrome (MIM 614337)	AD	E (1)
SERPINA1 (MIM 107400)	NP_000286.3: p.Glu366Lys NM_000295.4: c.1096G>A NC_000014.8: g.94844947C>T	rs28929474	alpha 1 antitrypsin deficiency (Z allele) (MIM 613490)	AR[b]	A (1)
SERPINA1 (MIM 107400)	NP_000286.3: p.Glu288Val NM_000295.4: c.863A>T NC_000014.8: g.94847262T>A	rs17580	alpha 1 antitrypsin deficiency (S allele) (MIM 613490)	AR[b]	A (1)

Abbreviations are as follows: AD, autosomal dominant; AR, autosomal recessive; E, European; and A, African.
[a]Based on classification reported by Myriad in the Breast Cancer Information Core database.
[b]Found in the same individual.

single genetic counselor and then discussed with a senior medical geneticist; this led to the movement of two variants among the classes pathogenic, likely pathogenic, and all other. One was upgraded from VUS to likely pathogenic, and one was downgraded from pathogenic to VUS. Of the 28 variants wholly double reviewed, three were discordant among the classes pathogenic, likely pathogenic, and all other. One was downgraded from pathogenic to VUS, one was downgraded from likely pathogenic VUS to VUS, and one was upgraded from VUS to likely pathogenic VUS. These data demonstrate that even expert reviewers can interpret the literature differently.

The allele frequency and literature review of the 239 unique variants took a total of 5,536 min (92.27 hr). Thus, the average time spent reviewing each variant was 23 min (range = 1–135 min) for just the literature review and categorization step, excluding the time to generate the list of potential variants, collect the references, and resolve questionable variants in a group setting. This time was substantially less than that required if all 585 variants had been reviewed on a per person basis.

The five predicted disruptive mutations that were not listed in HGMD as disease-causing mutations are listed in Table 6. These mutations were located within the first 90% (18%–57%) of the transcript and most likely lead to nonsense-mediated mRNA decay. The *BRCA2* variant was classified as pathogenic in ClinVar. The remaining variants were not found in ClinVar; however, return might be warranted because the premature truncations are likely pathogenic, although we did not find literature or ClinVar entries specifically identifying *TMEM43* truncations as pathogenic. Two additional predicted disruptive mutations were identified; however, these stops occurred in the final 10% of the transcript and were thus not included as likely pathogenic, given that such transcripts might not be affected by nonsense-mediated decay and might be functional. Notably, the disruptive mutations identified with a gained stop codon, as opposed to the HGMD listed disease-causing mutations from the literature, were more evenly split with three of the five mutations identified in the African-descent group.

Discussion

This work has several important findings. First, only 23 participants were identified as having pathogenic or likely pathogenic mutations in 114 medically actionable genes whose pathogenic mutations might not present clinically until adulthood (Table 7). In 18 of the participants, these

Table 4. Likely Pathogenic Actionable Variants Listed as Disease Causing in HGMD

Gene	Variant	Reference SNP ID	Primary Associated Condition(s)	Inheritance	Ancestry (n)
CACNB2 (MIM 600003)	NP_963884.2: p.Ser143Phe NM_201590.2: c.428C>T NC_000010.10: g.18789874C>T	rs150528041	Brugada syndrome (MIM 601144)	AD	E (1)
CDH1 (MIM 192090)	NP_004351.1: p.Val832Met NM_004360.3: c.2494G>A NC_000016.9: g.68867247G>A	rs35572355	hereditary diffuse gastric cancer (MIM 137215)	AD	A (1)
DSG2 (MIM 125671)	NP_001934.2: p.Gly812Cys NM_001943.3: c.2434G>T NC_000018.9: g.29125783G>T	rs121913010	arrhythmogenic right ventricular cardiomyopathy (MIM 610193)	AD	E (1)
KCNQ1 (MIM 607542)	NP_000209.2: p.Thr600Met NM_000218.2: c.1799C>T NC_000011.9: g.2869001C>T	rs34516117	long QT syndrome (MIM 192500)	AD	A (1)
LDLR (MIM 143890)	NP_000518.1: p.Ala606Ser NM_000527.4: c.1816G>T NC_000019.9: g.11227645G>T	rs72658865	familial hypercholesterolemia (MIM 143890)	AD	E (1)
MYBPC3 (MIM 600958)	NP_000247.2: p.Glu619Lys NM_000256.3: c.1855G>A NC_000011.9: g.47362731C>T	rs200352299	hypertrophic cardiomyopathy (MIM 115197)	AD	E (2)[a]
MYBPC3 (MIM 600958)	NP_000247.2: p.Gly490Arg NM_000256.3: c.1468G>A NC_000011.9: g.47364285C>T	rs200625851	hypertrophic cardiomyopathy (MIM 115197)	AD	E (1)
SCN5A (MIM 600163)	NP_932173.1: p.Thr1304Met NM_198056.2: c.3911C>T NC_000003.11: g.38603958G>A	rs199473603	long QT syndrome (MIM 603830)	AD	E (1)
TNNT2 (MIM 191045)	NP_001001430.1: p.Arg278Cys NM_001001430.1: c.832C>T NC_000001.10: g.201328373G>A	rs121964857	dilated and hypertrophic cardiomyopathy (MIM 601494 and 115195)	AD	E (1)

Abbreviations are as follows: AD, autosomal dominant; E, European; and A, African.
[a]Found in one participant of Ashkenazi descent.

pathogenic and likely pathogenic variants were identified through classification in HGMD as disease-causing mutations, and five were identified via predicted premature truncations. Second, the vast majority of variants classified by HGMD as disease causing did not meet rigorous criteria to be classified as high-penetrance pathogenic mutations. Of the 239 unique variants classified by HGMD as disease causing, only 7.5%—16 unique AD variants in 17 individuals and two unique AR variants in the same individual—were found to be pathogenic or likely pathogenic. Third, allele frequency was a strong predictor of pathogenicity. Whereas 51.4% (123/239) of the variants evaluated were seen only once in the 1,000 participants, 15 of the 16 pathogenic or likely pathogenic AD variants were only seen once in the cohort. All HGMD variants classified as pathogenic or likely pathogenic had an allele frequency $\leq$ 0.104%. Indeed, no adequate database currently exists for the purpose of rigorously identifying pathogenic mutations at a level sufficient for clinical return. Inclusion of filters on allele frequencies is expected to improve classification. Fourth, there was a deficit of pathogenic variants identified in the African-descent participants, which most likely reflects the deficit of genetic literature on variants in non-European populations. Fifth, review of any one unique variant took, on average, 23 min of expert review time when references were provided.

The ACMG recently made recommendations for mandatory return of variants related to 56 genes for children or adults.[1] Our 114-gene list overlaps the ACMG list by 52 genes relevant to adults (Table 1). The four genes on the ACMG list that do not overlap with our gene list (*RB1* [MIM 614041], *TSC1*, *TSC2*, and *WT1* [MIM 607102]) are related to conditions with pediatric onset only and do not apply to our adult population. Of the 23 participants we identified with pathogenic or likely pathogenic variants, 20 (8 with pathogenic and 12 with likely pathogenic mutations) had variants from the ACMG gene list, including *BRCA1*, *BRCA2*, *CACNA1S*, *DSC2*, *DSG2*, *KCNQ1*, *LDLR*, *MYBPC3*, *PMS2*, *RYR1*, *SCN5A*, *TMEM43*, and *TNNT2*. Three participants had pathogenic or likely pathogenic variants in other genes that our committee considered actionable: *CACNB2*, *CDH1*, and *SERPIN1A*. Thus, the ACMG list successfully identifies the more common actionable genes. The cohorts that provided the samples we analyzed must decide, in consultation with their participants and other stakeholders, whether and how sequencing results will be offered to participants. The data on which these analyses were based are available to the contributing cohorts.

Fault for misclassification of variants in HGMD falls largely on the weak primary literature rather than HGMD extraction errors. In one case, a paper proposed that a

Table 5. Number and Percent of Variants from HGMD by Ancestry

	African Descent	European Descent	Ashkenazi Descent	Total
Participants	500 (50.0%)	484 (48.4%)	16 (1.6%)	1,000
HGMD "disease-causing" variants	146 (25.0%)	419 (71.6%)	20 (3.4%)	585
Unique HGMD "disease-causing" variants	94 (39.3%)	125 (52.3%)	20 (8.4%)	239
Participants with pathogenic variants	1 (12.5%)	7 (87.5%)	0	8
Participants with likely pathogenic variants	2 (20%)	7 (70%)	1 (10%)	10

TP53 VUS with a MAF of 0.001 worsened the phenotype in an affected individual with a separate known pathogenic mutation, ignoring that the known pathogenic variant segregated with the disease on the maternal side and that the VUS was inherited from an apparently unaffected father.[11] Similarly, a second paper proposed that a paternally inherited *FBN1* VUS with a MAF of 0.001, which was found in a proband with a maternally inherited known *FBN1* mutation, contributed to the proband's skeletal features of the phenotype.[12] These reports should not have concluded that these variants were pathogenic. These VUSs were found in individuals with known pathogenic mutations, and their allele frequencies make them too common to be the cause of their associated diseases. More often, the unsupported conclusions rely on weak data such as allele frequency differences in small numbers of cases and controls or the simple identification of a VUS in an affected participant. Going forward, access to the allele frequencies for these variants in the 1000 Genomes Project or through the NHLBI EVS will help identify polymorphisms that occur too frequently to be highly penetrant pathogenic mutations. Occasionally, errors compound one another, e.g., a second publication cites an initial weak report.[13] Although HGMD is certainly nonspecific in its classification of a variant as disease causing, it has an important role in being oversensitive. This allows those interested in pathogenicity to use HGMD as a source of potentially pathogenic variants to review. However, a well-populated source of more specific data is a requirement for the reliable, efficient, and widespread application of genomic medicine.

With regard to the reporting of pathogenic mutations in the literature, we endorse standards that could aid in informatic and manual review for determining the pathogenicity of variants. Clinical laboratories and many journals have adopted the variant nomenclature proposed by the Human Genome Variation Society. Additionally, researchers should detail all segregation data in the pedigree(s), including affected and unaffected individuals, along with the likelihood that the segregation happened by chance rather than rely on uninformative statements such as "the variant segregated with the disease." For example, co-occurrence of a variant with disease in an affected mother and proband pair would be described as having a 50% probability of happening by chance. Reporting information on the genotype and the affected status of the grandparent from whom a dominant allele arose and all other tested individuals would allow quantification of the probability that the segregation occurs by chance.

The literature is beginning to address the issue of actionable IFs. A prior report[14] identified eight pathogenic cancer syndrome variants in 572 ClinSeq participants; however, seven of these were *BRCA1* or *BRCA2* mutations, and four were in participants of Ashkenazi Jewish descent. That paper surveyed the literature for pathogenic variants, but other than having a MAF < 0.015, it only specified general (e.g., segregation) rather than quantitative criteria for inclusion. Of the 572 ClinSeq participants, 97 (17%) were Ashkenazi. Excluding these, 3 of 475 (0.6%) non-Ashkenazi participants had such pathogenic cancer variants. Only 16 of the 500 European-descent participants in the current report, or ~3.2% of the total sample, were Ashkenazi, and only 1 out of the 23 (4.3%) participants with pathogenic or likely pathogenic variants was Ashkenazi (Tables 3, 4, and 5). To our knowledge, this *MYBPC3* c.1855G>A (p.Glu619Lys) (rs200352299) variant is not an Ashkenazi founder mutation. Overall, 5 of our 1,000 participants had pathogenic variants, and another had a likely pathogenic actionable variant relevant to cancer, similar to the ClinSeq[14] experience (after the decreased numbers of Ashkenazi participants are considered).

An analysis by Xue et al. of 179 participants from the 1000 Genomes Project estimated that each individual would have on average two disease-causing mutations.[15] However, this work did not limit itself to highly penetrant, actionable variants. Further, although HGMD classification was reviewed in the primary literature, a single source or functional-study evidence appears to have been sufficient for classification as a pathogenic mutation. Given that Xue et al.'s goal was estimation of pathogenic-variant load and our goal was high confidence that returned variants would be clinically useful, it is understandable that they used a less stringent test than the criteria used here. As in the current study, these authors emphasize the need for better databases of disease alleles. This underscores our conclusion that the primary literature on which HGMD draws requires more stringent criteria to support that an allele is disease causing.

Variants that are pathogenic in individuals of European descent are expected to be pathogenic in participants of other ancestry groups,[16] but variants that do not occur in those of European descent are understudied. Fifty percent

Table 6. Disruptive Variants Not Listed as Disease Causing in HGMD

Gene	DNA Change	Protein Change	Amino Acid Length	Reference SNP ID	Primary Associated Condition(s)	Inheritance	Ancestry (n)
BRCA2[a] (MIM 600185)	c.5855T>A	p.Leu1952*	3,419	-	hereditary breast and ovarian cancer (MIM 612555)	AD	A (1)
CACNA1S (MIM 114208)	c.2707G>A	p.Arg903*	1,874	-	malignant hyperthermia (MIM 601887)	AD	E (1)
DSC2 (MIM 125645)	c.663A>T	p.Tyr221*	848; 902	rs145476705	arrhythmogenic right ventricular dysplasia (MIM 610476)	AD	A (1)
RYR1 (MIM 180901)	c.2662C>T	p.Gln888*	5,039; 5,034	rs141838633	malignant hyperthermia (MIM 145600)	AD	A (1)
TMEM43[b] (MIM 612048)	c.578C>A	p.Ser193*	401	rs140380494	arrhythmogenic right ventricular dysplasia (MIM 604400)	AD	E (1)

Abbreviations are as follows: AD, autosomal dominant; E, European; and A, African.
[a]Listed as pathogenic in ClinVar (submission accession number RCV000031583.1).
[b]No literature citing truncations in *TMEM43* as causative of arrhythmogenic right ventricular dysplasia was identified. No truncating mutations in *TMEM43* were present in HGMD or ClinVar.

of our study cohort was of European descent, but 83% (15/18) of the participants were found to have potentially returnable results from this group with the use of HGMD for identifying disease-causing mutations. The deficit in likely pathogenic or pathogenic variants identified in the African-descent group, which is expected to have more variation overall, is probably due primarily to the underrepresentation of these participants in the literature[17]-based reporting for disease variants; however, other reasons have been postulated.[18] When the five disruptive mutations not listed as disease causing in HGMD were added, a total of 74% (17/23) of subjects with returnable results were of European descent, which remains less than 50% (binomial $p = 0.012$; Table 7). The result is that genomic testing is more informative for those of European descent, causing a health-care disparity that is best addressed by active inclusion of non-European-descent individuals in studies that characterize variants.

Our study informs the types of efforts required of a clinical genomics lab to call pathogenic variants. It also informs the debate about return of IFs to research participants. Although there is some consensus that researchers should return the genetic IFs that they identify to their interested participants,[19,20] there is a debate about whether investigators are obligated to actively look for such results.[21] These data suggest that the search for and competent interpretation of genomic variants are not inconsequential tasks.

Limitations of the current work include (1) the study of only 500 each of European- and African-decent participants, (2) the exclusion of pediatric-onset conditions, (3) the reliance on HGMD and disruptive mutations for identifying potentially pathogenic mutations, and (4) the lack of access to phenotypes. These results might not be generalizable to all ethnic groups, but because European-decent groups are studied the most, the search for known pathogenic mutations might be most fruitful in this racial group. A larger number of possibly pathogenic actionable findings than we describe here might be identified in infants and young children, given that we excluded genes for conditions that would be uniformly diagnosed in childhood. We did include pediatric-onset conditions that might remain undiagnosed into adulthood, e.g., mild phenylketonuria (MIM 261600). Finally, it is possible that some pathogenic variants were missed because of their not being labeled as disease causing in HGMD or disruptive. However, it is unlikely that variants not identified by HGMD would meet our strict criteria for pathogenicity; rather, HGMD is oversensitive and not specific. Our results suggest that we did not markedly overcall or undercall variants. For example, *LDLR* mutations are expected to be found in 1/500 individuals,[22] and we found two pathogenic or likely pathogenic mutations in 1,000 people. Similarly, we found one pathogenic mutation for Lynch syndrome and three *BRCA1* or *BRCA2* mutations,[23,24] which are also each expected to be found in 1/350 to 1/1,000 people. Use of ESP data might have enriched for *LDLR* or *SERPINA1* mutations, given the inclusion of subjects with atherosclerosis and chronic obstructive pulmonary disease, although pathogenic variants in these conditions occur at the expected population frequencies. All other pathogenic or likely pathogenic variants appear unrelated to the ascertainment of the ESP cohorts.

The term IF is imperfect for describing genome or exome findings that are not related to the condition for which the test was ordered. IFs are generally considered to be unavoidable consequences of a test or clinical evaluation. However, a genomic test need not query for results in genes unrelated to the testing purpose, and indeed, these types of findings might be actively sought.[25] One alternative term, "secondary findings," has also been criticized; the term secondary has the specific meaning in medicine of being due to another cause, for example, secondary hyperparathyroidism as a response to hypocalcemia. Further, secondary might suggest to a patient that the results are less important than they truly are. "Unanticipated findings[26]" has

Table 7. Number of Individuals with Pathogenic or Likely Pathogenic Variants by Ancestry

Classification	European Ancestry	African Ancestry
Pathogenic variants from HGMD	7/500	1/500
Likely pathogenic variants from HGMD	8/500	2/500
Disruptive pathogenic variants	0/500	1/500
Disruptive likely pathogenic variants	2/500	2/500
Total	**17/500**	**6/500**

been criticized given that we do expect, and should plan for, such results. Reflecting on similarities to other types of medical opportunistic screening, "opportunistic findings" has been suggested to reflect that extra steps are required for identifying these findings but has also been criticized because of a perceived negative connotation of the word opportunistic. "Unrelated findings" has also been suggested[27] but does not fully capture the concept and does not have broad recognition. We chose to use the term IFs here given that it was the preferred term in the recent ACMG paper[1] and is commonly used in the genomics literature. Over time, consensus might develop around an alternative term for these genomic findings, such as "ancillary," but there is no commonly used or readily understandable alternative at the present time.

In summary, we found that ~3.4% of European-descent adults and ~1.2% of African-descent adults can be expected to have actionable highly penetrant pathogenic or likely pathogenic mutations identified by exome sequencing at this time. Twelve of the 1,000 participants had pathogenic mutations in genes for which the ACMG recommends mandatory review and return of IFs to adults. We suggest that consistent criteria should be developed for the reporting of pathogenic variants and that these criteria should be more stringent for IFs, where the prior probability that a variant is pathogenic is lower than for genes related to the indication for the test. Current databases do not adequately report pathogenicity for large numbers of variants, and these reviews are time consuming. Current literature also identifies fewer pathogenic variants in those of African descent, most likely because of the underrepresentation of these participants in clinical and research reports. Finally, we endorse standard reporting formats for pathogenic variants to aid in literature review.

Supplemental Data

Supplemental Data include Supplemental Acknowledgments and one table and can be found with this article online at http://www.cell.com/AJHG.

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Web Resources

The URLs for data presented herein are as follows:

Breast Cancer Information Core, http://research.nhgri.nih.gov/bic/
ClinVar, http://www.ncbi.nlm.nih.gov/clinvar/
dbSNP, http://www.ncbi.nlm.nih.gov/SNP/
Human Gene Mutation Database (HGMD) Professional, http://www.biobase-international.com/product/hgmd
Human Genome Variation Society, http://www.hgvs.org/mutnomen
InSiGHT, http://www.insight-group.org
Leiden Open Variant Database, http://www.lovd.nl/3.0/home
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

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