Original Investigation

Association of Arrhythmia-Related Genetic Variants With Phenotypes Documented in Electronic Medical Records

Sara L. Van Driest, MD, PhD; Quinn S. Wells, MD, PharmD, MSCI; Sarah Stallings, PhD; William S. Bush, PhD, MS; Adam Gordon, PhD; Deborah A. Nickerson, PhD; Jerry H. Kim, MD; David R. Crosslin, PhD; Gail P. Jarvik, MD, PhD; David S. Carrell, PhD; James D. Ralston, MD, MPH; Eric B. Larson, MD, MPH; Suzette J. Bielinski, PhD; Janet E. Olson, PhD; Zi Ye, MD, PhD; Iftikhar J. Kullo, MD; Noura S. Abul-Husn, MD, PhD; Stuart A. Scott, PhD; Erwin Bottinger, MD; Berta Almoguera, PhD; John Connolly, PhD; Rosetta Chiavacci, BSN, CCRC; Hakon Hakonarson, MD, PhD; Laura J. Rasmussen-Torvik, PhD, MPH; Vivian Pan, MS, CGC; Stephen D. Persell, MD, MPH; Maureen Smith, MS, CGC; Rex L. Chisholm, PhD; Terrie E. Kitchner, CCRP; Max M. He, PhD; Murray H. Brilliant, PhD; John R. Wallace, MS; Kimberly F. Doheny, PhD; M. Benjamin Shoemaker, MD, MCSI; Rongling Li, MD, PhD, MPH; Teri A. Manolio, MD, PhD; Thomas E. Callis, PhD; Daniela Macaya, MQC; Marc S. Williams, MD; David Carey, PhD; Jamie D. Kapplinger, BA; Michael J. Ackerman, MD, PhD; Marylyn D. Ritchie, PhD; Joshua C. Denny, MD, MS; Dan M. Roden, MD

IMPORTANCE Large-scale DNA sequencing identifies incidental rare variants in established Mendelian disease genes, but the frequency of related clinical phenotypes in unselected patient populations is not well established. Phenotype data from electronic medical records (EMRs) may provide a resource to assess the clinical relevance of rare variants.

OBJECTIVE To determine the clinical phenotypes from EMRs for individuals with variants designated as pathogenic by expert review in arrhythmia susceptibility genes.

DESIGN, SETTING, AND PARTICIPANTS This prospective cohort study included 2022 individuals recruited for nonantiarrhythmic drug exposure phenotypes from October 5, 2012, to September 30, 2013, for the Electronic Medical Records and Genomics Network Pharmacogenomics project from 7 US academic medical centers. Variants in *SCN5A* and *KCNH2*, disease genes for long QT and Brugada syndromes, were assessed for potential pathogenicity by 3 laboratories with ion channel expertise and by comparison with the ClinVar database. Relevant phenotypes were determined from EMRs, with data available from 2002 (or earlier for some sites) through September 10, 2014.

EXPOSURES One or more variants designated as pathogenic in *SCN5A* or *KCNH2*.

MAIN OUTCOMES AND MEASURES Arrhythmia or electrocardiographic (ECG) phenotypes defined by *International Classification of Diseases, Ninth Revision* (*ICD-9*) codes, ECG data, and manual EMR review.

RESULTS Among 2022 study participants (median age, 61 years [interquartile range, 56-65 years]; 1118 [55%] female; 1491 [74%] white), a total of 122 rare (minor allele frequency <0.5%) nonsynonymous and splice-site variants in 2 arrhythmia susceptibility genes were identified in 223 individuals (11% of the study cohort). Forty-two variants in 63 participants were designated potentially pathogenic by at least 1 laboratory or ClinVar, with low concordance across laboratories (Cohen κ = 0.26). An *ICD-9* code for arrhythmia was found in 11 of 63 (17%) variant carriers vs 264 of 1959 (13%) of those without variants (difference, +4%; 95% CI, −5% to +13%; P = .35). In the 1270 (63%) with ECGs, corrected QT intervals were not different in variant carriers vs those without (median, 429 vs 439 milliseconds; difference, −10 milliseconds; 95% CI, −16 to +3 milliseconds; P = .17). After manual review, 22 of 63 participants (35%) with designated variants had any ECG or arrhythmia phenotype, and only 2 had corrected QT interval longer than 500 milliseconds.

CONCLUSIONS AND RELEVANCE Among laboratories experienced in genetic testing for cardiac arrhythmia disorders, there was low concordance in designating *SCN5A* and *KCNH2* variants as pathogenic. In an unselected population, the putatively pathogenic genetic variants were not associated with an abnormal phenotype. These findings raise questions about the implications of notifying patients of incidental genetic findings.

JAMA. 2016;315(1):47-57. doi:10.1001/jama.2015.17701

Editorial page 33

Supplemental content at jama.com

Author Affiliations: Author affiliations are listed at the end of this article.

Corresponding Author: Dan M. Roden, MD, Vanderbilt University Medical Center, 2215B Garland Ave, Room 1285, Nashville, TN 37232-0575 (dan.roden@vanderbilt.edu).

Sequencing of selected gene sets, whole exomes, and whole genomes is increasingly used for research and clinical care. These approaches also identify incidental findings (also called secondary findings) of potential clinical relevance. Driven by the prospect for preclinical diagnosis and risk factor mitigation, the American College of Medical Genetics and Genomics (ACMG) has supported the return of medically actionable incidental findings and generated a list of genes in which known or predicted pathogenic variants should be returned to patients who undergo clinical sequencing.[1,2] These recommendations have been controversial because the frequency of clinical manifestations of these variants and their implications for diagnosis and management are poorly defined.[3-6]

The burden of rare, possibly deleterious variants in the human genome is much higher than previously assumed.[7,8] However, the frequency of clinical phenotypes in unselected individuals with these variants is unknown. Genomic and phenotypic data from large, unselected populations are required to define the true risk associated with genetic variants identified as incidental findings.

One approach is to examine phenotypes in electronic medical records (EMRs) in a large cohort unselected for any specific phenotype. The Electronic Medical Records and Genomics (eMERGE) network has developed and validated algorithms to extract phenotypes from EMRs and associate these with genotypes determined from banked DNA.[9-11] The eMERGE Pharmacogenomics (eMERGE-PGx) project is sequencing 84 genes involved in drug response (pharmacogenes).[12] Two of these genes, *SCN5A* (NG_008934.1) and *KCNH2* (NG_008916.1), were included because they encode ion channels with which many drugs interact. Rare variants in these genes can also cause congenital long QT syndrome, Brugada syndrome, and other genetic arrhythmias (aggregate population prevalence <1:1000).[13] Here, we report the arrhythmia and electrocardiographic (ECG) phenotypes determined through EMR phenotyping in participants with rare, potentially pathogenic variants in these 2 genes.[13]

Methods

Study Participants

The design and implementation of the eMERGE-PGx project have been reported.[12] In brief, select pharmacogenes were sequenced in a diverse cohort of approximately 9000 patients who consented to have genetic results included in their EMR for genome-informed clinical decision support. Participants were recruited based on their likelihood of being prescribed medications with relevant pharmacogenomic associations (eTable 1 in the Supplement).[12] The project was reviewed and approved by the institutional review board of each of the 10 participating sites. Written consent was obtained from study participants or their parent or guardian (for pediatric patients). Sequence and EMR data for the individuals consecutively enrolled between October 5, 2012, and September 30, 2013, in the eMERGE-PGx project were analyzed, which includes data from 7 sites.

DNA Sequencing, Variant Calling, and Variant Annotation

DNA samples were sequenced using PGRNseq, a custom sequencing assay designed to produce highly accurate DNA sequences of 84 selected pharmacogenes.[14] Single-nucleotide variants were called in accordance with the Genome Analysis Toolkit best practices and filtered for nonsynonymous and splice-site variants by the eMERGE coordinating center.[15] Copy-number variants were assessed using a read-depth–based algorithm. Minor allele frequency (MAF, number of alleles with the variant divided by the total number of sequenced alleles) was calculated for each variant based on study data and Exome Aggregation Consortium data, with rare variants defined as MAF less than 0.5% in the study cohort. Sequence data for all putatively pathogenic variant calls were reviewed manually and confirmed or refuted using Sanger sequencing as needed. Additional details are available in the eMethods in the Supplement.

Variant Classification

Two molecular diagnostic laboratories (GeneDx, Gaithersburg, Maryland, and Transgenomic, New Haven, Connecticut) and 1 research laboratory (the Mayo Clinic Windland Smith Rice Sudden Death Genomics Laboratory, Rochester, Minnesota), all with expertise in ion channel gene variant evaluation, assessed potential pathogenicity of all missense and splice-site polymorphisms in *SCN5A* and *KCNH2*. Each laboratory was blinded to the assessments by the other laboratories and to any demographic or clinical data. All variants designated as "pathogenic" or "likely pathogenic" by any 1 of the 3 laboratories or by ClinVar,[16] the submitter-driven public archive of variant-phenotype associations, were considered to be putatively pathogenic and are referred to as "designated variants" (eMethods in the Supplement). Each laboratory is deidentified by randomly assigned labels of laboratory 1, 2, or 3.

Identification of Arrhythmia and ECG Phenotypes

For all participants, demographic data; *International Classification of Diseases, Ninth Revision* (*ICD-9*) data for relevant arrhythmia and ECG phenotypes; and all available machine-read ECG intervals for all ECGs in the EMR were extracted from the oldest EMR available (2002 or earlier for all sites) through September 10, 2014 (eTable 2 in the Supplement). Length of EMR follow-up was calculated for each individual as the difference between the first and last EMR data point available. Collection of race and ethnicity data, used to calculate differences in variant frequency expected due to ancestry-specific variation, varied by site (self-report or EMR-derived); participants missing race/ethnicity data were coded as "unknown." Manual review of EMR data was performed for the subset of participants with designated variants and not those without variants (eFigure and eMethods in the Supplement). All available ECG tracings from individuals with designated variants as well as all available ECG tracings from age-, race-, and sex-matched individuals without pathogenic variants were manually reviewed by 2 independent cardiologists blinded to gene variant status (eTable 3 in the Supplement).

Table 1. Demographic and Arrhythmia Characteristics of Participants With and Without Designated Variants[a]

	Overall Cohort (N = 2022)	No Designated Variant (n = 1959)	Designated Variant Carrier (n = 63)	*P* Value
Age, median (IQR), y	61 (56-65)	61 (55-65)	60 (57-64)	.60[b]
Male sex, No. (%)	904 (45)	875 (45)	29 (46)	.90[c]
Race, No. (%)				
White	1491 (74)	1461 (75)	30 (48)	<.001[c]
Black or African American	283 (14)	271 (14)	12 (19)	
Asian	84 (4)	66 (3)	18 (29)	
American Indian or Alaska Native	14 (1)	14 (1)	0	
Native Hawaiian or other Pacific Islander	2 (0.1)	1 (0.1)	1 (2)	
Unknown	148 (7)	146 (7)	2 (3)	
Ethnicity, No. (%)				
Hispanic or Latino	143 (7)	141 (7)	2 (3)	.31[c]
Not Hispanic or Latino	1798 (89)	1741 (89)	57 (90)	
Unknown	81 (4)	77 (4)	4 (6)	
Site of recruitment, No. (%)				
Group Health, University of Washington	894 (44)	866 (44)	28 (44)	.61[c]
Mayo Clinic	296 (15)	281 (14)	15 (24)	
Mount Sinai	291 (14)	283 (14)	8 (13)	
Children's Hospital of Philadelphia	282 (14)	276 (14)	6 (10)	
Marshfield Clinic	91 (5)	89 (5)	2 (3)	
Northwestern University	89 (4)	87 (4)	2 (3)	
Vanderbilt University Medical Center	79 (4)	77 (4)	2 (3)	
Length of EMR follow-up, median (IQR), y[d]	18 (9-20)	18 (9-20)	19 (9-20)	.60[b]
Arrhythmia *ICD-9* codes in EMR, No. (%)				
AV block	83 (4)	78 (4)	5 (8)	.18[c]
Bundle-branch block	108 (5)	106 (5)	2 (3)	.77[c]
Long QT	2 (0.1)	2 (0.1)	0	>.99[c]
Atrial fibrillation	92 (5)	88 (4)	4 (6)	.53[c]
Conduction disorder	6 (0.3)	6 (0.3)	0	>.99[c]
Any arrhythmia code	275 (14)	264 (13)	11 (17)	.35[c]
ECG parameters from EMR, No. (%)	1270 (63)	1234 (63)	36 (57)	.36[c]
Maximum heart rate, median (IQR), /min	76 (65-90)	76 (65-90)	73 (65-82)	.11[b]
Maximum PR interval, median (IQR), ms	164 (150-182)	164 (150-182)	172 (151-193)	.24[b]
Maximum QRS interval, median (IQR), ms	92 (86-102)	92 (86-100)	92 (85-104)	.78[b]
Maximum QT interval, median (IQR), ms	420 (398-442)	420 (398-442)	416 (395-437)	.37[b]
Maximum QTc, median (IQR), ms[e]	443 (423-465)	439 (421-460)	429 (414-458)	.17[b]
PR >200 ms, No. (%)	135 (7)	130 (7)	5 (8)	.58[c]
QRS interval >120 ms, No. (%)	82 (4)	81 (4)	1 (2)	.73[c]
QTc >500 ms, No. (%)[e]	71 (4)	70 (4)	1 (2)	.72[c]

Abbreviations: AV, atrioventricular; ECG, electrocardiographic; EMR, electronic medical record; *ICD-9*, *International Classification of Diseases, Ninth Revision*; IQR, interquartile range; QTc, corrected QT interval.

[a] All variants designated as "pathogenic" or "likely pathogenic" by any 1 of the 3 laboratories or by ClinVar.[16]

[b] *P* value from Wilcoxon rank sum (Mann-Whitney) test comparing variant carriers with noncarriers.

[c] *P* value from Fisher exact test comparing variant carriers with noncarriers.

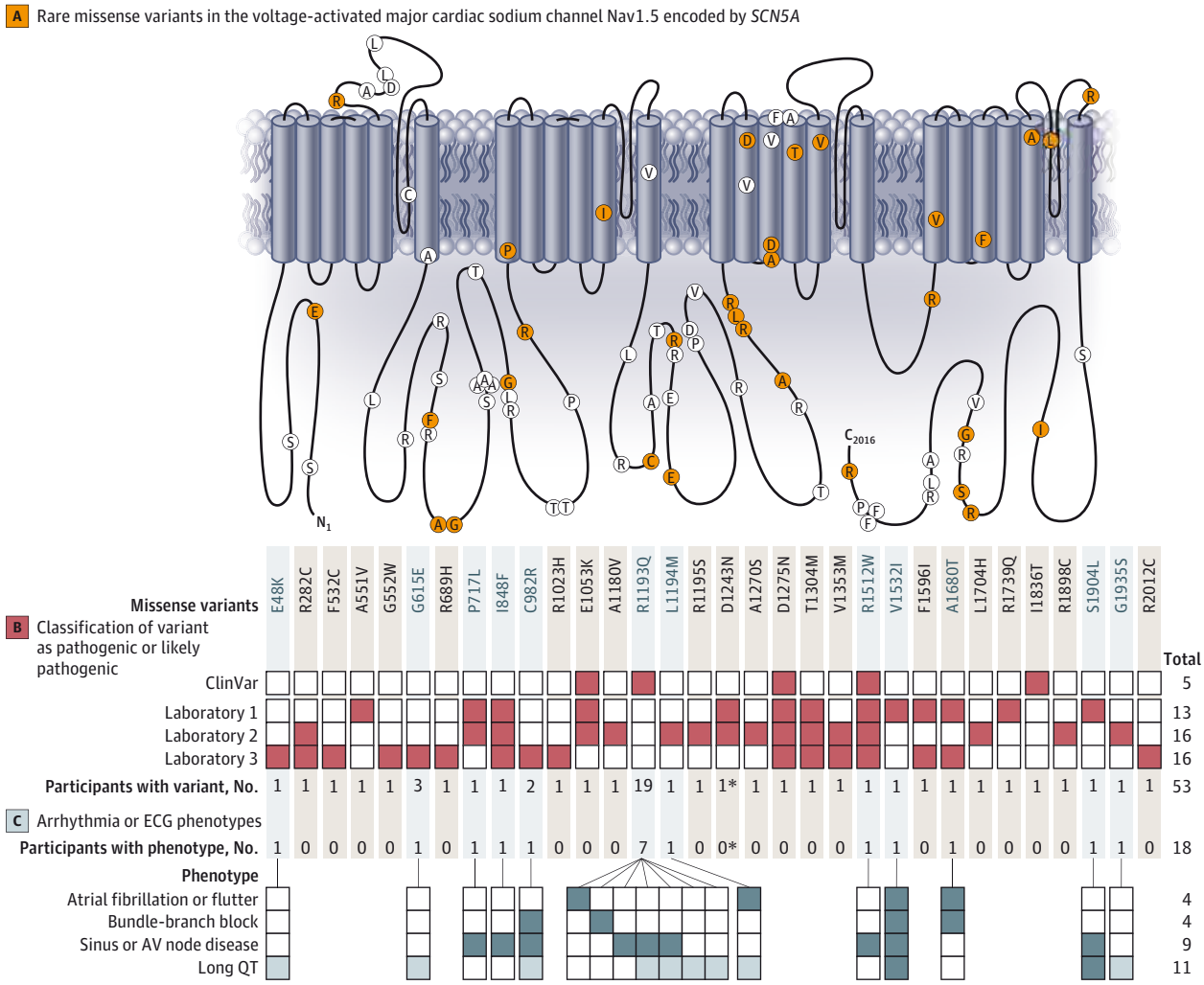
[d] Calculated as the difference in years between the first and last EMR data points available for assessment.

[e] Corrected for heart rate using Bazett formula (QTc = QT/$\sqrt{}$[RR in seconds]).

Statistical Analysis

Two-sided Pearson χ^2, Wilcoxon rank sum, or Fisher exact tests were performed; 95% confidence intervals were calculated; and unweighted Cohen κ for agreement across laboratories was calculated using Stata version 13.1 (StataCorp) or R version 3.1.2 (R Project for Statistical Computing), with statistical significance considered if $P < .05$. Stratified analyses by age (<30 years, 30-60 years, and >60 years at time of study enrollment) were performed to assess for survival bias.

Figure 1. Rare Variants Identified in *SCN5A*



A, All 81 rare (minor allele frequency <0.5%) missense variants in the study population are designated as circles with the single-letter amino acid code for the reference sequence on the protein structure[33,34] of the encoded voltage-activated sodium channel. Variants in orange (n = 32) were designated as pathogenic or likely pathogenic by ClinVar or at least 1 of the 3 expert laboratories.

B, Variant classification by ClinVar and the 3 independent expert laboratories, with pathogenic or likely pathogenic designation indicated in red, for the 32 rare variants with at least 1 such designation. The numbers of participants with each variant and phenotypes are shown below. Totals for each row are listed to the right. One participant had both *SCN5A*-D1243N and *KCNH2*-T983I, designated with an asterisk.

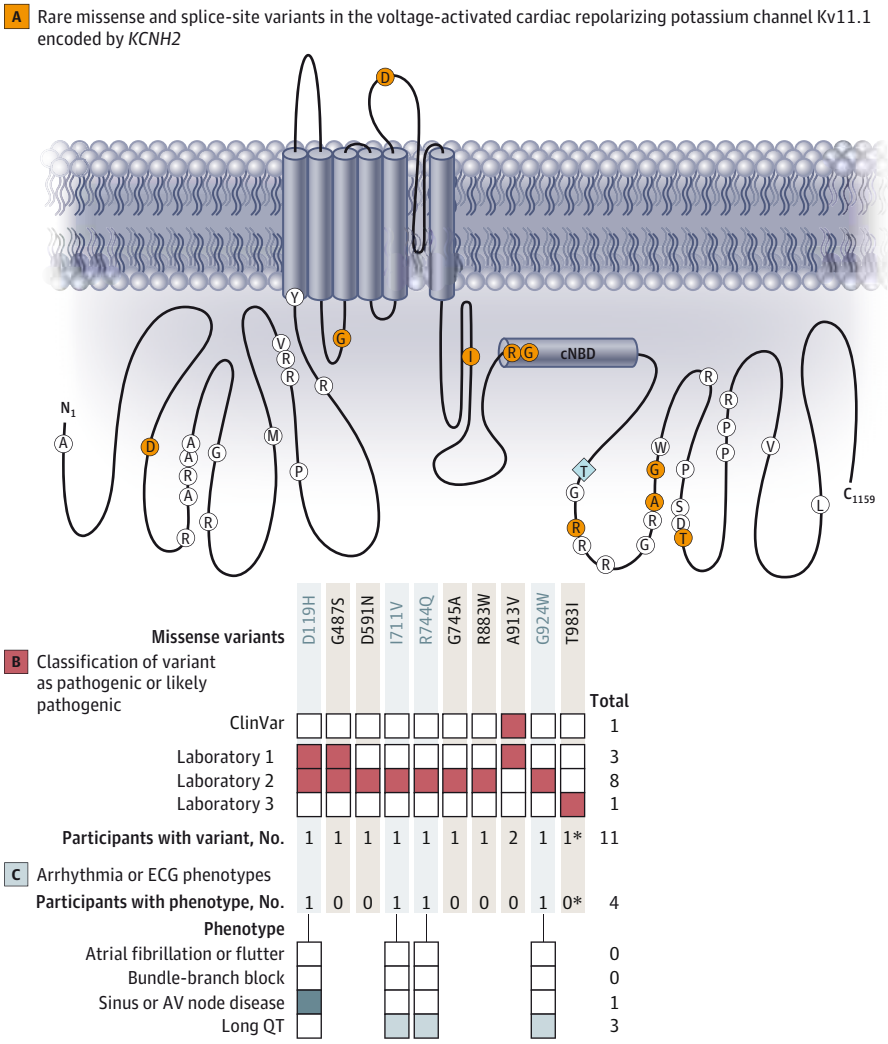
C, Arrhythmia and electrocardiographic (ECG) phenotypes, indicated in blue, identified through electronic medical record and ECG review of all participants with 1 or more putatively pathogenic variants; aggregated data from Tables 1, 2, and 3 are shown. Lines above join the phenotype to the variant identified. Electronic medical records were searched using *International Classification of Diseases, Ninth Revision* (*ICD-9*) codes and keywords for each phenotype (listed in eTable 2 in the Supplement), with manual review of the record to confirm accuracy. All available ECG tracings were reviewed by 2 cardiologists using the criteria in eTable 3. No individuals were identified with Brugada syndrome or cardiac pacing. For long QT syndrome, light blue denotes corrected QT interval (QTc) of 450-500 milliseconds for males or 460-500 milliseconds for females, while dark blue indicates at least 1 ECG with QTc longer than 500 milliseconds. AV indicates atrioventricular.

Results

Study Participants and Sequencing Data

The study cohort (**Table 1**) of 2022 participants had a median age of 61 years (range, 3-103 years, interquartile range [IQR], 56-65 years). About half were female (n = 1118, 55%). Most were white (n = 1491, 74%) and not Hispanic or Latino (n = 1798, 89%). Electrocardiographic data were available for 1270 participants (63%), and median length of EMR follow-up was 18 years (IQR, 9-20 years). The mean per-sample sequencing depth was 502× platform-wide (indicating that on average, 502 reads were available to assess the genotype at each sequenced position for each participant's DNA), with mean per-sample coverage of *SCN5A* and *KCNH2* of 578× and 400×, respectively.

Figure 2. Rare Variants Identified in *KCNH2*



A, All 41 rare (minor allele frequency <0.5%) variants in the study population are designated as circles (missense variants) or a blue diamond (splice-site variant at position 150645629) with the single-letter amino acid code for the reference sequence on the protein structure[33,35] of the encoded voltage-activated potassium channel, Kv11.1 (sometimes called HERG). Variants in orange (n = 10) were designated as pathogenic or likely pathogenic by ClinVar or at least 1 of the 3 expert laboratories.

B, Variant classification by ClinVar and the 3 independent expert laboratories, with pathogenic or likely pathogenic designation indicated in red, for the 10 rare variants with at least 1 such designation. The numbers of participants with each variant and phenotypes are shown below. Totals for each row are listed to the right. One participant had both *SCN5A*-D1243N and *KCNH2*-T983I, designated with an asterisk.

C, Arrhythmia and electrocardiographic (ECG) phenotypes, indicated in blue, identified through electronic medical record and ECG review of all participants with 1 or more putatively pathogenic variants; aggregated data from Tables 1, 2, and 3 are shown. Lines above join the phenotype to the variant identified. Electronic medical records were searched using *International Classification of Diseases, Ninth Revision* (*ICD-9*) codes and keywords for each phenotype (listed in eTable 2 in the Supplement), with manual review of the record to confirm accuracy. All available ECG tracings were reviewed by 2 cardiologists using the criteria in eTable 3. No individuals were identified with Brugada syndrome or cardiac pacing. For long QT syndrome, light blue denotes corrected QT interval of 450-500 milliseconds for males or 460-500 milliseconds for females. AV indicates atrioventricular; cNBD, cyclic nucleotide binding domain.

Variant Identification and Classification

We identified no copy-number variants, 1 splice-site variant, and 126 nonsynonymous variants (eTables 4 and 5 in the Supplement), including 5 common variants with MAFs ranging from 1% to 25%. The splice-site variant and 121 of the missense variants were rare (**Figure 1** and **Figure 2**). One or more of these rare variants were found in 223 participants (11% of the cohort). Manual review of sequencing data indicated potential false-positive calls for rs77331749 (*KCNH2*-A913V) in 5 of 7 samples; Sanger sequencing confirmed the absence of the variant in all 5 suspect samples.

At least 1 of the 3 expert laboratories designated 40 variants as pathogenic or likely pathogenic (eTable 6 in the Supplement); all 40 were rare. Laboratory 1 designated 16 variants; laboratory 2, 24 variants; and laboratory 3, 17 variants. There was agreement by at least 2 of the 3 labo-

Table 2. Participants With Designated Variants and Arrhythmia or Electrocardiographic Phenotypes

Age, y/Sex	Race and Ethnicity	Variant	No. of Laboratories Designating Variant Pathogenic[a]	ClinVar Annotation	Arrhythmia Phenotype	
					From EMR Review	From ECG Review[b]
61/M	White, NH	rs199473048 *SCN5A* E48K	1	None	None	Long QTc <500 ms
63/F	White, NH	rs12720452 *SCN5A* G615E	1	None	None	Long QTc <500 ms
59/M	Asian, NH	rs199473149 *SCN5A* P717L	2	None	None	AV node disease
63/M	African American, NH	rs199473166 *SCN5A* I848F	3	None	None	AV node disease
56/M	African American, NH	rs199473182 *SCN5A* C982R	1	None	Bundle-branch block	AV node disease, long QTc <500 ms
65/F	White, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	Atrial fibrillation	Atrial fibrillation
60/F	Asian, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	None	Bundle-branch block
67/F	Asian, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	Sinus or AV node disease	AV node disease
77/M	White, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	None	AV node disease, long QTc <500 ms
59/M	Asian, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	None	AV node disease, long QTc <500 ms
57/F	Asian, NH	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	None	Long QTc <500 ms
66/F	Asian, Unknown	rs41261344 *SCN5A* R1193Q	0	Pathogenic for Brugada, risk factor for long QT	None	Long QTc <500 ms
57/F	White, NH	(No rs ID) *SCN5A* L1194M	1	None	Atrial fibrillation	Long QTc <500 ms
20/M	White, NH	rs137854602 *SCN5A* R1512W	3	Pathogenic for Brugada	None	AV node disease
83/M	White, NH	rs199473618 *SCN5A* V1532I	1	None	Atrial fibrillation, bundle-branch block, and sinus or AV node disease	Atrial fibrillation, AV node disease, long QTc >500 ms
67/M	White, NH	rs199473294 *SCN5A* A1680T	2	None	Atrial fibrillation and bundle-branch block	Atrial fibrillation
64/F	African American, NH	rs150264233 *SCN5A* S1904L	1	None	Sinus or AV node disease and long QT	Long QTc >500 ms
58/F	White, NH	rs199473637 *SCN5A* G1935S	1	None	None	Long QTc <500 ms
61/M	African American, NH	(No rs ID) *KCNH2* D119H	2	None	None	AV node disease
60/M	White, NH	rs199473532 *KCNH2* I711V	1	None	None	Long QTc <500 ms
66/F	African American, NH	(No rs ID) *KCNH2* R744Q	1	None	Long QT	Long QTc <500 ms
23/F	African American, NH	(No rs ID) *KCNH2* G924W	1	None	None	Long QTc <500 ms

Abbreviations: AV, atrioventricular; ECG, electrocardiogram; EMR, electronic medical record; F, female; M, male; NH, not Hispanic; QTc, corrected QT interval.

[a] No. of the 3 laboratories designating variant as "pathogenic" or "likely pathogenic."

[b] QT interval corrected for heart rate using Bazett formula (QTc = QT/$\sqrt{}$[RR in seconds]).

ratories for 13 of 40 designated variants (33%), and only 4 variants (10%) were designated by all 3 laboratories (Cohen κ = 0.26). ClinVar designated 6 rare variants as pathogenic or likely pathogenic, 4 of which were designated by at least 1 expert laboratory and 2 of which were not. The 42 rare variants designated by at least 1 of the 3 laboratories or ClinVar were present in 63 participants. One participant had both rs199473599 (*SCN5A*-D1243N) and rs149955375 (*KCNH2*-T983I), while the remaining had 1 designated variant.

Arrhythmia and ECG Phenotypes

Based on the demographic, *ICD-9*, and ECG interval data extracted from the EMRs, the 63 participants with designated variants were not different from those without such variants with respect to age, sex, or site of recruitment (Table 1). Those with designated variants were less likely to be white (n = 30/63 [48%] vs 1461/1959 [75%]; difference, −27%; 95% CI, −39% to −14%; $P < .001$). Frequencies of *ICD-9* codes for arrhythmia diagnoses did not differ significantly between

groups (11/63 [17%] variant carriers vs 264/1959 [13%] without variants; difference, +4%; 95% CI, −5% to +13%; *P* = .35). The proportion of patients with ECG data available for analysis was not significantly different between groups (36/63 [57%] vs 1234/1959 [63%]; difference, −6%; 95% CI, −18% to 7%; *P* = .36), nor were machine-read ECG parameters (heart rate, PR, QRS, QT, or corrected QT [QTc] intervals as continuous or dichotomous traits). Specifically, median QTc in variant carriers vs those without variants was 429 vs 439 milliseconds (difference −10 milliseconds; 95% CI, −16 to +3 milliseconds; *P* = .17). Based on this review of *ICD-9* and machine-read ECG intervals, 1 participant with a designated variant was identified with QTc longer than 500 milliseconds, compared with 70 participants without such variants (difference, −2%; 95% CI, −5% to 1%; *P* = .72).

Manual review of the EMR data and review with remeasurement of intervals for 203 ECG tracings from the 63 participants with designated variants identified arrhythmia or ECG phenotypes in 22 participants (35%) (Figure 1, Figure 2, and **Table 2**). One additional participant with QTc longer than 500 milliseconds was identified. Additional phenotypes identified were QTc above normal (450-500 milliseconds for males, 460-500 milliseconds for females, n = 12), atrial fibrillation (n = 4), bundle-branch block (n = 4), and sinus or atrioventricular node disease (n = 10). None with designated variants had evidence of Brugada syndrome. A family history (based on clinical documentation) of atrial fibrillation was identified in 3 participants, 2 of whom were women with QTc between 460 and 500 milliseconds identified on manual review (**Table 3**). Family history of sudden cardiac death was identified in 1 individual with no personal history of arrhythmia or ECG abnormality. Expert review of 125 ECGs from 41 participants without designated variants found no differences in arrhythmia or ECG phenotypes compared with ECG findings from those with variants (**Table 4**).

We stratified participants by age (<30 years, n = 282; 30-60 years, n = 680; and >60 years, n = 1060); there were no differences across strata in the proportion of participants with a designated variant, nor in the proportions with ECG or arrhythmia phenotypes (eTable 7 in the Supplement). The youngest stratum had no machine-read ECG data for analysis, but there were no differences in demographic characteristics or *ICD-9* codes for arrhythmia in those with vs without designated variants (eTable 8 in the Supplement). In 30- to 60-year-olds, those with designated variants were less likely to be white, were more likely to have atrioventricular block, had longer PR intervals, and had longer QRS intervals (eTable 9 in the Supplement). Among those older than 60 years, designated variant carriers were less likely to be white and had shorter QTc (eTable 10 in the Supplement).

Table 3. Summary of Arrhythmia and Electrocardiographic Phenotypes Determined From Manual EMR and ECG Review for 63 Participants With Designated Variants[a]

Characteristic	No. of Participants (%)
Arrhythmia phenotype in EMR	
Long QT	2 (3.2)
Brugada	0
Bundle-branch block	3 (4.8)
Pacemaker or ICD	0
Sinus node or AV node disease	3 (4.8)
Atrial fibrillation	4 (6.3)
Sudden cardiac death	0
Any arrhythmia	8 (12.7)
Family history of arrhythmia	
Long QT	0
Brugada	0
Bundle-branch block	0
Pacemaker or ICD	0
Sinus node or AV node disease	0
Atrial fibrillation	3 (4.8)
Cardiac arrest[b]	1 (1.6)
Any arrhythmia family history	4 (6.3)
ECG traits[c]	
Long QTc[d]	14 (31.8)
QTc >500 ms[d]	2 (4.5)
Brugada	0
Bundle-branch block or interventricular conduction delay	1 (2.3)
Pacing present	0
AV node disease	9 (20.5)
Atrial fibrillation or atrial flutter	3 (6.8)

Abbreviations: AV, atrioventricular; ECG, electrocardiogram; EMR, electronic medical record; ICD, implantable cardioverter/defibrillator; QTc, corrected QT interval.

[a] All variants designated as "pathogenic" or "likely pathogenic" by any 1 of the 3 laboratories or by ClinVar.[16]

[b] Father of individual, at age 44 years.

[c] Ascertained from the 44 individuals with an ECG tracing available from the EMR.

[d] QT interval corrected by Bazett method (QTc = QT/$\sqrt{}$[RR in seconds]). Long QTc defined as >450 milliseconds in males or >460 milliseconds in females.

Discussion

In this cohort of 2022 participants in a study of pharmacogenomic sequencing, we identified 122 rare nonsynonymous or splice-site variants in 2 arrhythmia disease genes. Expert laboratory review of these variants designated 42 as potentially pathogenic, and these classifications were discordant across the laboratories. Review of EMR and ECG data revealed no difference in prevalence of arrhythmia diagnoses or ECG phenotypes among participants with the designated variants compared with those without. After we performed a manual review of EMR data and an ECG review, the majority of participants with a designated variant in either *SCN5A* or *KCNH2* had no identifiable arrhythmia or ECG phenotype. Among patients with designated variants, 35% had evidence of any arrhythmia or ECG phenotype; the most common finding was a QTc above normal; however, only 2 participants had QTc values greater than 500 milliseconds. One individual had variants in each gene (*SCN5A*-D1243N

Table 4. Summary of Phenotypes Identified in Expert ECG Review of Participants With or Without Designated Variants[a]

	No Designated Variant With ECG (n = 41)	Designated Variant Carrier With ECG (n = 44)	*P* Value
Age, median (IQR), y	66 (61-68)	61 (57-65)	<.001[b]
Male sex, No. (%)	19 (46.3)	17 (38.6)	.62[c]
Race, No. (%)			
White	24 (58.5)	24 (54.5)	
Black or African American	5 (12.2)	9 (20.5)	
Asian	11 (26.8)	9 (20.5)	
American Indian or Alaska Native	0	0	.78[c]
Native Hawaiian or other Pacific Islander	0	1 (2.3)	
Unknown	1 (2.4)	1 (2.3)	
Ethnicity, No. (%)			
Hispanic or Latino	1 (2.4)	2 (4.5)	
Not Hispanic or Latino	40 (97.6)	40 (91.0)	.62[c]
Unknown	0	2 (4.5)	
Site of recruitment, No. (%)			
Group Health, University of Washington	22 (53.7)	19 (43.2)	
Mayo Clinic	9 (22.0)	9 (20.5)	
Mount Sinai	5 (12.2)	7 (15.9)	
Children's Hospital of Philadelphia	0	3 (6.8)	.75[c]
Marshfield Clinic	2 (4.9)	2 (4.5)	
Northwestern University	2 (4.9)	2 (4.5)	
Vanderbilt University Medical Center	1 (2.4)	2 (4.5)	
ECG availability			
Total No. of ECGs reviewed	125	203	
No. of ECGs per individual, median (range)	3 (1-14)	3 (1-31)	.31[b]
ECG traits, No. (%)			
Long QTc[d]	6 (14.6)	14 (31.8)	.08[c]
QTc >500 ms[d]	0	2 (4.5)	.49[c]
Brugada pattern	2 (4.9)	0	.23[c]
Bundle-branch block or interventricular conduction delay	1 (2.4)	1 (2.3)	>.99[c]
Pacing present	0	0	>.99[c]
AV node disease	6 (14.6)	9 (20.5)	.57[c]
Atrial fibrillation or atrial flutter	2 (4.9)	3 (6.8)	>.99[c]

Abbreviations: AV, atrioventricular; ECG, electrocardiogram; IQR, interquartile range; QTc, corrected QT interval.

[a] All variants designated as "pathogenic" or "likely pathogenic" by any 1 of the 3 laboratories or by ClinVar.[16]

[b] *P* value from Wilcoxon rank sum (Mann-Whitney) test comparing variant carriers with ECGs to noncarriers with ECGs.

[c] *P* value from Fisher exact test comparing variant carriers with ECGs to noncarriers with ECGs.

[d] QT interval corrected by Bazett method (QTc = QT/$\sqrt{}$[RR in seconds]). Long QTc defined as >450 milliseconds in males or >460 milliseconds in females.

and *KCNH2*-T983I) and no apparent arrhythmia phenotype. One variant, *SCN5A*-R1193Q, designated pathogenic by ClinVar but by none of the 3 laboratories, was found in 19 of 2022 participants; 7 of 19 participants (5 with Asian ancestry) had arrhythmia phenotypes, predominantly long QTc but less than 500 milliseconds. This variant is so common among a general East Asian population (MAF, 7%) that it is unlikely to be a cause of the congenital long QT syndrome (eTable 4 in the Supplement). In vitro functional studies, which likely account for its ClinVar pathogenic classification, suggest it may predispose to longer QT intervals, Brugada syndrome, or both.[17,18]

There are several potential explanations for the paucity of clinical manifestations among participants with designated ion channel variants. First, some participants may have clinically manifest disease that was not documented in the EMR. Additional evaluation and directed history may reveal personal or family history of syncope or other relevant symptoms. Electronic medical record data including *ICD-9* codes were available for all participants with median EMR follow-up time of 18 years, and ECG tracings were available and reviewed for 44 of 63 participants (70%) with designated variants, indicating that only a minority of patients have not had a diagnostic test for long QT (an ECG). Second, these variants may have low penetrance or cause subclinical disease except in the setting of additional genetic or environmental influences. Some patients with familial long QT syndrome may have normal ECGs,[19] and

provocative testing or medication use may unmask the latent syndrome. Third, this cohort may not represent individuals at risk for the phenotype; this cohort includes a wide spectrum of age groups with differential risk for some of the phenotypes assessed (eg, atrial fibrillation has age-related penetrance, while long QT syndrome does not). Age-stratified analysis identified the same frequency of phenotypes in those with designated variants across age strata, indicating that the low frequency of phenotypes was not age-related in this cohort. Fourth, some of these designated variants may confer little or no increased risk for either arrhythmias or ECG abnormalities; ie, they may be of low penetrance for disease or benign variants.

Another important potential reason for the paucity of clinical findings is the unselected nature of the study population. The laboratories evaluated identified variants using classification methods developed for analysis of variants in patients with manifest clinical phenotypes. In patients with phenotypes, there is a much higher a priori probability that a detected rare variant contributes to the disease under study. The prevalence of arrhythmias associated with these genes is low (eg, 1/2500 for long QT syndrome[20-23] and 1/8000 for Brugada syndrome[24]), so it is in fact unlikely that any variant found in this cohort of 2022 unselected participants is pathogenic. Nevertheless, similar to other early efforts to characterize phenotypes in unselected cohorts, we found a high frequency of potentially pathogenic mutations without a high burden of disease.[25,26] Variant classification methods require significant recalibration to assess incidental findings in unselected cohorts before results can be returned to patients and participants, a process that will be facilitated by large sequencing and phenotyping efforts.

A secondary observation in this study is the discordance in variant classification among the 3 different expert laboratories and ClinVar. There was consensus across all 3 laboratories and ClinVar for only 2 of the 42 rare pathogenic variants. Each of the 3 laboratories has developed specific methodology for evaluating the clinical effect of variants, and none of the 3 classification systems yielded similar results. Potential reasons for discordance include use of different reference databases and annotation tools, different criteria for comparing annotation sources, and different thresholds for defining pathogenicity. Developing standards for variant classification is an ongoing and critical area of study, as evidenced by recent ACMG guidelines and ongoing efforts by ClinGen.[27,28] Ensuring all clinical laboratories have access to the most extensive variant and phenotype information available and that these data are shared across laboratories through resources such as ClinVar will likely improve variant interpretation.[29,30] Of note, 2 participants harbored variants that were designated pathogenic by all 4 sources: one a 20-year-old with atrioventricular nodal disease and the other a 59-year-old with no arrhythmia or ECG phenotype, indicating that consensus across variant classification methods may not increase specificity.

We also observed fewer white participants among those with designated variants, which may reflect increased burden of variants[31,32] or difficulties in variant annotation as a result of a relative lack of sequence data in nonwhite populations. Higher MAF in any ancestry group is evidence of low penetrance or nonpathogenicity. Focused initiatives to engage, enroll, sequence, and phenotype individuals from a wide variety of ancestral backgrounds have the potential to greatly accelerate efforts toward comprehensive variant classification.

These data also highlight the potential power of coupling sequencing data with EMRs. Efforts to refine and validate rare variant classification are dependent on the gathering of accurate phenotype data from very large cohorts who are unselected for any one specific diagnosis. Although the population of patients who present to any health system is not perfectly representative of the global community, it does mirror those individuals who present for clinical care. If the goal of precision medicine is to enhance that care, the population of individuals represented in large EMR data sets is a practical starting point.

Our study approach has several limitations. This study focused on just 2 ion channel genes. Participants with or without designated variants may indeed harbor genetic variants in other genes contributing to long QTc or other phenotypes, although these are expected to be very rare. This study included 2022 participants; given the rarity of pathogenic variants and the long QT phenotype, we did not have adequate power to demonstrate statistically significant differences between variant carriers and those without variants. Much larger cohorts and complementary methods are required to definitively determine associations of ion channel variants to disease. All rare variants identified as pathogenic or likely pathogenic by any one of the 3 expert laboratories or ClinVar were included as designated variants, which enabled us to search for phenotypes among the greatest number of participants but potentially reduced the specificity of the designated variant classification. Because the median age at enrollment was 61 years, the cohort may experience survival bias, with participants with severe long QT syndrome or life-threatening arrhythmias at an early age being underrepresented. A large prospective study (for example, of a cohort established at birth) may reveal additional variants and increased burden of phenotypes. This study used existing EMRs to ascertain phenotypes. Some participants with genetic variants may have undocumented or subclinical disease, which may be realized in focused evaluation or manifest in time due to age, additional pathology, or drug challenge (eg, drug-induced long QT).

Conclusions

Among laboratories experienced in genetic testing for cardiac arrhythmia disorders, there was low concordance in designating *SCN5A* and *KCNH2* variants as pathogenic. In an unselected population, the putatively pathogenic genetic variants were not associated with an abnormal phenotype. These findings raise questions about the implications of notifying patients of incidental genetic findings.

ARTICLE INFORMATION

Author Affiliations: Vanderbilt University Medical Center, Nashville, Tennessee (Van Driest, Wells, Stallings, Bush, Shoemaker, Denny, Roden); Case Western Reserve University, Cleveland, Ohio (Bush); University of Washington, Seattle (Gordon, Nickerson, Kim, Crosslin, Jarvik); Group Health Research Institute, Seattle, Washington (Carrell, Ralston, Larson); Mayo Clinic, Rochester, Minnesota (Bielinski, Olson, Ye, Kullo, Kapplinger, Ackerman); Icahn School of Medicine at Mount Sinai, New York, New York (Abul-Husn, Scott, Bottinger); The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania (Almoguera, Connolly, Chiavacci, Hakonarson); Perelman School of Medicine, University of Pennsylvania, Philadelphia (Hakonarson); Northwestern University Feinberg School of Medicine, Chicago, Illinois (Rasmussen-Torvik, Pan, Persell, Smith, Chisholm); Marshfield Clinic Research Foundation, Marshfield, Wisconsin (Kitchner, He, Brilliant); Pennsylvania State University, University Park (Wallace, Ritchie); Johns Hopkins School of Medicine, Baltimore, Maryland (Doheny); National Human Genome Research Institute, National Institutes of Health, Bethesda, Maryland (Li, Manolio); Transgenomic, New Haven, Connecticut (Callis); GeneDx, Gaithersburg, Maryland (Macaya); Geisinger Health System, Danville, Pennsylvania (Williams, Carey, Ritchie).

Author Contributions: Dr Roden had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Van Driest, Wells, Stallings, Gordon, Smith, Chisholm, Li, Manolio, Williams, Denny, Roden.
Acquisition, analysis, or interpretation of data: Van Driest, Wells, Stallings, Bush, Gordon, Nickerson, Kim, Crosslin, Jarvik, Carrell, Ralston, Larson, Bielinski, Olson, Ye, Kullo, Abul-Husn, Scott, Bottinger, Almoguera, Connolly, Chiavacci, Hakonarson, Rasmussen-Torvik, Pan, Persell, Chisholm, Kitchner, He, Brilliant, Wallace, Doheny, Shoemaker, Callis, Macaya, Carey, Kapplinger, Ackerman, Ritchie, Denny, Roden.
Drafting of the manuscript: Van Driest, Wells, Jarvik, He, Brilliant, Denny, Roden.
Critical revision of the manuscript for important intellectual content: Van Driest, Wells, Stallings, Bush, Gordon, Nickerson, Kim, Crosslin, Jarvik, Carrell, Ralston, Larson, Bielinski, Olson, Ye, Kullo, Abul-Husn, Scott, Bottinger, Almoguera, Connolly, Chiavacci, Hakonarson, Rasmussen-Torvik, Pan, Persell, Smith, Chisholm, Kitchner, Wallace, Doheny, Shoemaker, Li, Manolio, Callis, Macaya, Williams, Carey, Kapplinger, Ackerman, Ritchie, Denny, Roden.
Statistical analysis: Van Driest, Wells, Stallings, Bush, Gordon, Jarvik, Kapplinger, Ritchie.
Obtained funding: Stallings, Jarvik, Ralston, Larson, Kullo, Scott, Bottinger, Connolly, Hakonarson, Rasmussen-Torvik, Chisholm, He, Brilliant, Doheny, Williams, Carey, Denny, Roden.
Administrative, technical, or material support: Stallings, Ralston, Larson, Olson, Almoguera, Connolly, Hakonarson, Pan, Smith, Chisholm, Kitchner, Brilliant, Wallace, Shoemaker, Li, Callis, Carey, Denny, Roden.
Study supervision: Bottinger, Brilliant, Doheny, Li, Williams, Ackerman, Denny, Roden.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Larson reported having received royalties from UpToDate. Dr Persell reported having received grants from Pfizer. Dr Ackerman reported having consulted for Boston Scientific, Gilead Sciences, Medtronic, and St Jude Medical and having received along with Mayo Clinic Ventures sales-based royalties from Transgenomic for their FAMILION-LQTS and FAMILION-CPVT genetic tests.

Funding/Support: The eMERGE Network was initiated and funded by the National Human Genome Research Institute (NHGRI) through the following grants: U01HG006828 (Cincinnati Children's Hospital Medical Center/Boston Children's Hospital); U01HG006830 (Children's Hospital of Philadelphia); U01HG006389 (Essentia Institute of Rural Health, Marshfield Clinic Research Foundation, and Pennsylvania State University); U01HG006382 (Geisinger Clinic); U01HG006375 (Group Health Cooperative, University of Washington); U01HG006379 (Mayo Clinic); U01HG006380 (Icahn School of Medicine at Mount Sinai); U01HG006388 (Northwestern University); U01HG006378 (Vanderbilt University Medical Center); and U01HG006385 (Vanderbilt University Medical Center serving as the Coordinating Center). Additional support was provided by National Center for Advancing Translational Sciences (NCATS) grant UL1TR000427 (University of Wisconsin, Marshfield Clinic); National Institute on Aging grant U01AG006781 (Group Health Cooperative, University of Washington); NHGRI grant U01HG004438 (Johns Hopkins School of Medicine); NCATS grant UL1TR000445 (Vanderbilt University Medical Center); National Institute of General Medical Sciences grants U19GM61388, R01GM28157, U01HG005137, R01CA138461, R01AG034676; and the Windland Smith Rice Comprehensive Sudden Cardiac Death Program (Mayo Clinic). The sequencing platform was developed by the next-generation sequencing centers of the Pharmacogenomics Research Network supported by National Institutes of Health grants U19GM61388, U19HL069757, and U01GM097119.

Role of the Funder/Sponsor: NHGRI program staff participated with eMERGE investigators in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication and are accordingly included as authors.

Additional Contributions: We thank Arvindh Kanagasundram, MD (Vanderbilt University Medical Center), who assisted with ECG adjudication without receiving compensation, and Alfred L. George Jr, MD (Northwestern University Feinberg School of Medicine), and Kunal N. Karmali, MD (Northwestern University Feinberg School of Medicine), who reviewed patient ECG and clinical data without receiving compensation.

REFERENCES

1. Green RC, Berg JS, Grody WW, et al; American College of Medical Genetics and Genomics. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genet Med*. 2013;15(7):565-574.

2. American College of Medical Genetics and Genomics. Incidental findings in clinical genomics: a clarification. *Genet Med*. 2013;15(8):664-666.

3. Green RC, Berg JS, Berry GT, et al. Exploring concordance and discordance for return of incidental findings from clinical sequencing. *Genet Med*. 2012;14(4):405-410.

4. Wolf SM, Crock BN, Van Ness B, et al. Managing incidental findings and research results in genomic research involving biobanks and archived data sets. *Genet Med*. 2012;14(4):361-384.

5. Clayton EW, McGuire AL. The legal risks of returning results of genomics research. *Genet Med*. 2012;14(4):473-477.

6. Jarvik GP, Amendola LM, Berg JS, et al; eMERGE Act-ROR Committee and CERC Committee; CSER Act-ROR Working Group. Return of genomic results to research participants: the floor, the ceiling, and the choices in between. *Am J Hum Genet*. 2014;94(6):818-826.

7. Amendola LM, Dorschner MO, Robertson PD, et al. Actionable exomic incidental findings in 6503 participants: challenges of variant classification. *Genome Res*. 2015;25(3):305-315.

8. Tabor HK, Auer PL, Jamal SM, et al; NHLBI Exome Sequencing Project. Pathogenic variants for Mendelian and complex traits in exomes of 6,517 European and African Americans: implications for the return of incidental results. *Am J Hum Genet*. 2014;95(2):183-193.

9. Pathak J, Kho AN, Denny JC. Electronic health records-driven phenotyping: challenges, recent advances, and perspectives. *J Am Med Inform Assoc*. 2013;20(e2):e206-e211.

10. Newton KM, Peissig PL, Kho AN, et al. Validation of electronic medical record-based phenotyping algorithms: results and lessons learned from the eMERGE network. *J Am Med Inform Assoc*. 2013;20(e1):e147-e154.

11. Denny JC. Chapter 13: Mining electronic health records in the genomics era. *PLoS Comput Biol*. 2012;8(12):e1002823.

12. Rasmussen-Torvik LJ, Stallings SC, Gordon AS, et al. Design and anticipated outcomes of the eMERGE-PGx project: a multicenter pilot for preemptive pharmacogenomics in electronic health record systems. *Clin Pharmacol Ther*. 2014;96(4):482-489.

13. Schwartz PJ, Ackerman MJ, George AL Jr, Wilde AAM. Impact of genetics on the clinical management of channelopathies. *J Am Coll Cardiol*. 2013;62(3):169-180.

14. Crosslin DR, Robertson PD, Carrell DS, et al. Prospective participant selection and ranking to maximize actionable pharmacogenetic variants and discovery in the eMERGE Network. *Genome Med*. 2015;7(1):67.

15. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491-498.

16. Landrum MJ, Lee JM, Riley GR, et al. ClinVar: public archive of relationships among sequence variation and human phenotype. *Nucleic Acids Res*. 2014;42(Database issue):D980-D985.

17. Wang Q, Chen S, Chen Q, et al. The common *SCN5A* mutation R1193Q causes LQTS-type electrophysiological alterations of the cardiac sodium channel. *J Med Genet*. 2004;41(5):e66.

18. Hwang HW, Chen JJ, Lin YJ, et al. R1193Q of *SCN5A*, a Brugada and long QT mutation, is a common polymorphism in Han Chinese. *J Med Genet*. 2005;42(2):e7.

19. Vincent GM, Timothy KW, Leppert M, Keating M. The spectrum of symptoms and QT intervals in carriers of the gene for the long-QT syndrome. *N Engl J Med*. 1992;327(12):846-852.

20. Goldenberg I, Zareba W, Moss AJ. Long QT syndrome. *Curr Probl Cardiol*. 2008;33(11):629-694.

21. Earle N, Crawford J, Smith W, et al. Community detection of long QT syndrome with a clinical registry: an alternative to ECG screening programs? *Heart Rhythm*. 2013;10(2):233-238.

22. Schwartz PJ, Stramba-Badiale M, Crotti L, et al. Prevalence of the congenital long-QT syndrome. *Circulation*. 2009;120(18):1761-1767.

23. Quaglini S, Rognoni C, Spazzolini C, Priori SG, Mannarino S, Schwartz PJ. Cost-effectiveness of neonatal ECG screening for the long QT syndrome. *Eur Heart J*. 2006;27(15):1824-1832.

24. Patel SS, Anees S, Ferrick KJ. Prevalence of a Brugada pattern electrocardiogram in an urban population in the United States. *Pacing Clin Electrophysiol*. 2009;32(6):704-708.

25. Ng D, Johnston JJ, Teer JK, et al; NIH Intramural Sequencing Center (NISC) Comparative Sequencing Program. Interpreting secondary cardiac disease variants in an exome cohort. *Circ Cardiovasc Genet*. 2013;6(4):337-346.

26. Lahtinen AM, Havulinna AS, Noseworthy PA, et al. Prevalence of arrhythmia-associated gene mutations and risk of sudden cardiac death in the Finnish population. *Ann Med*. 2013;45(4):328-335.

27. Richards S, Aziz N, Bale S, et al; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.

28. Rehm HL, Berg JS, Brooks LD, et al; ClinGen. ClinGen: the Clinical Genome Resource. *N Engl J Med*. 2015;372(23):2235-2242.

29. Korf BR, Rehm HL. New approaches to molecular diagnosis. *JAMA*. 2013;309(14):1511-1521.

30. MacArthur DG, Manolio TA, Dimmock DP, et al. Guidelines for investigating causality of sequence variants in human disease. *Nature*. 2014;508(7497):469-476.

31. Ackerman MJ, Tester DJ, Jones GS, Will ML, Burrow CR, Curran ME. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. *Mayo Clin Proc*. 2003;78(12):1479-1487.

32. Ackerman MJ, Splawski I, Makielski JC, et al. Spectrum and prevalence of cardiac sodium channel variants among black, white, Asian, and Hispanic individuals: implications for arrhythmogenic susceptibility and Brugada/long QT syndrome genetic testing. *Heart Rhythm*. 2004;1(5):600-607.

33. UniProt Consortium. UniProt: a hub for protein information. *Nucleic Acids Res*. 2015;43(Database issue):D204-D212.

34. Wang Q, Li Z, Shen J, Keating MT. Genomic organization of the human *SCN5A* gene encoding the cardiac sodium channel. *Genomics*. 1996;34(1):9-16.

35. Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: *KVLQT1*, *HERG*, and *KCNE1*. *Genomics*. 1998;51(1):86-97.