Interpreting Incidentally Identified Variants in Genes Associated With Catecholaminergic Polymorphic Ventricular Tachycardia in a Large Cohort of Clinical Whole-Exome Genetic Test Referrals

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Background—The rapid expansion of genetic testing has led to increased utilization of clinical whole-exome sequencing (WES). Clinicians and genetic researchers are being faced with assessing risk of disease vulnerability from incidentally identified genetic variants which is typified by variants found in genes associated with sudden death-predisposing catecholaminergic polymorphic ventricular tachycardia (CPVT). We sought to determine whether incidentally identified variants in genes associated with CPVT from WES clinical testing represent disease-associated biomarkers.

Methods and Results—CPVT-associated genes *RYR2* and *CASQ2* variants were identified in one of the world's largest collections of clinical WES referral tests (N=6517, Baylor Miraca Genetics Laboratories) and compared with a control cohort of ostensibly healthy individuals (N=60 706) and a case cohort of CPVT cases (N=155). Within the WES cohort, the rate of rare variants in CPVT-associated genes was 8.8% compared with 6.0% among controls and 60.0% among cases. There was a predominance of variants of undetermined significance (97.7%). After protein topology mapping, WES variants colocalized more frequently to residues with variants found in controls compared with cases. Retrospective clinical evaluation of individuals referred to our institution with WES-positive variants demonstrated no evidence of clinical CPVT in individuals with a low pretest clinical suspicion for CPVT.

Conclusions—The prevalence of incidentally identified CPVT-associated variants is ≈9% among WES tests. Variants of undetermined significances in CPVT-associated genes in WES genetic testing, in the absence of clinical suspicion for CPVT, are unlikely to represent markers of CPVT pathogenicity. **(*Circ Arrhythm Electrophysiol*. 2017;10:e004742. DOI: 10.1161/CIRCEP.116.004742.)**

Key Words: Casq2 ■ catecholamingeric polymorphic ventricular tachycardia ■ exome ■ genetic testing ■ genetics ■ RyR2 ■ variant of undetermined significance

In the modern era of genetic testing, the ability to interrogate the human genome in search of the cause of presumed genetic disease has greatly expanded. Whole-exome sequencing (WES), the high-throughput sequencing of all coding exons in the genome, has led to a renewed ability to identify the genetic underpinnings of patients previously phenotypically indistinguishable or clinically heterogeneous. Despite the clear diagnostic benefit of WES, there has been a simultaneous explosion in the identification of genetic variants of unclear clinical significance (VUSs).[1,2] This problem is typified by the incidental finding of VUSs in genes that cause an arrhythmia that carries a high risk of fatality—catecholaminergic polymorphic ventricular tachycardia (CPVT).

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CPVT is a heritable arrhythmia syndrome that classically manifests with adrenergically induced syncope or sudden death secondary to ventricular tachyarrhythmias (VT).[3,4] Clinically, CPVT can present with premature ventricular contractions (PVCs) and bidirectional VT, particularly with exercise. A prototypical cardiac channelopathy, CPVT is caused by mutations in genes encoding components of the macromolecular intracellular Ca^{2+} release channel complex within the sarcoplasmic reticulum of the cardiocyte. Heterozygous mutations in the *RYR2*-encoded cardiac ryanodine receptor 2 (RyR2) represent the most common genetic subtype of CPVT, accounting

Received October 11, 2016; accepted February 15, 2017.

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The Data Supplement is available at http://circep.ahajournals.org/lookup/suppl/doi:10.1161/CIRCEP.116.004742/-/DC1.

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WHAT IS KNOWN

- Pathogenic mutations in *RYR2* and *CASQ2* are associated with the development of catecholaminergic polymorphic ventricular tachycardia (CPVT), a sudden death predisposing arrhythmia.
- Rapid expansion of genetic testing technologies has increased the utilization of clinical whole-exome sequencing (WES) and, with it, the incidental identification of VUSs in *RYR2* and *CASQ2*.

WHAT THE STUDY ADDS

- Clinically reported incidentally identified variants in *RYR2* and *CASQ2* are found in ≈9% of WES tests.
- The location of WES-identified variants more closely resembles healthy variation than pathogenic mutations.
- For those WES referral subjects who underwent cardiac evaluation with a low pretest suspicion for channelopathic disease, there was no clinical evidence of CPVT.

for ≈50% to 60% of cases.[3] Classically, these mutations affect a single amino acid (missense) and are associated with autosomal dominant pattern of disease inheritance. Pathological mutations cause leakage of Ca^{2+} stored within the sarcoplasmic reticulum (so-called store Ca^{2+}) into the cytosol, particularly during sympathetic stimulation. This store Ca^{2+} leak triggers delayed afterdepolarization and Ca^{2+} overload-induced VT and ventricular fibrillation. *RYR2* is one of the largest genes in the human genome, containing 105 coding exons, and clinical genetic testing has traditionally been focused on 3 hotspot domains. Recently, genetic evaluation of *RYR2* has broadened to encompass the entire coding sequence of this gene. Although this offers an expanded ability to identify clinically relevant biomarkers, there is a paucity of understanding on the background genetic variation inherent in the gene.[5] A second genetic subtype of CPVT is *CASQ2*-encoded calsequestrin 2 (Casq2) which accounts for a minority of CPVT cases.[6] Mutations in this 11 exon gene have been widely linked to the pathogenesis of *RYR2*-negative CPVT and are classically inherited in an autosomal recessive manner. In addition, rare causes of CPVT have been associated with mutations in *TRDN*-encoded triadin (Trdn) and *CALM1*-encoded calmodulin type 1 (Calm1) which account for ≈1% of CPVT-associated mutations.[7]

To this end, we systematically evaluated a large database of WES report genetic variants and compared it to a large cohort of ostensibly healthy control individuals and clinically robust cases of CPVT. We describe the spectrum and prevalence of genetic variation within *RYR2* and *CASQ2* among individuals undergoing WES testing, and demonstrate statistical similarities between WES genetic variation and healthy background variation.

Methods

Study Cohorts

The study included 4 cohorts. The clinical WES cohort (also referred to as the referral cohort) comprised individuals referred for genetic testing to the Baylor Miraca Genetics Laboratories (Houston, TX; previously Baylor College of Medicine Medical Genetics Laboratories) independent of referral diagnosis or indication for genetic testing. Demographic and clinical referral information was abstracted. Genetic information from samples derived for platform validation studies, or from oncological samples, was excluded. Probands, and biological parents of probands in so-called trio studies, were included to maximize the ability to detect variants. Variants included in this study were (1) identified in the coding nucleotide sequence, or predicted splice junction, of *RYR2* or *CASQ2*, (2) deemed likely pathological or VUS at the time of genetic testing, (3) included on the clinical report sent to the referring provider or an expanded reported provided to the clinician on request, and (4) heterozygous mutations in *CASQ2* were included that were deemed likely pathological or VUSs. Because of the low frequency of *TRDN* and *CALM1* mutations among patients with CPVT, these genes were not analyzed. Variants excluded from this study were (1) interpreted as not pathogenic at the time of genetic testing or (2) synonymous variants.

The CPVT case cohort was based on a compendium established in the literature as previously described.[8] This cohort consisted of 155 individuals clinically diagnosed with CPVT. Among this cohort, 33 were deemed clinically robust, who demonstrated exertional syncope and documented biventricular or polymorphic VT. Individuals with a possible CPVT diagnosis demonstrated exertional syncope and stress test–induced ventricular ectopy without VT.

The control cohort comprised genetic findings derived from the ExAC database which consisted of individuals of African/Black (N=5203), Latino (N=5789), East Asian (N=4327), Finnish (N=3307), Non-Finnish European (N=33 370), South Asian (N=8256), and other individuals (N=454) for a total of 60 706 largely ostensibly healthy individuals.[9] Genetic variants associated with *RYR2* and *CASQ2* with a minor allele frequency <0.01 were included.

The Texas Children's Hospital (TCH) cohort comprised individuals within the clinical WES cohort who hosted rare variants in *RYR2* and *CASQ2* who were referred from TCH, Baylor College of Medicine (Houston, TX). These individuals were identified based on referral records and cross-referencing with clinical records. Anonymous clinical information, including basic demographics, clinical evaluation and diagnoses, 12-lead ECG, 24-hour Holter, cardiac stress test, and transthoracic echocardiographic findings, was reviewed retrospectively and abstracted when available. Patients referred for evaluation by pediatric cardiology were noted, and all records were reviewed for clinical suspicion, or diagnosis, of CPVT. This research study was approved by the Baylor College of Medicine Institutional Review Board.

Whole-Exome Sequencing

From October 2011 through October 2015, clinical WES was completed in the Exome Laboratory at Baylor Miraca Genetics Laboratories, and sequencing and data analyses were conducted as previously described.[10] Extracted DNA, subjected to an in-house exome capture platform, VCRome version 2.1 (targeting ≈20 000 genes, including the coding and untranslated region exons), was sequenced using a HiSeq. This platform has a minimum depth of coverage of 20× with a practical detection rate of ≈95% of all single-nucleotide variants and insertion/deletions. Samples were additionally analyzed by an Illumina HumanExome-12 v1 cSNP array for quality control assessment of exome data, as well as for detecting large copy-number variants and regions of absence of heterozygosity.

Nomenclature

Missense variants were defined as those that altered a single amino acid. Radical mutations were defined as variants that are predicted to cause more than a single amino acid change, including nonsense (early termination), insertion/deletion (both in-frame and out-of-frame), and predicted canonical splice site mutations. Pathological, likely pathological, or VUS was based on the designation ascribed by the WES test.

Primary Sequence Localization

Variant localization was conducted using the *RYR2* consensus sequence from Ensembl browser.[11] Specifically, the RyR2 (NP_001026.2)

primary sequence was used, and variants across cohorts localizing to RyR2 were mapped along the primary sequence.

Statistics

Statistical results were expressed as mean with variance expressed as SD or median and interquartile range (brackets), as appropriate. Variance of prevalence/proportion was expressed as the exact 95% confidence interval around proportion when statistical comparisons were made (brackets). Comparisons were made by Student *t* Test, Fisher exact test, χ^2 with Yates Correction, as appropriate (GraphPad, La Jolla, CA). Statistical significance threshold was set at $P < 0.05$.

Results

Prevalence of *RYR2* and *CASQ2* Variants in the WES Cohort

To establish the background rate of *RYR2* and *CASQ2* variants, a large cohort of clinical whole-exome genetic testing referrals was interrogated. The clinical whole-exome cohort consisted of 6517 individuals, derived from 6477 families, with 3529 male (54.2%), 2941 female (45.1%), and 47 (0.7%) fetal subjects (Figure 1A; Table 1). These individuals were referred from various outside institutions. Overall, 91.1% of the referrals were from the United States with 47.4% from the state of TX, whereas 8.9% were international referral samples. A total of 6359 individuals (97.6%) were probands, whereas 158 individuals (2.4%) were biological parents of probands included as part of trio sequencing. The median age at genetic testing was 6.1 years [2.5–12.3] with a range of fetal life to 90.1 years.

RYR2 and *CASQ2* variants deemed either likely pathological or VUS on WES testing were present in 573 individuals (8.8% [8.1–9.5]) undergoing clinical WES (Figure 1B). This frequency was unchanged when only unrelated probands were included. This group of variant-positive individuals were unrelated and consisted of 547 individuals with a single variant (8.4% [7.7–9.1] of total cases and 95.5% [93.4–97.0] of variant-positive individuals), 25 individuals who hosted 2 variants (0.4% [0.2–0.6] and 4.4% [2.8–6.4], respectively), and 1 individual who hosted 3 variants (0.02% [0.0–0.09] and 0.2% [0.0–1.0], respectively). This last individual demonstrated 3 variants in *RYR2*, including R2793Q (c.8378G>A) and 2 variants which may disrupt the canonical splicing sequence IVS70-4T>C (c.10231-4T>C) and IVS104+3A>G (c.14808+3A>G). Among these variant-positive individuals, a total of 599 individual *RYR2* and *CASQ2* coding variants were identified. Among these variants, 14 (2.3% [1.3–3.9]) were deemed likely pathological and 585 (97.7% [96.1–98.7]) were deemed VUSs at the time of WES. Taken together, these results suggest that there is a significant background frequency of CPVT-associated gene variants, particularly VUSs, among individuals undergoing WES testing. These results are summarized in Table 1. A complete list of variants identified is provided in Tables I and II in the Data Supplement.

Gene-Specific Variant Prevalence

To determine the gene-specific prevalence of variants in *RYR2* and *CASQ2* and the number of variants deemed likely pathological or VUS for each gene, subset analysis of the variants was conducted. Among all variants, 506 (84.5% [81.3–87.3]) localized to the coding exons of *RYR2* with 6 (1.2% [0.4–2.6] of *RYR2* variants) deemed likely pathological and 500 (98.8% [97.4–99.6]) deemed VUSs. Conversely, 93 variants (15.5% [12.7–18.7]) localized to coding exons of *CASQ2* with 8 (8.6% [3.8–16.3] of *CASQ2* variants) interpreted as likely pathological and 85 (91.4% [83.8–96.2]) deemed VUSs. The frequency of pathological *CASQ2* variants was significantly higher than *RYR2* variants (P=0.0005). All of the pathological variants identified in the WES cohort were heterozygous. Four subjects, 0.7% [0.2–1.9] of the variant-positive cases hosted homozygous VUSs comprised Casq2-D310N, RyR2-S151N,

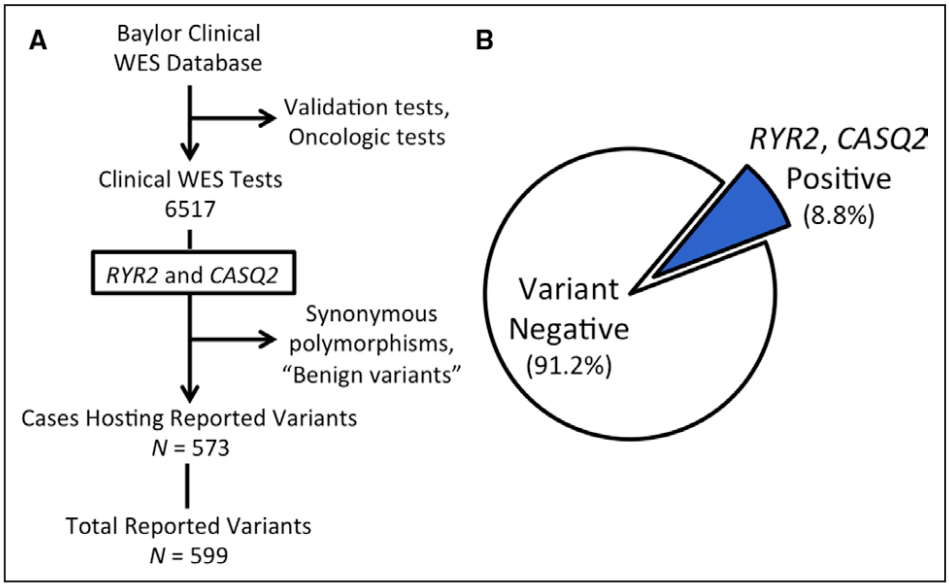

Figure 1. A, Schematic representation of the study methodology. The clinical whole-exome sequencing (WES) cohort comprised variants eligible for clinical reporting excluding test validation studies and oncological samples. Synonymous variants, known polymorphisms, and variants interpreted as benign were excluded. **B**, Pie chart of WES cohort demonstrating individuals with no *RYR2* or *CASQ2* variants (negative, white fill) and individuals who are variant positive (blue fill).

Table 1. Clinical WES Cohort Demographics and Characteristics

WES Cohort	N
Total cases	6517
Total families	6477
Probands	6359
Parents from Trio	158
Male	3529 (54.2%)
Female	2941 (45.1%)
Fetal	47 (0.7%)
Age at genetic test, y	6.1 [2.5–12.3]
RYR2 and *CASQ2* variants	599
Pathological	14 (2.3% [1.3–3.9])
VUS	585 (97.7% [96.1–98.7])
Variant-positive cases	573 (8.8% [8.1–9.5])
Single	547 (95.5% [93.4–97.0])
Double	25 (4.4% [2.8–6.4])
Triple	1 (0.2% [0.0–1.0])

VUS indicates variant of undetermined/unknown significance; and WES, whole-exome sequencing.

and 2 individuals with RyR2-S1756C. These results are summarized in Figure 2.

Mutation Class-Specific Variant Prevalence

To determine the prevalence of radical and missense mutations among the WES cohort, subset analysis of the variants based on mutation class was conducted. Among all variants, 101 (16.9% [14.0–20.1]) were radical variants, and 498 (83.1% [79.9–86.1]) were missense variants. Among *RYR2*-specific variants, 91 (18.0% [14.7–21.6] of *RYR2* variants) were radical variants, and 415 (82.0% [78.4–85.3]) were missense variants. Conversely, among *CASQ2*-specific variants, 10 (10.8% [5.3–18.9] of *CASQ2* variants) were radical, and 83 (89.2% [81.1–94.7]) were missense.

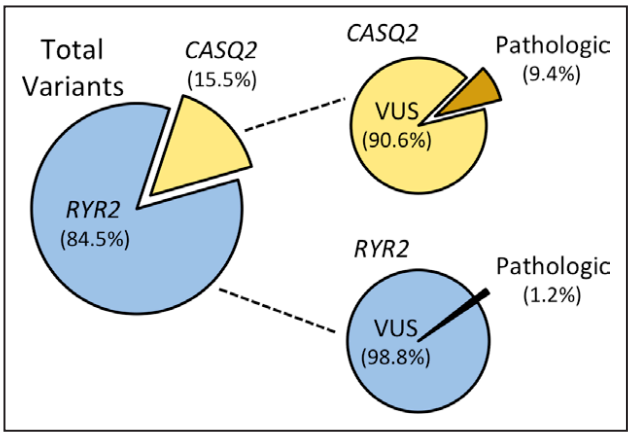

Figure 2. Gene-specific variant prevalence. **Left**, Pie chart of all variants divided into variants localizing to *RYR2* (blue fill) and *CASQ2* (yellow fill). **Right**, *CASQ2*- (upper) and *RYR2*-specific (lower) variants divided into those interpreted as likely pathological vs variants of undetermined significance (VUS).

Among the likely pathological *RYR2*-specific variants, 4 (0.8% [0.2–2.0] of *RYR2* variants) were radical mutations, and 2 (0.4% [0.05–1.4]) were missense mutations. The higher frequency of likely pathological variants identified in *CASQ2* was driven by a higher number of missense pathological variants with 7 (7.5% [3.1–14.9] of *CASQ2* variants) localizing the gene (P<0.0001). There was a similar frequency of radical pathological variants in *CASQ2* with 1 (1.1% [0.03–5.9%] compared with *RYR2*). Among VUSs, there were similar numbers of radical mutations (87, 17.2% [14.0–20.8] versus 9, 9.7% [4.5–17.6]) and missense mutations (413, 81.6% [78.0–84.9] versus 76, 81.7% [72.4–89.0]) between *RYR2* and *CASQ2*, respectively. These results are summarized in Figure 3.

Comparison of WES Cohort Prevalence Versus CPVT Cases and Healthy Controls

To compare the *RYR2* and *CASQ2*-associated rare variant frequency among the WES cohort and known CPVT cases, a compendium of CPVT-associated mutations was used.[8] Among a cohort of 155 individuals clinically diagnosed with CPVT, 73 of 155 (47.1% [39.0–55.3]) were found to be mutation positive, hosting a rare variant in either *RYR2* or *CASQ2*. Further, among cases that demonstrated clinically robust CPVT, 20 of 33 (60.1% [42.1–77.1]) were mutation-positive.[8,12] The prevalence of mutation-positive individuals in robust CPVT cases was over 6-fold higher than the 8.8% of individuals hosting *RYR2* and *CASQ2* variants among the clinical WES cohort (P<0.0001). To compare the variant frequency between the WES cohort and healthy individuals, the frequency of variants with a minor allele frequency <0.01 among ostensibly healthy control subjects was analyzed. Among these individuals, the rate of *RYR2* and *CASQ2* variants was 6.0% [5.8–6.2] which was statistically lower than the 8.8% prevalence among the clinical WES cohort (P<0.0001).

The higher rate of CPVT case variants was driven by a higher prevalence of missense variants. The rate of radical variants among clinically robust CPVT cases was 2.6% [0.71–6.5]. This prevalence was statistically similar the prevalence of radical mutations in the control cohort (0.9% [0.82–0.97]) and the WES cohort (1.6% [1.3–1.9]). Conversely, the rate of missense variants among CPVT cases was 57.4% [49.2–65.3] which was markedly higher than in both control (5.2% [5.0–5.3]) and WES (7.6% [7.0–8.3]) cohorts (P<0.0001, respectively). Further, the frequency of *RYR2* and *CASQ2* variants was higher among WES than control cohorts (P<0.0001). These results are summarized in Figure 4.

Variant Colocalization Between Cohorts and Mutation Hotspots

To determine whether variants identified among the clinical WES cohort more frequently altered amino acids affected by control or case variants, we next mapped each of the variants along the primary sequence of RyR2. Variants identified in the WES cohort localized to 278 individual RyR2 residues, whereas case and control variants localized to 140 and 973 individual residues, respectively. There was a background level of overlap between these 3 cohorts with 41 RyR2

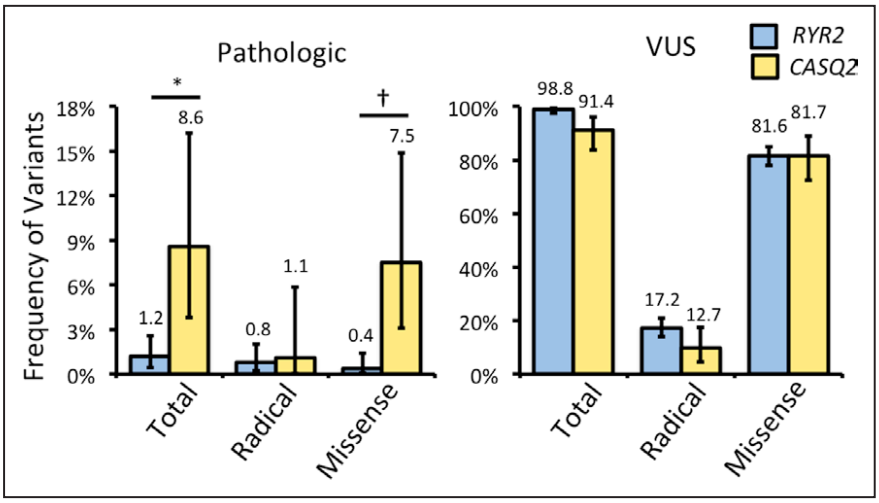

Figure 3. Variant class-specific prevalence. Bar graph of the frequency of *RYR2* (blue fill) and *CASQ2* (yellow fill) variants among whole-exome sequencing subjects separated by variants designated as likely pathological (**left**) versus variants of undetermined significance (VUS; **right**). Error bars denote 95% confidence interval. **P*=0.0003; †*P*<0.0001.

residues common to the case and control cohorts. Whereas 22 RyR2 residues were shared between the WES cohort and the CPVT case cohort, 168 residues were shared between the WES cohort and the control cohort. This represented an overlap of 7.9% [5.0–11.7] of variants between WES and cases and 60.4% [54.4–66.2] between WES and controls. In this way, WES cohort variants were ≈7.5-fold more likely to colocalize to a residue found in controls versus CPVT cases. These findings are demonstrated in Figure 5.

Classically, RyR2 variants have clustered to mutation hotspots within RyR2; however, recently expanded genetic studies have identified several putatively disease-associated variants that are located outside of these regions. Among CPVT cases, 80.2% [72.3–86.6] of mutated residues were located within 1 of 3 canonical mutation hotspots, whereas 32.2% [29.3–35.3] of altered residues among control cohort individuals localized to a hotspot (*P*<0.0001). Among WES cohort individuals, 10.5% [8.6–12.6] of altered residuals located within a canonical hotspot which was significantly less than both case and control variants (*P*<0.0001, respectively).

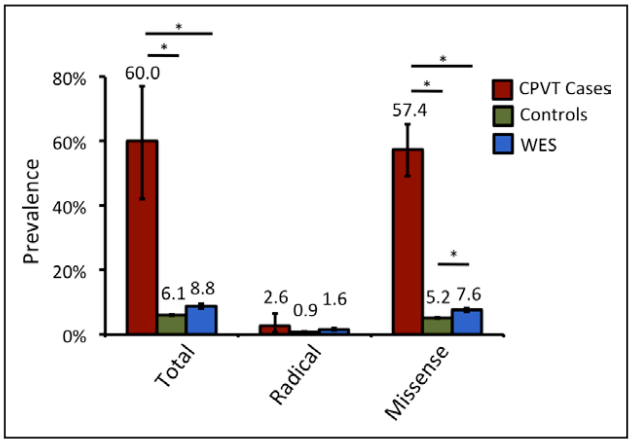

Figure 4. Cohort-specific variant prevalence. Bar graph of the frequency of all *RYR2* and *CASQ2* variants among catecholaminergic polymorphic ventricular tachycardia (CPVT)–positive cases (red fill), ostensibly healthy control individuals derived from the ExAC database (green fill), and whole-exome sequencing (WES) cohort subjects (blue fill). Frequencies for all variants, radical variants, and missense variants are noted. Error bars denote 95% confidence interval. **P*<0.0001.

Further, WES cohort variants seemed to be distributed across the linear topology of RyR2 without apparent clustering in a manner similar to control variants. These findings are depicted in Figure 6.

Pretest Clinical Suspicion of CPVT Among WES Referrals

To evaluate whether children referred for WES genetic testing demonstrated pre-WES suspicion for CPVT, or a post-test diagnosis of CPVT other occult channelopathic arrhythmias, we evaluated the WES referrals from our institution (TCH). A total of 140 individual probands from 136 families were identified who were referred for WES testing and who hosted either likely pathological or VUSs of *RYR2* and *CASQ2*. The median age of this cohort was 5.5 years [2.1–9.2] at the time of genetic testing with a range of 0.01 to 52.0 years. Seventy-four (52.9%) were male, 66 (47.1%) female, 18 (12.9%) Black/African, 7 (5.0%) Asian American/Asian, 46 (32.5%) White/Non-Hispanic, and 69 (49.6%) were Hispanic. The results are summarized in Table 2.

In this subgroup, the indication for genetic testing was concern for cardiovascular disease in only a small minority of referrals. Within the TCH cohort, 85.7% of the cohort was referred for WES because of neurological disease with 66.4% having a referral of developmental delay and 16.4% with abnormal muscular tone. About 27.1% of referrals had an indication of seizures or epilepsy; however, only 1.4% of the cohort had this as the sole indication for genetic testing. About 12.9% of the WES cohort carried an indication of cardiac disease as any part of the clinical phenotype. About 5% of the cohort was referred for solely cardiovascular disease diagnoses, and all cardiovascular only referrals were for structural cardiac disease or concern for cardiomyopathy with the exception of a single referral with a structurally normal heart. This individual also had a history of syncope and a family history of sudden cardiac death and was ultimately found to have a RyR2-G3946S mutation identified as likely pathogenic by WES (detailed below). With the exception of this individual, no other subject had family history of sudden death (99.3% negative [96.1–100]), and only one other had a personal history of syncope (98.5% negative [94.9–99.8]). None had a history of clinical CPVT. Taken together, these findings suggest a low pre-WES testing concern for CPVT among

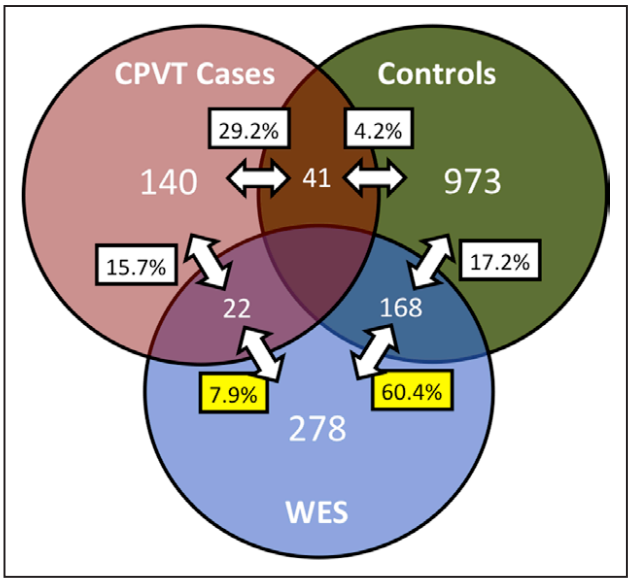

Figure 5. Variant position overlap between study cohorts. Venn diagram of the colocalization of variants to residues common to the catecholaminergic polymorphic ventricular tachycardia (CPVT) case (red fill), control (ExAC database, green fill), and whole-exome sequencing (WES; blue fill) cohorts. The numbers within the circles demonstrate number of amino acid residues on RyR2 which host variants/mutations within each cohort. Numbers within overlapping portions of the circles reflect the number of residues with variants shared between respective cohorts. The proportion of shared variants, out of the cohort total, is noted within each box.

the TCH cohort. These results are summarized in Table 2 and Figure 7A.

Among these 140 variant-positive individuals, all hosted a single *RYR2* or *CASQ2* variant. One-hundred thirty-nine (99.3% [96.1–100]) variants localized to *RYR2*, and 1 (0.7% [0.02–3.9%]) variant localized to *CASQ2*. Further, all individuals hosted heterozygous mutations. The vast majority of these variants, 97.1% [92.9–99.2], were designated as VUSs, whereas 2.9% [0.8–7.2%] were labeled as likely pathological variants. These results are summarized in Table 2 and Figure 7B.

Clinical Evaluation of WES Cohort Referrals With CPVT-Associated Variants

To determine whether WES referrals incidentally found to host *RYR2* and *CASQ2* variants had clinical evidence of CPVT, the available initial cardiac evaluation and subsequent visits for the TCH cohort were reviewed. ECG, 24-hour Holter, and treadmill stress tests were reviewed for presence of ventricular ectopy (PVCs) or VT. Among those evaluated, 2 (1.4%) individuals had evidence of ventricular ectopy. One individual, a 4-year-old male with macrocephaly, hemimegalencephaly, and capillary malformations with resultant severe developmental delay, had frequent PVCs. WES analysis for this constellation demonstrated a *RAB23* mutation that was felt to be pathological and numerous VUSs, including RyR2-R3190Q. Multiple 24 Holters confirmed frequent PVCs without evidence of VT or CPVT. The second individual found to have ventricular ectopy was a 7-year-old male who initially presented with syncope and had a family history of sudden death mentioned previously. He was found to have a structurally normal heart. ECG demonstrated normal sinus rhythm with a normal QTc, and a treadmill stress testing demonstrated PVCs. He has no history of VT with exercise or at rest. WES analysis demonstrated a RyR2-G3946S variant. He holds a diagnosis of possible CPVT. One-hundred and thirty-eight (98.6% [94.9–99.8]) individuals had no clinical evidence of ventricular ectopy and no clinical evidence of CPVT. These results are summarized in Figure 7C.

To evaluate for the presence of additional signs/symptoms of CPVT, we evaluated the subjects in the TCH cohort for history of syncope or a family history of sudden death. Two (1.4% [0.2–5.1%]) had a history of syncope, whereas 1 (0.7% [0.0–3.9]) had a family history of sudden death. The 7-year-old male described above demonstrated both characteristics. About 98.6% [94.9–99.8] of individuals had no history of syncope, and 99.3% [96.1–100.0] had no family history of sudden death. Overall, these findings suggest that among individuals with little or no clinical suspicion of occult arrhythmia, none demonstrated clinical evidence of CPVT despite the presence of variants on WES.

Discussion

Paramount to the interpretation of any clinical test is an understanding of the spectrum of healthy variation. Because the field of genetic testing, and the science behind genomic sequencing, has rapidly advanced, the ability to genetically interrogate individuals with clinical disease has rapidly outstripped our knowledge of the genetic background in the healthy population.[13] This has led to significant confusion over what clinical

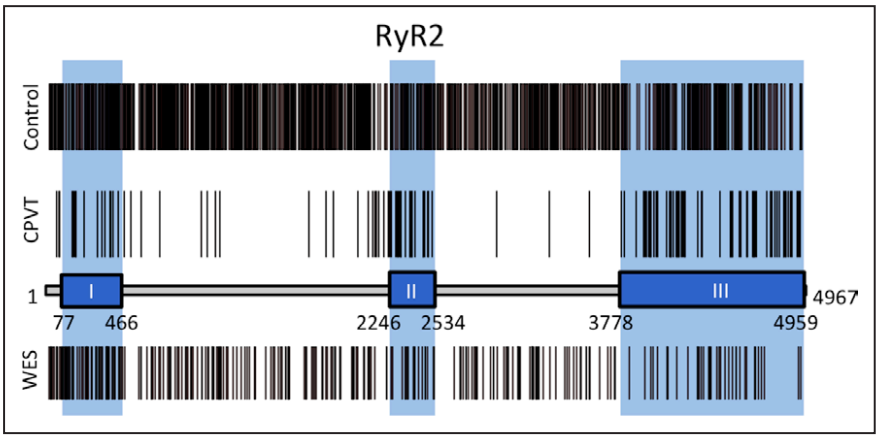

Figure 6. Topological variant mapping between cohorts. Schematic of control (ExAC), case (catecholaminergic polymorphic ventricular tachycardia [CPVT]), and whole-exome sequencing (WES) cohort variants illustrated as vertical lines mapped along the linear topology of RyR2. Three canonical RyR2 mutation hotspots (I, II, and III) are designated (blue fill/background).

Table 2. Variant-Positive Texas Children's Hospital Cohort Demographics and Characteristics

Referral Cohort	N
Individuals	140
Families	138
Age at genetic test, y	5.5 [2.1–9.2]
Male	74 (52.9%)
Female	66 (47.1%)
Black/African	18 (12.9%)
Asian American/Asian	7 (5.0%)
White, Non-Hispanic	46 (32.5%)
Hispanic	69 (49.6%)
History	
Syncope	2 (1.5% [0.2–5.1])
Sudden death (family)	1 (0.7% [0.0–3.9])
Diagnosis of CPVT	0 (0.0% [0.0–2.6])
Total variants	140
RYR2	139 (99.3% [96.1–100.0])
CASQ2	1 (0.7% [0.0–3.9])
Heterozygous	140 (100% [97.4–100.0])
Homozygous	0 (0.0% [0.0–2.6])

CPVT indicates catecholaminergic polymorphic ventricular tachycardia.

risk incidentally identified variants may confer, particularly variants that are not clearly linked to disease. Compounding this is the recommendation by the American College of Medical Genetics and Genomics to report any known pathological variant associated with CPVT that is incidentally found.[14] Although early studies have demonstrated the spectrum and prevalence of rare background variation in cardiomyopathic diseases, such as hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy, as well as channelopathic diseases, such as long QT syndrome, CPVT has lagged behind.[15–17] Our finding that ≈9% of individuals undergoing clinical WES will have a variant of either undetermined significance or deemed likely pathological that is eligible for clinical reporting highlights this problem. In the postgenomic era of science and medicine, the challenge has shifted away from developing genetic sequencing techniques to the clinical interpretation of the findings.

Classically, the interpretation of a genetic test result, or any other clinical test, has been done in the context of pretest clinical suspicion. Higher diagnostic value may be placed on a VUS in the setting of proband with a high suspicion for clinical disease with a positive family history and cosegregation of that variant with incidence of disease in the family. Conversely, lower diagnostic value may be placed on a variant identified in an individual who has low clinical suspicion of disease. Although ≈9% of individuals among the clinical WES cohort were positive for variants, a small minority of the referrals from our institution had clinical suspicion for occult channelopathic disease. Indeed, slightly <90% of the referral cohort was sent for genetic testing because of neurological disease, and only 5% had cardiac disease as the sole indication for genetic testing. Among these cardiac only referrals, only 1 patient had a structurally normal heart and a personal history concerning for channelopathic disease. Interestingly, the only subject to demonstrate any evidence of ventricular ectopy and may hold a diagnosis of CPVT is the individual with a high pre-WES clinical suspicion for occult channelopathy.

In an attempt to broadly guide interpretation of genetic testing findings, the American College of Medical Genetics and Genomics has published guidelines for classifying a variety of genetic variants along the spectrum of pathogenicity.[18] Within these guidelines is recognition that variant colocalization to an amino acid residue previously shown to contain a known pathological mutation is associated with a higher risk of pathogenicity. We find that there is overlap between the variant location of RyR2 variants associated with control individuals and CPVT cases with a notable 29% of variants in cases overlapping with residues with healthy variants. Conversely, 4% of variants found in healthy individuals genetically overlap with cases, implying inherent risk of overcalling the pathogenicity of healthy variation if indiscriminately applied. We also find that there is significant overlap in the WES variants and healthy individuals, 60%, which is significantly higher than with CPVT cases (8%). These support the conclusion that WES-identified variants are less likely to represent biomarkers of disease predisposition. At the same time, there remains many WES variants which, even in the context of a low clinical suspicion, may be ascribed elevated pathological status based solely on variant location or type. As such, caution should be given to ascribing increased likelihood of pathogenicity based solely on an incidentally identified variant in the absence of clinical suspicion for disease.[19]

Signs and symptoms of CPVT most often manifest in childhood. In a cohort-based study of CPVT, the first syncopal event occurred at a mean of ≈7.8 years with a diagnosis made at a mean age of ≈10 years. Further, approximately one third of CPVT patients had a family history of syncope or sudden death.[20] By nature of a demographic referral bias, we find that the subjects comprising the TCH cohort are youthful with a mean age of ≈6.6±6.3 years. Clinical evaluation for syncope, heritable sudden death, or ventricular ectopy identified a single individual with clinical suspicion for CPVT. Although we find that subjects with incidentally identified CPVT-associated variants demonstrate a low index of suspicion for occult channelopathic disease, we cannot exclude the possibility that clinical manifestations of CPVT may develop later in life.

There is recent evidence implicating *RYR2* mutations in the development of seizures, epilepsy, and sudden death in epilepsy.[21] Interestingly, although the majority of individuals referred for WES testing had a neurological diagnosis, epilepsy/seizures as the sole indication was rare (≈1.4%). While limited by the documentation sent by the referring provider, these findings support the conclusion that incidentally identified *RYR2* variants are not common among individuals with a sole referral diagnosis of seizures or epilepsy. In addition, several studies have linked mutations in *RYR2* to the development

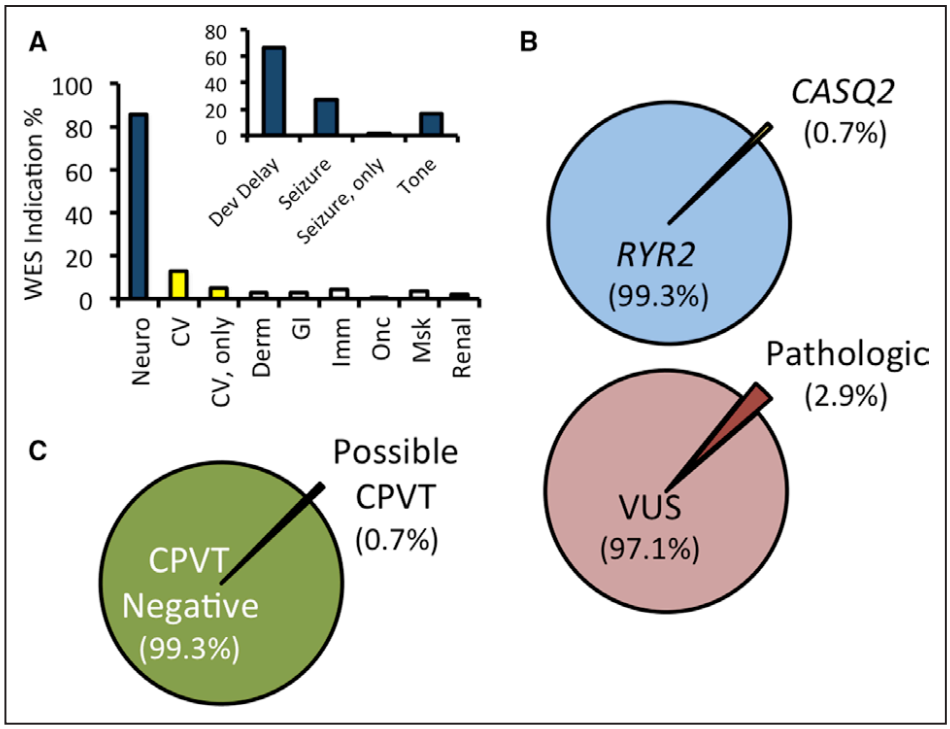

Figure 7. Pre- and post-test suspicion for catecholaminergic polymorphic ventricular tachycardia (CPVT) among Texas Children's Hospital (TCH) whole-exome sequencing (WES) referrals. **A**, Bar graph of the frequency of indication by organ systems for WES referral. Individuals referred for neurological diagnoses (neuro, blue fill), cardiovascular (CV, yellow fill), dermatologic (derm), gastrointestinal (GI), immunologic (imm), oncological (onc), musculoskeletal (MSK), and renal. **A**, Bar graph of neuro referrals subdivided into indications of developmental delay (dev delay), seizures, and hyper- or hypotonia. **B**, **top**, Pie chart of the proportion of referral cohort variants localizing to *RYR2* and *CASQ2*. **B**, **bottom**, Pie chart of the proportion of referral cohort variants interpreted as variants of unclear clinical significance (VUS) versus likely pathological. **C**, Pie chart of the proportion of referral cohort individuals with no clinical suspicion for CPVT versus possible CPVT.

of primary myocardial diseases such as arrhythmogenic right ventricular cardiomyopathy.[22] It is possible that some of these variants may represent susceptibility alleles for the development of cardiomyopathy in certain individuals.

Traditionally, CPVT-associated variants localizing to *RYR2* are missense mutations affecting a single amino acid, although the functional impact of radical mutations and their association with arrhythmia development are not well defined. We observe a low frequency of radical mutations among CPVT cases (2.6%) with a statistically similar frequency among control (0.9%) and WES (1.6%) subjects. In contrast, radical mutations in genes encoding the cardiac sarcomere and cardiac desmosome lead to a high likelihood of pathogenicity in hypertrophic cardiomyopathy and arrhythmogenic right ventricular cardiomyopathy, respectively.[16,17] One possibility is that this divergence represents an intolerance of the cardiomyocyte to loss-of-function mutations in large structural and mechanical proteins resulting in pathological myocardial remodeling, although there may be expression compensation of the wild-type *RYR2* allele preventing CPVT. Additional investigation will be needed to determine the functional impact of these radical *RYR2* variants. Conversely, CPVT-associated *CASQ2* mutations are inherited in an autosomal recessive fashion and cause disease when homozygous. We identified only a single individual hosting a homozygous *CASQ2* mutation, suggesting that the vast majority of variants within the WES cohort were variants with a presumed autosomal dominant inheritance pattern. Overall, this supports the conclusion that these variants either reflect potential unaffected genetic carriers or do not reflect a mode of inheritance which is classically associated with CPVT pathogenesis.

We observe that the background rare variant rate among ostensibly healthy volunteers is 6.0% which is similar to the background variant rate identified in long QT syndrome (≈5%) and hypertrophic cardiomyopathy (≈5%) and is significantly lower than the background rate of the arrhythmogenic right ventricular cardiomyopathy genetic test (≈16%).[15–17] This observed frequency is slightly lower than a previous estimate of CPVT background variation on a more limited cohort of ostensibly healthy individuals within the National Heart, Lung, and Blood Institute Exome Sequencing Project of ≈11%.[23] Given the observed yield of likely pathogenic mutations in 60% of individuals with phenotypically robust CPVT, this gives a signal:noise ratio of ≈10:1. This ratio is similar to long QT syndrome (≈15:1) and hypertrophic cardiomyopathy (≈12:1) which underscores the diagnostic use of the CPVT genetic test despite the background rate of genetic variation.[24] Given the high yield of CPVT genetic testing and the high signal:noise ratio, the targeted gene testing (ie, CPVT gene panel testing) remains the optimal diagnostic genetic testing tool. In this way, WES testing during the diagnostic evaluation of patient with suspected CPVT is unlikely to provide additional diagnostic use as an initial genetic testing platform.

Sources of Funding

Dr Landstrom is supported by National Institutes of Health grant NIH-L40 (HL129273-01), the Pediatric and Congenital

Electrophysiology Society Paul C. Gillette Award, and pilot grant funding from the Baylor College of Medicine Department of Pediatrics.

Disclosures

J.A. Rosenfeld and Dr Yang receive salary support from Baylor Miraca Genetics Laboratories. The other authors report no conflicts.

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Circulation
Arrhythmia and Electrophysiology

Interpreting Incidentally Identified Variants in Genes Associated With Catecholaminergic Polymorphic Ventricular Tachycardia in a Large Cohort of Clinical Whole-Exome Genetic Test Referrals

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Circ Arrhythm Electrophysiol. 2017;10:
doi: 10.1161/CIRCEP.116.004742
Circulation: Arrhythmia and Electrophysiology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1941-3149. Online ISSN: 1941-3084

SUPPLEMENTAL MATERIAL

Supplemental Table 1: Compendium of variants identified by WES testing associated with *RYR2* and *CASQ2*

Position	Desig	Gene	Location	Nucleotide	Amino Acid	Zygosity
Chr1: 116311152	VUS	*CASQ2*	Exon 1	c.11C>T	p.T4I	Het
Chr1: 116311119	VUS	*CASQ2*	Exon 1	c.44C>G	p.S15C	Het
Chr1: 116311107	VUS	*CASQ2*	Exon 1	c.56C>A	p.A19E	Het
Chr1: 116311054	VUS	*CASQ2*	Exon 1	c.109C>A	p.L37I	Het
Chr1: 116311015	VUS	*CASQ2*	Exon 1	c.148G>A	p.D50N	Het
Chr1: 116310993	VUS	*CASQ2*	Exon 1	c.170A>G	p.H57R	Het
Chr1: 116310990	VUS	*CASQ2*	Exon 1	c.173A>T	p.E58V	Het
Chr1: 116310958	VUS	*CASQ2*	Exon 1	c.205C>G	p.Q69E	Het
Chr1: 116287522	VUS	*CASQ2*	Exon 2	c.246G>C	p.Q82H	Het
Chr1: 116287503	VUS	*CASQ2*	Exon 2	c.265A>G	p.I89V	Het
Chr1: 116287481	VUS	*CASQ2*	Exon 2	c.287C>G	p.A96G	Het
Chr1: 116283431	VUS	*CASQ2*	Exon 3	c.338G>A	p.S113N	Het
Chr1: 116283408	VUS	*CASQ2*	Exon 3	c.361C>T	p.R121C	Het
Chr1: 116283366	VUS	*CASQ2*	Exon 3	c.G403A	p.V135M	Het
Chr1: 116283360	VUS	*CASQ2*	Exon 3	c.409T>C	p.F137L	Het
Chr1: 116283356	VUS	*CASQ2*	Exon 3	c.413T>C	p.L138P	Het
Chr1: 116280902	VUS	*CASQ2*	Exon 4	c.475G>A	p.E159K	Het
Chr1: 116280898	VUS	*CASQ2*	Exon 4	c.479G>A	p.R160H	Het
Chr1: 116280896	VUS	*CASQ2*	Exon 4	c.481A>G	p.I161V	Het
Chr1: 116280896	VUS	*CASQ2*	Exon 4	c.481A>C	p.I161L	Het
Chr1: 116280895	VUS	*CASQ2*	Exon 4	c.482T>C	p.I161T	Het
Chr1: 116280886	VUS	*CASQ2*	Exon 4	c.491A>G	p.Y164C	Het
Chr1: 116275561	VUS	*CASQ2*	Exon 5	c.567C>G	p.F189L	Het
Chr1: 116275525	VUS	*CASQ2*	Exon 5	c.603A>G	p.K201K	Het
Chr1: 116269620	VUS	*CASQ2*	Exon 6	c.730C>T	p.H244Y	Het
Chr1: 116268164	VUS	*CASQ2*	Exon 7	c.748C>T	p.R250C	Het
Chr1: 116268164	VUS	*CASQ2*	Exon 7	c.748C>G	p.R250G	Het
Chr1: 116268160	VUS	*CASQ2*	Exon 7	c.752G>A	p.R251H	Het
Chr1: 116268154	VUS	*CASQ2*	Exon 7	c.758G>A	p.R253H	Het
Chr1: 116247890	VUS	*CASQ2*	Exon 9	c.862C>G	p.L288V	Het
Chr1: 116247824	VUS	*CASQ2*	Exon 9	c.928G>A	p.D310N	Het
Chr1: 116247824	VUS	*CASQ2*	Exon 9	c.928G>A	p.D310N	Hom
Chr1: 116245613	VUS	*CASQ2*	Exon 10	c.G943A	p.V315I	Het
Chr1: 116245571	VUS	*CASQ2*	Exon 10	c.985C>T	p.P329S	Het
Chr1: 116245562	VUS	*CASQ2*	Exon 10	c.994G>A	p.G332R	Het
Chr1: 116245539	VUS	*CASQ2*	IVS	c.1014+3G>A	N/A	Het
Chr1: 116244010	VUS	*CASQ2*	Exon 11	c.1052A>G	p.D351G	Het

Chr1: 116243931	VUS	*CASQ2*	Exon 11	c.1131A>T	p.E377D	Het
Chr1: 116243926	VUS	*CASQ2*	Exon 11	c.1136A>T	p.D379V	Het
Chr1: 116243912	VUS	*CASQ2*	Exon 11	c.1150A>C	p.N384H	Het
Chr1: 116243912	VUS	*CASQ2*	Exon 11	c.1147_1149del	p.383_383del	Het
Chr1: 116243876	VUS	*CASQ2*	Exon 11	c.1186G>A	p.D396N	Het
Chr1: 116243874	VUS	*CASQ2*	Exon 11	c.1185_1187del	p.395_396del	Het
Chr1: 116243866	VUS	*CASQ2*	Exon 11	c.1196A>C	p.E399A	Het
Chr1: 237205857	VUS	*RYR2*	Exon 1	c.36G>C	p.Q12H	Het
Chr1: 237433824	VUS	*RYR2*	Exon 2	c.76G>A	p.A26T	Het
Chr1: 237538090	VUS	*RYR2*	Exon 7	c.458C>T	p.T153I	Het
Chr1: 237550653	VUS	*RYR2*	Exon 9	c.649A>G	p.I217V	Het
Chr1: 237551392	VUS	*RYR2*	Exon 10	c.682C>T	p.L228F	Het
Chr1: 237580357	VUS	*RYR2*	Exon 11	c.782A>G	p.H261R	Het
Chr1: 237580423	VUS	*RYR2*	Exon 11	c.848C>T	p.A283V	Het
Chr1: 237586469	VUS	*RYR2*	Exon 12	c.926T>C	p.M309T	Het
Chr1: 237586493	VUS	*RYR2*	Exon 12	c.950T>C	p.M317T	Het
Chr1: 237604635	VUS	*RYR2*	Exon 13	c.1022G>C	p.G341A	Het
Chr1: 237604683	VUS	*RYR2*	Exon 13	c.1070G>A	p.G357D	Het
Chr1: 237604748	VUS	*RYR2*	Exon 13	c.1135G>A	p.V379M	Het
Chr1: 237604748	VUS	*RYR2*	Exon 13	c.G1135A	p.V379M	Het
Chr1: 237604757	VUS	*RYR2*	Exon 13	c.1144G>A	p.V382M	Het
Chr1: 237604767	VUS	*RYR2*	Exon 13	c.1154G>C	p.G385A	Het
Chr1: 237608702	VUS	*RYR2*	Exon 14	c.1172C>G	p.A391G	Het
Chr1: 237608704	VUS	*RYR2*	Exon 14	c.1174A>G	p.I392V	Het
Chr1: 237608779	VUS	*RYR2*	Exon 14	c.1249C>G	p.R417G	Het
Chr1: 237608798	VUS	*RYR2*	Exon 14	c.1268T>C	p.V423A	Het
Chr1: 237608827	VUS	*RYR2*	IVS	c.1292+5T>C	N/A	Het
Chr1: 237617694	VUS	*RYR2*	Exon 15	c.1296C>A	p.G432G	Het
Chr1: 237617716	VUS	*RYR2*	Exon 15	c.1318G>A	p.A440T	Het
Chr1: 237617790	VUS	*RYR2*	Exon 15	c.1392C>A	p.H464Q	Het
Chr1: 237617794	VUS	*RYR2*	Exon 15	c.1396C>G	p.P466A	Het
Chr1: 237617795	VUS	*RYR2*	Exon 15	c.1397C>A	p.P466Q	Het
Chr1: 237617821	VUS	*RYR2*	Exon 15	c.1423A>C	p.K475Q	Het
Chr1: 237617822	VUS	*RYR2*	Exon 15	c.1424A>G	p.K475R	Het
Chr1: 237617852	VUS	*RYR2*	Exon 15	c.1454G>A	p.R485Q	Het
Chr1: 237617877	VUS	*RYR2*	IVS	c.1476+3C>T	N/A	Het
Chr1: 237617878	VUS	*RYR2*	IVS	c.1476+4C>T	N/A	Het
Chr1: 237617879	VUS	*RYR2*	IVS	c.1476+5G>A	N/A	Het
Chr1: 237619934	VUS	*RYR2*	Exon 16	c.1511G>A	p.R504H	Het
Chr1: 237655181	VUS	*RYR2*	Exon 18	c.1784A>G	p.K595R	Het
Chr1: 237655219	VUS	*RYR2*	Exon 18	c.1822C>T	p.H608Y	Het
Chr1: 237656251	VUS	*RYR2*	IVS	c.1828-3C>A	N/A	Het
Chr1: 237656254	VUS	*RYR2*	Exon 19	c.1828G>T	p.V610F	Het

Chr1: 237656365	VUS	*RYR2*	Exon 19	c.1939C>T	p.R647C	Het
Chr1: 237659884	VUS	*RYR2*	Exon 20	c.2035G>A	p.V679M	Het
Chr1: 237659888	VUS	*RYR2*	Exon 20	c.2039A>G	p.D680G	Het
Chr1: 237659906	VUS	*RYR2*	Exon 20	c.2057T>G	p.V686G	Het
Chr1: 237659953	VUS	*RYR2*	Exon 20	c.2104G>A	p.G702R	Het
Chr1: 237660023	VUS	*RYR2*	Exon 20	c.2174A>G	p.Y725C	Het
Chr1: 237660057	VUS	*RYR2*	IVS	c.2203+5G>A	N/A	Het
Chr1: 237664023	VUS	*RYR2*	Exon 21	c.2216G>A	p.R739H	Het
Chr1: 237664074	VUS	*RYR2*	Exon 21	c.2267G>A	p.S756N	Het
Chr1: 237664164	VUS	*RYR2*	Exon 21	c.2357G>A	p.G786D	Het
Chr1: 237664194	VUS	*RYR2*	Exon 21	c.2387C>T	p.A796V	Het
Chr1: 237666591	VUS	*RYR2*	Exon 22	c.T2399C	p.V800A	Het
Chr1: 237666602	VUS	*RYR2*	Exon 22	c.2410C>T	p.L804F	Het
Chr1: 237666702	VUS	*RYR2*	Exon 22	c.2510G>C	p.S837T	Het
Chr1: 237666737	VUS	*RYR2*	Exon 22	c.2545G>A	p.D849N	Het
Chr1: 237666765	VUS	*RYR2*	Exon 22	c.2573C>T	p.T858M	Het
Chr1: 237666809	VUS	*RYR2*	IVS	c.2613+4C>G	N/A	Het
Chr1: 237670026	VUS	*RYR2*	Exon 23	c.2630A>C	p.H877P	Het
Chr1: 237670107	VUS	*RYR2*	Exon 23	c.2711A>G	p.Y904C	Het
Chr1: 237670113	VUS	*RYR2*	Exon 23	c.C2717T	p.P906L	Het
Chr1: 237675024	VUS	*RYR2*	Exon 24	c.2755G>A	p.V919M	Het
Chr1: 237675076	VUS	*RYR2*	Exon 24	c.2807C>T	p.S936L	Het
Chr1: 237693732	VUS	*RYR2*	Exon 25	c.2828T>C	p.L943S	Het
Chr1: 237693755	VUS	*RYR2*	Exon 25	c.2851G>C	p.G951R	Het
Chr1: 237711759	VUS	*RYR2*	Exon 26	c.2935G>T	p.A979S	Het
Chr1: 237711849	VUS	*RYR2*	Exon 26	c.3025C>T	p.R1009W	Het
Chr1: 237711861	VUS	*RYR2*	Exon 26	c.3037C>T	p.R1013W	Het
Chr1: 237711862	VUS	*RYR2*	Exon 26	c.3038G>A	p.R1013Q	Het
Chr1: 237713884	VUS	*RYR2*	Exon 27	c.3107C>G	p.T1036S	Het
Chr1: 237713928	VUS	*RYR2*	Exon 27	c.3151C>T	p.R1051C	Het
Chr1: 237713929	VUS	*RYR2*	Exon 27	c.3152G>A	p.R1051H	Het
Chr1: 237713934	VUS	*RYR2*	Exon 27	c.3157G>T	p.A1053S	Het
Chr1: 237729882	VUS	*RYR2*	Exon 28	c.3230T>C	p.V1077A	Het
Chr1: 237729882	VUS	*RYR2*	Exon 28	c.3230T>G	p.V1077G	Het
Chr1: 237729890	VUS	*RYR2*	Exon 28	c.3238G>A	p.G1080S	Het
Chr1: 237729903	VUS	*RYR2*	Exon 28	c.3251G>A	p.R1084K	Het
Chr1: 237729918	VUS	*RYR2*	Exon 28	c.3266G>A	p.R1089H	Het
Chr1: 237729972	VUS	*RYR2*	Exon 28	c.3320C>T	p.T1107M	Het
Chr1: 237729972	VUS	*RYR2*	Exon 28	c.C3320T	p.T1107M	Het
Chr1: 237730008	VUS	*RYR2*	Exon 28	c.3356G>A	p.R1119H	Het
Chr1: 237730013	VUS	*RYR2*	Exon 28	c.3361G>C	p.G1121R	Het
Chr1: 237730032	VUS	*RYR2*	Exon 28	c.3380A>G	p.E1127G	Het
Chr1: 237730032	VUS	*RYR2*	Exon 28	c.A3380G	p.E1127G	Het

Chr1: 237730050	VUS	*RYR2*	Exon 28	c.3398G>A	p.R1133H	Het
Chr1: 237730059	VUS	*RYR2*	Exon 28	c.C3407T	p.A1136V	Het
Chr1: 237732481	VUS	*RYR2*	Exon 29	c.3460C>A	p.R1154S	Het
Chr1: 237732543	VUS	*RYR2*	Exon 29	c.3522G>A	p.M1174I	Het
Chr1: 237732563	VUS	*RYR2*	Exon 29	c.3542T>C	p.I1181T	Het
Chr1: 237753147	VUS	*RYR2*	Exon 30	c.3653G>A	p.G1218E	Het
Chr1: 237753215	VUS	*RYR2*	Exon 30	c.3721G>A	p.V1241I	Het
Chr1: 237753299	VUS	*RYR2*	Exon 30	c.3805G>C	p.E1269Q	Het
Chr1: 237754022	VUS	*RYR2*	Exon 31	c.3890C>G	p.T1297S	Het
Chr1: 237754064	VUS	*RYR2*	Exon 31	c.3932C>T	p.A1311V	Het
Chr1: 237754105	VUS	*RYR2*	Exon 31	c.3973G>C	p.A1325P	Het
Chr1: 237754201	VUS	*RYR2*	Exon 31	c.4069G>C	p.D1357H	Het
Chr1: 237754226	VUS	*RYR2*	Exon 31	c.4094C>T	p.A1365V	Het
Chr1: 237756794	VUS	*RYR2*	Exon 33	c.4294A>C	p.I1432L	Het
Chr1: 237756903	VUS	*RYR2*	Exon 33	c.4403C>T	p.T1468I	Het
Chr1: 237758806	VUS	*RYR2*	Exon 34	c.4445G>A	p.R1482H	Het
Chr1: 237758826	VUS	*RYR2*	Exon 34	c.4465T>C	p.C1489R	Het
Chr1: 237758905	VUS	*RYR2*	Exon 34	c.4544G>A	p.S1515N	Hom
Chr1: 237758946	VUS	*RYR2*	Exon 34	c.4585A>C	p.T1529P	Het
Chr1: 237765349	VUS	*RYR2*	Exon 35	c.4621C>T	p.P1541S	Het
Chr1: 237774070	VUS	*RYR2*	Exon 36	c.4692G>A	p.M1564I	Het
Chr1: 237774071	VUS	*RYR2*	Exon 36	c.4693C>G	p.P1565A	Het
Chr1: 237774113	VUS	*RYR2*	Exon 36	c.4735G>A	p.V1579M	Het
Chr1: 237774124	VUS	*RYR2*	Exon 36	c.4746C>G	p.C1582W	Het
Chr1: 237774125	VUS	*RYR2*	Exon 36	c.4747C>T	p.P1583S	Het
Chr1: 237774138	VUS	*RYR2*	Exon 36	c.4760A>G	p.H1587R	Het
Chr1: 237774291	VUS	*RYR2*	IVS	c.4910+3C>T	N/A	Het
Chr1: 237777335	VUS	*RYR2*	IVS	c.4911-4A>G	N/A	Het
Chr1: 237777488	VUS	*RYR2*	Exon 37	c.5060A>C	p.Y1687S	Het
Chr1: 237777509	VUS	*RYR2*	Exon 37	c.5081T>C	p.M1694T	Het
Chr1: 237777530	VUS	*RYR2*	Exon 37	c.5102G>C	p.G1701A	Het
Chr1: 237777626	VUS	*RYR2*	Exon 37	c.5198C>T	p.T1733M	Het
Chr1: 237777663	VUS	*RYR2*	Exon 37	c.5235A>C	p.K1745N	Het
Chr1: 237777694	VUS	*RYR2*	Exon 37	c.T5266G	p.S1756A	Het
Chr1: 237777722	VUS	*RYR2*	Exon 37	c.5294C>G	p.S1765C	Het
Chr1: 237777722	VUS	*RYR2*	Exon 37	c.5294C>G	p.S1765C	Hom
Chr1: 237777722	VUS	*RYR2*	Exon 37	c.C5294G	p.S1765C	Het
Chr1: 237777832	VUS	*RYR2*	Exon 37	c.5404G>A	p.G1802S	Het
Chr1: 237777847	VUS	*RYR2*	Exon 37	c.5419C>T	p.R1807W	Het
Chr1: 237777937	VUS	*RYR2*	Exon 37	c.5509G>A	p.E1837K	Het
Chr1: 237778016	VUS	*RYR2*	Exon 37	c.5588C>T	p.T1863M	Het
Chr1: 237778042	VUS	*RYR2*	Exon 37	c.5614G>A	p.D1872N	Het
Chr1: 237778082	VUS	*RYR2*	Exon 37	c.G5654A	p.G1885E	Het

Chr1: 237778088	VUS	*RYR2*	Exon 37	c.5660A>G	p.K1887R	Het
Chr1: 237778108	VUS	*RYR2*	Exon 37	c.5680C>A	p.L1894I	Het
Chr1: 237780626	VUS	*RYR2*	Exon 38	c.5756G>A	p.R1919Q	Het
Chr1: 237780631	VUS	*RYR2*	Exon 38	c.5761C>T	p.R1921W	Het
Chr1: 237780644	VUS	*RYR2*	Exon 38	c.5774T>C	p.I1925T	Het
Chr1: 237787153	VUS	*RYR2*	Exon 39	c.6005A>G	p.D2002G	Het
Chr1: 237787161	VUS	*RYR2*	Exon 39	c.6013A>G	p.T2005A	Het
Chr1: 237787167	VUS	*RYR2*	Exon 39	c.6019T>A	p.C2007S	Het
Chr1: 237787167	VUS	*RYR2*	Exon 39	c.T6019A	p.C2007S	Het
Chr1: 237787168	VUS	*RYR2*	Exon 39	c.6020G>T	p.C2007F	Het
Chr1: 237787175	VUS	*RYR2*	IVS	c.6022+5G>A	N/A	Het
Chr1: 237788984	VUS	*RYR2*	Exon 40	c.6046T>A	p.S2016T	Het
Chr1: 237789017	VUS	*RYR2*	Exon 40	c.6079G>A	p.G2027R	Het
Chr1: 237789053	VUS	*RYR2*	Exon 40	c.6115C>A	p.L2039M	Het
Chr1: 237791175	VUS	*RYR2*	Exon 41	c.6235G>A	p.E2079K	Het
Chr1: 237791185	VUS	*RYR2*	Exon 41	c.6245G>C	p.R2082T	Het
Chr1: 237791260	VUS	*RYR2*	Exon 41	c.6320C>T	p.T2107M	Het
Chr1: 237791277	VUS	*RYR2*	Exon 41	c.6337G>A	p.V2113M	Het
Chr1: 237791277	VUS	*RYR2*	Exon 41	c.G6337A	p.V2113M	Het
Chr1: 237791286	VUS	*RYR2*	Exon 41	c.6346A>G	p.T2116A	Het
Chr1: 237791298	VUS	*RYR2*	Exon 41	c.6358_6359insTGGCAT	p.L2120delinsLAL	Het
Chr1: 237791323	VUS	*RYR2*	Exon 41	c.6383C>G	p.S2128C	Het
Chr1: 237794731	VUS	*RYR2*	Exon 42	c.6445A>G	p.I2149V	Het
Chr1: 237798193	VUS	*RYR2*	Exon 44	c.6693C>G	p.S2231S	Het
Chr1: 237798195	VUS	*RYR2*	Exon 44	c.6695C>A	p.P2232Q	Het
Chr1: 237798272	VUS	*RYR2*	Exon 44	c.C6772T	p.R2258C	Het
Chr1: 237801654	VUS	*RYR2*	IVS	c.6793-3C>T	N/A	Het
Chr1: 237801675	VUS	*RYR2*	Exon 45	c.6811G>T	p.G2271C	Het
Chr1: 237802380	VUS	*RYR2*	Exon 46	c.6994G>T	p.G2332C	Het
Chr1: 237802446	VUS	*RYR2*	Exon 46	c.7060G>T	p.A2354S	Het
Chr1: 237802449	VUS	*RYR2*	Exon 46	c.7063G>A	p.E2355K	Het
Chr1: 237802480	VUS	*RYR2*	Exon 46	c.7094A>G	p.N2365S	Het
Chr1: 237802500	VUS	*RYR2*	Exon 46	c.7114C>A	p.L2372I	Het
Chr1: 237806629	VUS	*RYR2*	Exon 48	c.7224G>A	p.L2408L	Het
Chr1: 237806639	VUS	*RYR2*	Exon 48	c.7234G>A	p.G2412R	Het
Chr1: 237806713	VUS	*RYR2*	Exon 48	c.7309delA	p.S2437fs	Het
Chr1: 237806726	VUS	*RYR2*	Exon 48	c.7321C>G	p.Q2441E	Het
Chr1: 237811851	VUS	*RYR2*	Exon 49	c.7450C>T	p.L2484F	Het
Chr1: 237811859	VUS	*RYR2*	Exon 49	c.T7458G	p.H2486Q	Het
Chr1: 237811894	VUS	*RYR2*	Exon 49	c.7493C>T	p.A2498V	Het
Chr1: 237813283	VUS	*RYR2*	Exon 50	c.7619A>G	p.H2540R	Het
Chr1: 237813285	VUS	*RYR2*	Exon 50	c.7621C>A	p.H2541N	Het
Chr1: 237817572	VUS	*RYR2*	IVS	c.7825-2A>G	N/A	Het

Chr1: 237821259	VUS	*RYR2*	Exon 54	c.8145G>T	p.E2715D	Het
Chr1: 237821261	VUS	*RYR2*	Exon 54	c.8147A>T	p.K2716I	Het
Chr1: 237821276	VUS	*RYR2*	Exon 54	c.8162T>C	p.I2721T	Het
Chr1: 237821318	VUS	*RYR2*	Exon 54	c.8204A>C	p.D2735A	Het
Chr1: 237823338	VUS	*RYR2*	Exon 55	c.8262G>C	p.Q2754H	Het
Chr1: 237824120	VUS	*RYR2*	Exon 56	c.8309T>C	p.I2770T	Het
Chr1: 237824189	VUS	*RYR2*	Exon 56	c.8378G>A	p.R2793Q	Het
Chr1: 237824230	VUS	*RYR2*	Exon 56	c.8419A>G	p.I2807V	Het
Chr1: 237829807	VUS	*RYR2*	IVS	c.8437-5T>G	N/A	Het
Chr1: 237829818	VUS	*RYR2*	Exon 57	c.8443G>T	p.V2815L	Het
Chr1: 237829845	VUS	*RYR2*	Exon 57	c.8470C>T	p.R2824W	Het
Chr1: 237829857	VUS	*RYR2*	Exon 57	c.A8482C	p.M2828L	Het
Chr1: 237831240	VUS	*RYR2*	Exon 58	c.8573_8578del	p.2858_2860del	Het
Chr1: 237837397	VUS	*RYR2*	Exon 59	c.8592A>G	p.G2864G	Het
Chr1: 237838065	VUS	*RYR2*	Exon 60	c.8749A>G	p.I2917V	Het
Chr1: 237838114	VUS	*RYR2*	Exon 60	c.8798T>G	p.V2933G	Het
Chr1: 237838122	VUS	*RYR2*	Exon 60	c.8806G>A	p.A2936T	Het
Chr1: 237838150	VUS	*RYR2*	IVS	c.8830+4G>A	N/A	Het
Chr1: 237841389	VUS	*RYR2*	Exon 61	c.8872C>A	p.Q2958K	Het
Chr1: 237843864	VUS	*RYR2*	Exon 62	c.9004G>C	p.E3002Q	Het
Chr1: 237850787	VUS	*RYR2*	Exon 63	c.9050A>G	p.H3017R	Het
Chr1: 237862291	VUS	*RYR2*	Exon 64	c.9094T>G	p.C3032G	Het
Chr1: 237863590	VUS	*RYR2*	Exon 65	c.9190G>A	p.A3064T	Het
Chr1: 237863684	VUS	*RYR2*	Exon 65	c.9284A>G	p.N3095S	Het
Chr1: 237863690	VUS	*RYR2*	Exon 65	c.9290C>T	p.T3097I	Het
Chr1: 237863693	VUS	*RYR2*	Exon 65	c.9293C>T	p.T3098I	Het
Chr1: 237863695	VUS	*RYR2*	Exon 65	c.9295G>C	p.V3099L	Het
Chr1: 237863710	VUS	*RYR2*	Exon 65	c.9310A>G	p.M3104V	Het
Chr1: 237863731	VUS	*RYR2*	Exon 65	c.9331C>T	p.H3111Y	Het
Chr1: 237868510	VUS	*RYR2*	IVS	c.9450-3T>C	N/A	Het
Chr1: 237868581	VUS	*RYR2*	Exon 67	c.9518C>T	p.T3173I	Het
Chr1: 237868623	VUS	*RYR2*	Exon 67	c.9560A>G	p.K3187R	Het
Chr1: 237868632	VUS	*RYR2*	Exon 67	c.9569G>A	p.R3190Q	Het
Chr1: 237868634	VUS	*RYR2*	Exon 67	c.9571G>C	p.E3191Q	Het
Chr1: 237870253	VUS	*RYR2*	Exon 68	c.9585C>T	p.L3195L	Het
Chr1: 237870269	VUS	*RYR2*	Exon 68	c.9601G>A	p.V3201M	Het
Chr1: 237870309	VUS	*RYR2*	Exon 68	c.9641T>A	p.L3214H	Het
Chr1: 237870323	VUS	*RYR2*	Exon 68	c.9655G>A	p.V3219M	Het
Chr1: 237870341	VUS	*RYR2*	Exon 68	c.9673G>A	p.G3225S	Het
Chr1: 237870360	VUS	*RYR2*	Exon 68	c.9692T>C	p.M3231T	Het
Chr1: 237870369	VUS	*RYR2*	Exon 68	c.9701T>C	p.V3234A	Het
Chr1: 237870446	VUS	*RYR2*	Exon 68	c.9778C>T	p.R3260W	Het
Chr1: 237872179	VUS	*RYR2*	Exon 69	c.9923A>G	p.N3308S	Het

Chr1: 237872291	VUS	*RYR2*	Exon 69	c.10035G>C	p.R3345S	Het
Chr1: 237872402	VUS	*RYR2*	IVS	c.10142+4T>C	N/A	Het
Chr1: 237875040	VUS	*RYR2*	IVS	c.10231-5C>T	N/A	Het
Chr1: 237875041	VUS	*RYR2*	IVS	c.10231-4T>C	N/A	Het
Chr1: 237875072	VUS	*RYR2*	Exon 71	c.10258G>A	p.V3420I	Het
Chr1: 237875118	VUS	*RYR2*	Exon 71	c.10304C>T	p.T3435I	Het
Chr1: 237875140	VUS	*RYR2*	IVS	c.10323+3A>G	N/A	Het
Chr1: 237880494	VUS	*RYR2*	IVS	c.10324-4A>G	N/A	Het
Chr1: 237880555	VUS	*RYR2*	Exon 72	c.10381A>G	p.M3461V	Het
Chr1: 237880618	VUS	*RYR2*	Exon 72	c.10444G>A	p.A3482T	Het
Chr1: 237881790	VUS	*RYR2*	Exon 73	c.10523T>C	p.I3508T	Het
Chr1: 237881795	VUS	*RYR2*	Exon 73	c.10528C>T	p.R3510C	Het
Chr1: 237881795	VUS	*RYR2*	Exon 73	c.10528C>A	p.R3510S	Het
Chr1: 237881796	VUS	*RYR2*	Exon 73	c.10529G>A	p.R3510H	Het
Chr1: 237886504	VUS	*RYR2*	Exon 74	c.10631C>T	p.P3544L	Het
Chr1: 237886504	VUS	*RYR2*	Exon 74	c.C10631G	p.P3544R	Het
Chr1: 237886513	VUS	*RYR2*	Exon 74	c.10640C>T	p.T3547M	Het
Chr1: 237886566	VUS	*RYR2*	IVS	c.10689+4A>G	N/A	Het
Chr1: 237889582	VUS	*RYR2*	Exon 75	c.10699C>T	p.R3567C	Het
Chr1: 237889592	VUS	*RYR2*	Exon 75	c.10709G>A	p.R3570Q	Het
Chr1: 237890503	VUS	*RYR2*	IVS	c.10838+4G>A	N/A	Het
Chr1: 237893618	VUS	*RYR2*	Exon 77	c.10897G>A	p.E3633K	Het
Chr1: 237897028	VUS	*RYR2*	Exon 79	c.11063A>G	p.Y3688C	Het
Chr1: 237905620	VUS	*RYR2*	Exon 80	c.11116G>A	p.D3706N	Het
Chr1: 237919639	VUS	*RYR2*	Exon 81	c.11197G>A	p.D3733N	Het
Chr1: 237919649	VUS	*RYR2*	Exon 81	c.C11207T	p.A3736V	Het
Chr1: 237934114	VUS	*RYR2*	Exon 85	c.11484G>T	p.K3828N	Het
Chr1: 237941995	VUS	*RYR2*	Exon 88	c.11805G>C	p.L3935F	Het
Chr1: 237944903	VUS	*RYR2*	Exon 89	c.11919T>G	p.D3973E	Het
Chr1: 237946976	VUS	*RYR2*	Exon 90	c.11964T>A	p.G3988G	Het
Chr1: 237947059	VUS	*RYR2*	Exon 90	c.12047T>A	p.F4016Y	Het
Chr1: 237947256	VUS	*RYR2*	Exon 90	c.12244G>A	p.E4082K	Het
Chr1: 237947261	VUS	*RYR2*	Exon 90	c.12249C>A	p.F4083L	Het
Chr1: 237947439	VUS	*RYR2*	Exon 90	c.12427A>C	p.K4143Q	Het
Chr1: 237947659	VUS	*RYR2*	Exon 90	c.12647C>G	p.A4216G	Het
Chr1: 237947676	VUS	*RYR2*	Exon 90	c.12665_12667del	p.4222_4223del	Het
Chr1: 237947847	VUS	*RYR2*	Exon 90	c.12835A>G	p.M4279V	Het
Chr1: 237947854	VUS	*RYR2*	Exon 90	c.12842C>T	p.T4281M	Het
Chr1: 237947877	VUS	*RYR2*	Exon 90	c.12865A>G	p.S4289G	Het
Chr1: 237947931	VUS	*RYR2*	Exon 90	c.12919C>T	p.R4307C	Het
Chr1: 237947976	VUS	*RYR2*	Exon 90	c.12964G>A	p.A4322T	Het
Chr1: 237948073	VUS	*RYR2*	Exon 90	c.13061T>G	p.L4354R	Het
Chr1: 237948081	VUS	*RYR2*	Exon 90	c.13069G>A	p.A4357T	Het

Chr1: 237948115	VUS	*RYR2*	Exon 90	c.13103A>G	p.E4368G	Het
Chr1: 237949275	VUS	*RYR2*	Exon 91	c.13267A>C	p.K4423Q	Het
Chr1: 237949299	VUS	*RYR2*	Exon 91	c.13291G>A	p.E4431K	Het
Chr1: 237951285	VUS	*RYR2*	IVS	c.13329-3T>C	N/A	Het
Chr1: 237951338	VUS	*RYR2*	Exon 92	c.13379A>G	p.Q4460R	Het
Chr1: 237951416	VUS	*RYR2*	Exon 92	c.13457C>G	p.A4486G	Het
Chr1: 237954787	VUS	*RYR2*	Exon 93	c.13535C>G	p.A4512G	Het
Chr1: 237955441	VUS	*RYR2*	Exon 94	c.13600C>T	p.P4534S	Het
Chr1: 237955445	VUS	*RYR2*	Exon 94	c.13604C>G	p.T4535R	Het
Chr1: 237955513	VUS	*RYR2*	Exon 94	c.13672C>T	p.H4558Y	Het
Chr1: 237955615	VUS	*RYR2*	Exon 94	c.13774T>C	p.C4592R	Het
Chr1: 237961356	VUS	*RYR2*	Exon 97	c.13976A>G	p.E4659G	Het
Chr1: 237961422	VUS	*RYR2*	Exon 97	c.14042A>G	p.D4681G	Het
Chr1: 237961432	VUS	*RYR2*	Exon 97	c.14053_14055del	p.4685_4685del	Het
Chr1: 237965166	VUS	*RYR2*	Exon 98	c.14101A>G	p.I4701V	Het
Chr1: 237965217	VUS	*RYR2*	IVS	c.14151+1G>A	N/A	Het
Chr1: 237969452	VUS	*RYR2*	Exon 99	c.14167G>A	p.A4723T	Het
Chr1: 237993933	VUS	*RYR2*	IVS	c.14756+3G>A	N/A	Het
Chr1: 237995874	VUS	*RYR2*	Exon 105	c.14831A>G	p.Y4944C	Het
Chr1: 237995918	VUS	*RYR2*	Exon 105	c.14875C>T	p.R4959W	Het