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1 **Classification and correlation of *RYR2* missense variants in individuals with catecholaminergic**  
2 **polymorphic ventricular tachycardia reveals phenotypic relationships**

3

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31

32 **Abstract**

33 Catecholaminergic polymorphic ventricular tachycardia (CPVT) is predominantly caused by  
34 heterozygous missense variants in the cardiac ryanodine receptor, *RYR2*. However, many *RYR2*  
35 missense variants are classified as variants of uncertain significance (VUS). We systematically re-  
36 evaluated all *RYR2* variants in healthy individuals and those with CPVT or arrhythmia using the 2015  
37 American College of Medical Genomics guidelines. *RYR2* variants were identified by the NW  
38 Genomic Laboratory Hub, from the published literature and databases of sequence variants. Each  
39 variant was assessed based on minor allele frequencies, *in silico* prediction tools and appraisal of  
40 functional studies and classified according to the ACMG-AMP guidelines. Phenotype data was  
41 collated where available. Of the 326 identified *RYR2* missense variants, 55 (16.9%), previously  
42 disease-associated variants were re-classified as benign. Application of the gnomAD database of  
43 >140,000 controls allowed reclassification of 11 variants more than the ExAC database. CPVT-  
44 associated *RYR2* variants clustered predominantly between amino acid positions 3949-4332 and  
45 4867-4967 as well as the RyR and IP3R homology associated and ion transport domains ( $P < 0.005$ ).  
46 CPVT-associated *RYR2* variants occurred at more conserved amino acid positions compared to  
47 controls, and variants associated with sudden death had higher conservation scores ( $P < 0.005$ ).  
48 There were **five potentially pathogenic** *RYR2* variants associated with sudden death during sleep  
49 which were located almost exclusively in the C-terminus of the protein. In conclusion, control  
50 sequence databases facilitate reclassification of *RYR2* variants but the majority remain as VUS.  
51 Notably, **pathogenic** variants in *RYR2* are associated with death in sleep.

52

53 KEYWORDS: Catecholaminergic ventricular tachycardia, cardiac ryanodine receptor, variant  
54 classification, arrhythmia.

55

56

57 **Introduction**

58 The rare monogenic arrhythmogenic disorder catecholaminergic polymorphic ventricular  
59 tachycardia (CPVT, MIM 604772) is characterised by episodic ventricular dysrhythmia triggered by  
60 exercise or emotion in individuals without structural cardiac defects (1). CPVT can be inherited in  
61 both an autosomal dominant form caused by heterozygous pathogenic variants in the cardiac  
62 ryanodine receptor gene (*RYR2*) (MIM 180902)(2), less frequently in *CALM1* (MIM 114180)(3)  
63 encoding calmodulin 1 and autosomal recessive form due to biallelic variants in *CASQ2* (MIM  
64 114251)(4) encoding calsequestrin, *TRDN* (MIM 603283)(5) encoding triadin and *TECRL* (MIM  
65 617242)(6). Furthermore, CPVT can also be caused by rare deletions in exon 3 of *RYR2* (7). However,  
66 in such cases CPVT is often accompanied by left ventricular non-compaction (7). It is estimated that  
67 one in 10,000 people are clinically affected by the condition, with sudden cardiac death being the  
68 first manifestation in some individuals (1, 8, 9). The phenotypic heterogeneity of CPVT can delay or  
69 obscure diagnosis. It has been reported that almost one in three individuals with CPVT are initially  
70 diagnosed with long QT syndrome (LQTS) despite a normal QT interval (2). Consequently, combined  
71 approaches of cardiac assessment including exercise stress testing and genetic analysis are used to  
72 confirm a diagnosis of CPVT.

73 The large coding region of the cardiac ryanodine receptor (105 exons) previously made genetic  
74 testing costly and time consuming using conventional DNA sequencing methods. As a result, it  
75 became common practice to only screen the four regions considered to be mutation hotspots in  
76 *RYR2* (10). Next generation sequencing has now become more widely available and all coding exons  
77 of *RYR2* can be screened rapidly and cheaply. This has led to an increase in the number of *RYR2*  
78 variants being reported in individuals with cardiac dysrhythmia or associated symptoms of  
79 palpitations, syncope or sudden unexplained death. Concomitant with this has been the increase in  
80 *RYR2* variants identified in apparently healthy individuals collated through international resources,  
81 including the Genome Aggregation Database (gnomAD)(11). The majority of *RYR2* variants are

82 missense changes. When assayed in functional experiments a number of these lead to increased  
83 channel activity consistent with pathogenicity. However, the rarity of these variants and complexity  
84 of functional assays makes it difficult to determine their pathogenicity, and so the majority are  
85 classified as variants of uncertain significance (VUS). Recently the American College of Medical  
86 Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) proposed  
87 guidelines to standardise the classification of genetic variants (12).

88 In this study we collate *RYR2* variants reported in individuals with, or suspected of having CPVT, and  
89 classify them according to these ACMG guidelines and correlate the variants with clinical features.

## 90 **Methods**

91 A comprehensive search for *RYR2* variants identified in individuals undergoing genetic testing for  
92 CPVT or an associated arrhythmia was performed. A total of 326 different *RYR2* variants was  
93 obtained from the North West Genomic Laboratory Hub, UK (a service that has been undertaking  
94 clinical diagnostic testing of *RYR2* for >10 years), the published literature and clinical variant  
95 databases, including ClinVar and the Human Gene Mutation Database (HGMD) (Supplementary  
96 Table 1) (13, 14). Allele frequencies of *RYR2* variants in apparently healthy individuals were obtained  
97 from gnomAD as a comparator, accessed online from <https://gnomad.broadinstitute.org> (11).

## 98 **Phenotype-Genotype analysis**

99 All *RYR2* variants both in control and CPVT populations were mapped to the domains, structural  
100 motifs and regions in which they are located in the RyR2 protein (using the universal protein  
101 resource (UniProt) accession number Q92736) with Mutation Mapper from the cBio Cancer  
102 Genomics Portal. For the purpose of our analysis *RYR2* regions that were not associated with any  
103 known functional or structural domains were individually labelled as 'no domain' followed by a  
104 number ranging from 1 to 9 corresponding to their location. The proportion of missense variants in  
105 each region/domain of *RYR2* in control and CPVT populations were compared using the Fisher's

106 exact test using GraphPad prism. To account for multiple testing a p-value < 0.005 was considered  
107 significant.

#### 108 **Variant Classification**

109 *RYR2* variants were classified based on the 2015 ACMG-AMP guidelines. As reported by Denham *et*  
110 *al.* (2019) 12 of the 27 criteria listed in the ACMG-AMP guidelines were excluded from this study as  
111 they were considered non-applicable (reasons for the exclusion of these criteria are included in  
112 Supplementary Table 2) (15). The application of these guidelines has been previously reported  
113 (Supplementary Table 3 and 4)(15).

#### 114 **Criteria for segregation**

115 The criteria for a variant to qualify for variant segregation (PP1) required that the variant was  
116 present in two or more members of the same family with a CPVT-like phenotype (arrhythmias,  
117 syncope, bradycardia or sudden death). The occurrence of affected individuals in whom the putative  
118 variant did not segregate was considered strong evidence for a benign classification (BS4).

#### 119 **Criteria for functional studies**

120 Robust functional studies, including animal models, calcium imaging, cellular electrophysiology and  
121 single channel analysis showing either a significant reduction or gain of function, were considered  
122 strong evidence for pathogenicity (PS3). Conflicting functional data on variants was not considered  
123 as positive evidence of pathogenicity. Functional studies that reported no change in channel  
124 function were considered evidence for a benign classification (BS3).

#### 125 **Criteria for variant frequency**

126 Variants that were absent from gnomAD were considered ultra-rare (PM2) and variants with an  
127 allele count of below 4 were considered rare.

128 The statistical framework used to identify variants that occur too frequently in the gnomAD  
129 database to be pathogenic has been described by Whiffin et al. (2017) (16). To summarize, it  
130 involves the determination of the maximum credible population allele frequency for a missense  
131 variant in *RYR2* that causes CPVT. This was calculated based on CPVT as a dominant condition with a  
132 penetrance of approximately 60% (8). A binomial distribution of the maximum credible allele  
133 frequency was generated for our sample of CPVT cases (observed allele number) and the upper  
134 boundary of the 95% confidence interval (the maximum tolerated allele count) was used as the cut  
135 off frequency. Variants that occurred more frequently than the maximum tolerated allele count in  
136 gnomAD were considered common and this was strong evidence for a benign classification (BS1).

#### 137 **Criteria for variant enrichment in CPVT cases**

138 Ultra-rare and rare variants were considered for variant enrichment analysis. The presence of an  
139 ultra-rare or rare variant in at least five or ten CPVT cases, respectively, was considered as strong  
140 evidence (PS4).

#### 141 **Criteria for computational evidence**

142 To remain consistent with previously reported variant classification methods, five protein-level in  
143 silico prediction tools: SIFT, PolyPhen, Mutation Taster, Mutation assessor, FATHMM and three  
144 conservation tools GERP++, PhyloP conservation and SiPhy were used for the computational analysis  
145 of variants **where DNA positional information was provided** (GERP++, PhyloP, and SiPhy scores of  
146 4.4, 1.6, and 12.17, respectively were set as thresholds for conservation)(15). In addition, Consurf  
147 (<http://consurf.tau.ac.il/>), which uses advanced probabilistic evolutionary models to distinguish true  
148 conservation resulting from purifying selection and produces estimates for the credibility of the  
149 results, was used to measure conservation of amino acid positions of variants in controls and CPVT  
150 patients. The Consurf scores of the amino acid positions of control, CPVT and sudden death cases  
151 were compared using the Mann-Whitney test using GraphPad prism, p-value <0.005 was considered  
152 significant.

153 **Criteria for critical functional domain**

154 The location of a missense variant in the transmembrane 4-6 region or ion-transport domain of the  
155 protein was considered as moderate evidence for a pathogenic classification, as the functional  
156 significance of these regions has been established (17, 18).

157 **Results**

158 **Collation of RYR2 missense variants**

159 A total of 326 independent *RYR2* single nucleotide, non-synonymous variants associated with CPVT  
160 or arrhythmia were identified. Of these variants 97 were present in both control and CPVT  
161 populations. Importantly, 104 (31.9%) of the CPVT-associated *RYR2* variants were located outside of  
162 regions previously considered mutation hotspots. The hotspot regions with the most *RYR2* variants  
163 were domains III and IV, where 21.5% and 21.2% of variants were located, respectively. The numbers  
164 of male and female cases were similar (Table 1).

165 The most common amino acid position at which missense changes occurred was Arg420. Nineteen  
166 (19) of 440 (4.3%) independent cases were reported to have a missense change at this amino acid  
167 position, 10 cases carried the Arg420Trp variant and 9 carried the Arg420Gln variant. The second  
168 most common protein position for missense changes was Arg176, 8 of 440 (1.8%) cases carried the  
169 Arg176Gln variant.

170 **Genotype-Phenotype analysis**

171 Several domains or regions within *RYR2* contained a significantly higher proportion of CPVT-  
172 associated missense variants compared with controls (Figure 1 and Table 2). CPVT-associated  
173 missense variants occurred more frequently than expected between amino acid positions 3949-4332  
174 and 4867-4967 (No Domain regions 5 and 7). CPVT-associated *RYR2* variants were also enriched in  
175 the RyR and IP3R homology-associated and ion transport domains when compared to control  
176 variants ( $p < 0.005$ ). In contrast, control variants clustered between amino acid positions 2906-3826

177 and the SPRY and RYR domain when compared to CPVT-associated variants ( $p < 0.005$ ). There was  
178 no clear relationship between sudden death and the location of CPVT-associated *RYR2* variants  
179 (Figure 1A, Supplementary Table 5). However, five of the nine *RYR2* variants associated with sudden  
180 death during sleep occurred in the C-terminus of the protein (Figure 1A, Supplementary Table 6).

### 181 **Conservation analysis**

182 CPVT variants occurred at positions with significantly higher ConSurf scores than controls ( $P <$   
183  $0.0001$ ), and variants identified in individuals or families with a history of sudden death had higher  
184 ConSurf scores compared to variants in individuals and families without a history of sudden death ( $P$   
185  $< 0.0001$ ) (Figure 2). This suggests variants with ConSurf scores above 7 are more likely to be CPVT-  
186 associated and of these variants those with ConSurf scores above 8 are more likely to be associated  
187 with sudden death.

### 188 **Classification of *RYR2* variants**

189 *RYR2* variants were classified according to the ACMG-AMP guidelines and statistical methods were  
190 used to identify those variants that occurred too frequently in controls to be pathogenic, these  
191 variants were classified as benign (Supplementary Table 7). Using the statistical method described by  
192 Whiffin et al. (2017)(16), data on the most common CPVT-associated *RYR2* variant c.1258C>T  
193 (Arg420Trp) and control populations from the ExAC or gnomAD databases, the maximum tolerated  
194 allele count for CPVT associated *RYR2* variants was calculated (Supplementary Tables 8 and 9).

195 The maximum tolerated allele count for pathogenic *RYR2* variants was calculated to be two when  
196 using the ExAC database as a control population and three for the gnomAD database. Using the  
197 gnomAD database and the maximum tolerated allele count as a frequency threshold for  
198 pathogenicity 55 of 326 previously putative disease associated variants were re-classified as benign  
199 according to the ACMG guidelines, 11 fewer variants (44) were reclassified as benign using the ExAC  
200 database. A further 245 variants were classified as variants of uncertain significance, 14 as likely

201 pathogenic and 12 as pathogenic using gnomAD as the control comparator (Table 3). Both benign  
202 and pathogenic variants occurred most frequently outside of known functional domains. The ion  
203 transport domain contained the most (7 of the 26) pathogenic or likely pathogenic variants. The  
204 SPRY domain was found to be the domain containing the most benign variants; this domain did not  
205 contain any pathogenic variants.

206 Sufficient functional data to aid classification was available for 50 of the 326 variants  
207 (Supplementary Table 10). The classification of the 26 variants deemed to be pathogenic/likely  
208 pathogenic was driven by absence from the gnomAD database (92%, P6), computational evidence  
209 (88%, P9), functional data (73%, P3) and *de novo* status (50%, P2). The classification of the 55  
210 variants classified as benign was largely driven by variant frequency in gnomAD exceeding the  
211 maximum tolerated allele count (100%, B2) and only one or none of the computational prediction  
212 tools indicating pathogenicity (11%, B5).

### 213 Reason for referral and genetic testing outcome

214 Cases referred for genetic testing at MCGM with a more confident diagnosis of CPVT based on  
215 clinical evaluation were tested using the CPVT genetic panel, whereas those cases with less  
216 diagnostic certainty were tested using either the arrhythmia panel (including 37 genes associated  
217 with inherited arrhythmia) or the molecular autopsy panel (61 genes associated with sudden cardiac  
218 death). The proportion of patients referred for genetic testing with the CPVT panel that carried *RYR2*  
219 variants was significantly greater than that of the patients tested with the arrhythmia panel ( $P <$   
220  $0.05$ ) or molecular autopsy panel ( $P < 0.0005$ ). Furthermore, these patients were more likely to carry  
221 pathogenic *RYR2* variants ( $P < 0.05$ ) (Table 4, Figure 3).

### 222 Discussion

223 The availability of sequence variant databases like gnomAD (11) and a statistical threshold to aid in  
224 the classification of pathogenicity for genetic variants (16) is aiding the robust classification of

225 sequence variants as associated, or not, with disease. The maximum tolerated allele count method  
226 was validated in individuals with hypertrophic cardiomyopathy using previous variant assessments  
227 and reports of pathogenicity in ClinVar (14). In the present study we used this method to calculate a  
228 maximum tolerated allele count for CPVT-associated *RYR2* missense variants. Using this frequency  
229 threshold, 55 of 326 (16.9%) CPVT associated *RYR2* variants were re-classified as benign. Thus, our  
230 data show a sizeable number of *RYR2* variants are not disease-causing, in which case the proportion  
231 of CPVT cases attributable to *RYR2* variants is likely to be over-estimated and the proportion of cases  
232 attributable to changes in other genes or to post-translational modifications is likely to be  
233 underestimated. The reclassification of VUS as benign variants is important as family members  
234 previously cascade tested to carry these variants may not be at increased risk and those without  
235 these variants may have been falsely reassured and remain at risk of arrhythmia or sudden cardiac  
236 death. Furthermore, this classification of benign variants offers the opportunity to find the real  
237 explanation for the CPVT phenotype in affected individuals.

238 In the present study the maximum tolerated allele count for CPVT associated *RYR2* missense variants  
239 was calculated using both the ExAC and gnomAD databases as control populations. Variants that  
240 occurred above the frequency threshold in each population were then reclassified accordingly. The  
241 ExAC database contains exome data from 60,706 unrelated apparently healthy individuals, whereas  
242 the gnomAD database contains combined exome and genome variant data from 141,456 individuals.  
243 Importantly 1600 of the 1975 (81%) *RYR2* missense variants reported in gnomAD have a minor allele  
244 count below four. This not only highlights that a number of potentially healthy individuals have rare  
245 variants in *RYR2* which may have a consequence in the context of a particular trigger e.g. exercise or  
246 emotion, but also that many benign *RYR2* variants are rare. The utility of larger control datasets in  
247 reclassifying *RYR2* variants was exemplified in this study. Comparison with the larger gnomAD  
248 database as a control population allowed the reclassification of 11 additional *RYR2* variants as  
249 benign compared to ExAC. Further reclassification of VUSs may be achieved with larger sequence

250 datasets and by using data from individuals with more phenotype data and of older age to reduce  
251 the effects of non-penetrance.

252 Applying Consurf, we found *RYR2* variants present in CPVT patients occurred at amino acid positions  
253 that were significantly more conserved than those of control variants and the conservation of  
254 residues where CPVT sudden death variants occurred was even greater (Figure 2). The application of  
255 a frequency threshold in control datasets with the consideration of Consurf may be more  
256 informative than using each method independently and may be particularly useful for determining  
257 the probability of a rare variant being pathogenic or benign.

258 Both *RYR2* variants and CPVT are commonly associated with arrhythmias and/or sudden death  
259 triggered by exercise or stress. However, we noted *RYR2* variants in individuals who died or  
260 experienced cardiac arrest while asleep. In these patients almost all of the *RYR2* variants that were  
261 not classified as benign resulted in changes within the C-terminal of the protein, with the exception  
262 of one variant that occurred in the central domain. Although, limited by the small number of cases  
263 this data suggests pathogenic C-terminal *RYR2* variants may pose a greater risk of sudden death at  
264 rest, particularly during sleep. This relationship between C-terminal variants and sudden death in  
265 sleep is novel and requires independent validation. Sleep is considered a restful period but during  
266 rapid eye movement (REM) sleep, which accounts for approximately 20% of sleep time, sympathetic  
267 activity is increased and intense emotional states occur (19). Thus, sudden death during sleep in  
268 patients with *RYR2* variants may be due to episodes triggered by increased sympathetic activity  
269 comparable to exercise or emotional stress. Contrastingly, specific *RYR2* variants may exhibit  
270 properties that increase their sensitivity to other sleep related triggers like rises in hormones such as  
271 melatonin which has been shown to induce ventricular arrhythmias (20, 21). Importantly in a recent  
272 prospective study of sudden cardiac death the majority of deaths occurred during sleep (22) and  
273 CPVT should be considered as a potential cause in this setting.

274 Our data shows that CPVT-associated *RYR2* variants predominantly cluster in four regions/domains,  
275 namely the RyR and IP3R homology-associated domain; the ion transport domain; and two regions  
276 outside of known domains (No domain regions 5 and 7). Generally, these regions correspond to the  
277 previously-reported mutation hotspots. However, more than 30% of CPVT-associated *RYR2* variants  
278 occurred outside of mutation hotspots, emphasising the importance of screening the entire coding  
279 region of *RYR2* in patients suspected of having CPVT.

280 The presence of functional data was a major driver of pathogenic classifications. However functional  
281 data was only available for 50 of the 326 CPVT associated variants. In addition to this the threshold  
282 of at least a 50% effect on channel function required for pathogenicity as applied by Denham et al.  
283 (2019) may not be applicable for *RYR2* as there is no direct correlation between the magnitude of  
284 variant functional effect and disease phenotype in CPVT (15). Computational evidence and absence  
285 from control datasets were also major contributors to pathogenic classifications, similar to Denham  
286 et al. (2019), we used eight computational tools and applied a threshold of six tools predicting a  
287 pathogenic effect for pathogenic classification (15). This method was found to be more stringent and  
288 require more evidence for a pathogenic classification when compared to previous systems (15).

289 A limitation of this study was the lack of systematically collected phenotype data and this will be  
290 required prospectively to identify effective means of combining clinical and genetic information to  
291 make accurate CPVT diagnoses. Nonetheless, based on the clinical indications considered our data  
292 shows specificity of testing (a surrogate for confidence in the underlying phenotype) correlates with  
293 the likelihood of identifying a relevant variant. Thus, although genetic testing is a useful aid in the  
294 diagnosis of CPVT rigorous clinical evaluations and the establishment of additional common  
295 phenotypic traits for CPVT is likely to increase the efficiency of genetic testing, identification of  
296 pathogenic variants and possibly improve the management of the condition.

297 In summary, CPVT-associated *RYR2* variants cluster in specific domains/regions, many of which are  
298 within, but not confined to, previously established mutation hotspots. CPVT-associated variants

299 occur at residues that are more evolutionarily conserved than controls, and *RYS2* variants associated  
300 with sudden death occur at positions which are even more highly conserved. The application of a  
301 frequency threshold for pathogenicity, amino acid conservation scores and functional data aid  
302 distinguishing pathogenic and benign variants. However, the majority of CPVT variants remain  
303 classified as VUS. Therefore, additional approaches are required, including sharing of sequence data  
304 from affected individuals through Clinvar and other resources, generation of additional sequence  
305 data from healthy controls and use of sensitive high-throughput functional assays like saturation  
306 genome editing, with sufficient weight to drive pathogenic or benign classifications (23).

307

308 The authors have no conflict of interest to declare.

309

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318 Supplementary information is available at the Journal of Human Genetics website.

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405 Titles and legends to figures

406 Figure 1A. The distribution of missense variants in RYR2 in control population from the gnomAD  
407 database (A), CPVT (B), sudden death (C) and sudden death in sleep (D) populations.

408

409 Figure 1B. Proportion of RYR2 variants in grouped domains in controls from the gnomAD database,  
410 CPVT, sudden death and sleep-associated sudden death populations. The number of cases in the  
411 sudden death and sleep-associated sudden death groups was limited, there were 47 sudden death  
412 cases and 7 sleep-associated sudden death cases.

413

414 Figure 1C. Grouped domains of RYR2 in which the proportion of RYR2 variants was significantly  
415 different in controls from gnomAD compared to CPVT patients. \*\*\*\* and \*\*\* represent  $P < 0.0001$   
416 and  $P < 0.005$ , respectively.

417

418 Figure 2. A) ConSurf scores of amino acid positions of CPVT variants compared to controls from  
419 gnomAD. B) ConSurf scores of amino acid positions of non-sudden death CPVT variants compared to  
420 sudden death CPVT variants. \*\*\*\* represents  $P < 0.0001$ .

421

422 Figure 3. A) Number of patients referred for genetic testing using the CPVT, arrhythmia or molecular  
423 autopsy panel with an RYR2 variant detected. B) Number of patients referred for genetic testing  
424 using the CPVT, arrhythmia or molecular autopsy panel with a pathogenic RYR2 variant detected.

**Table 1.** Pre-established RyR2 variant hotspot regions in CPVT.

<b>Variant hotspot region</b>	<b>Residues (amino acids)</b>	<b>variants (% of total) n=326</b>	<b><i>de novo</i> variants (%) n=40</b>	<b>Male:Female Ratio (%)</b>
I	77-466	35 (10.7)	7 (17.5)	56:44
II	2246-2534	48 (14.7)	6 (15)	50:50
III	3778-4201	70 (21.5)	13 (32.5)	42:58
IV	4497-4959	69 (21.2)	10 (25)	41:59
Non-hotspot regions		104 (31.9)	4 (10)	47:53

**Table 2.** Proportion of *RYR2* variants in individual *RYR2* domains or regions in controls from gnomAD, CPVT, sudden death and sleep-associated sudden death populations.

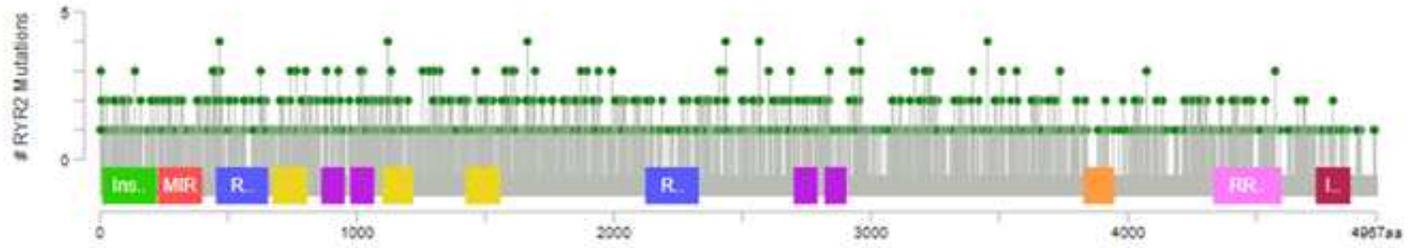
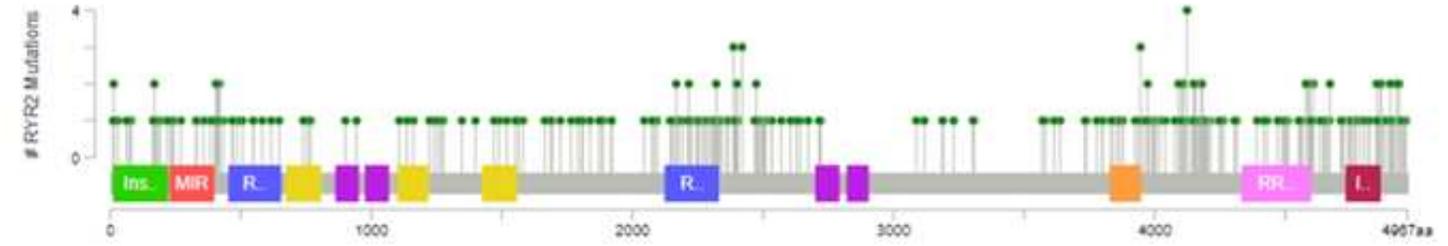
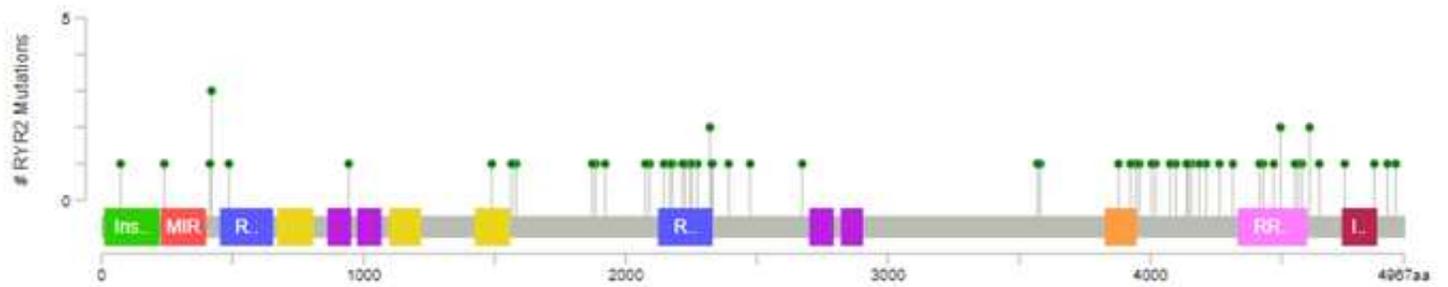
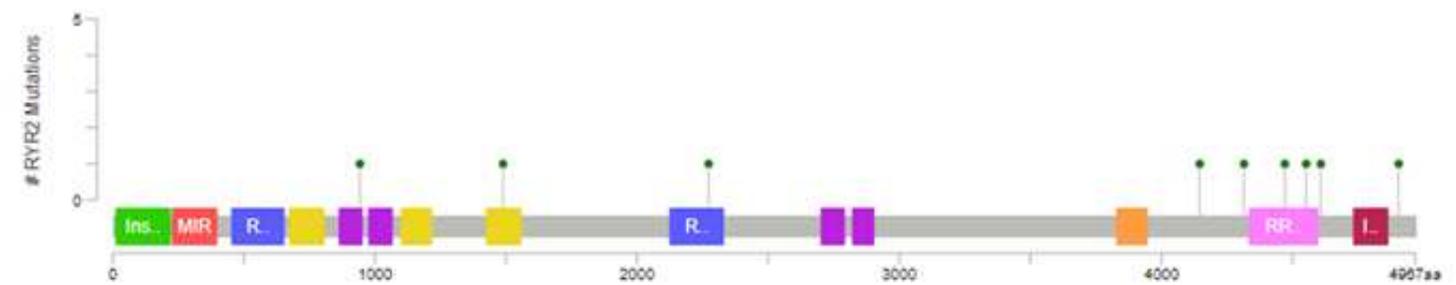
Protein domain or region	Length of region (delimiting amino acids)	Control population from gnomAD database (%)	CPVT (%)	Sudden death (%)	Sleep (%)
Inositol 1,4,5-trisphosphate/ryanodine receptor	212 (10-222)	100 (5.1)	18 (5.5)	1 (1.6)	0
MIR domain	173 (226-399)	78 (3.9)	9 (2.8)	1 (1.6)	0
No domain #1	51 (400-451)	31 (1.6)	15 (4.6)	4 (6.3)	0
RYDR_ITPR domain #1	203 (452-655)	89 (4.5)	9 (2.8)	1 (1.6)	0
SPRY domain #1	137 (671-808)	75 (3.8)	4 (1.2)	0	0
RyR domain #1	91 (862-953)	39 (2.0)	2 (0.6)	1 (1.6)	1 (11.1)
RyR domain #2	93 (975 - 1068)	46 (2.3)	0	0	0
SPRY domain #2	120 (1099 - 1219)	60 (3.0)	3 (0.9)	0	0
SPRY domain #3	135 (1424 - 1559)	59 (3.0)	4 (1.2)	1 (1.6)	1 (11.1)
No domain #2	564 (1560-2122)	282 (14.3)	21 (6.4)	5 (7.9)	0
RYDR_ITPR domain #2	208 (2123 - 2331)	52 (2.6)	29 (8.9)	12 (19)	1 (11.1)
No domain #3	367 (2332-2699)	160 (8.1)	38 (11.7)	3 (4.7)	0
RyR domain #3	93 (2700 - 2793)	34 (1.7)	3 (0.9)	0	0
RyR domain #4	85 (2820 - 2905)	26 (1.3)	0	0	0
No domain #4	920 (2906-3826)	352 (17.8)	16 (4.9)	2 (3.2)	0
RyR and IP3R Homology associated	121 (3827 - 3948)	19 (1.0)	11 (3.4)	4 (6.3)	0
No domain #5	383 (3949-4332)	133 (6.7)	64 (19.6)	10 (15.9)	1 (11.1)
Ryanodine Receptor TM 4-6	266 (4333 - 4599)	96 (4.9)	20 (6.1)	8 (12.7)	2 (22.2)
No domain #6	130 (4600-4730)	38 (1.9)	16 (4.9)	3 (4.7)	2 (22.2)
Ion_transport domain	135 (4731 - 4866)	19 (1.0)	18 (5.5)	2 (3.2)	0
No domain #7	100 (4867-4967)	10 (0.5)	21 (6.4)	1 (1.6)	1 (11.1)

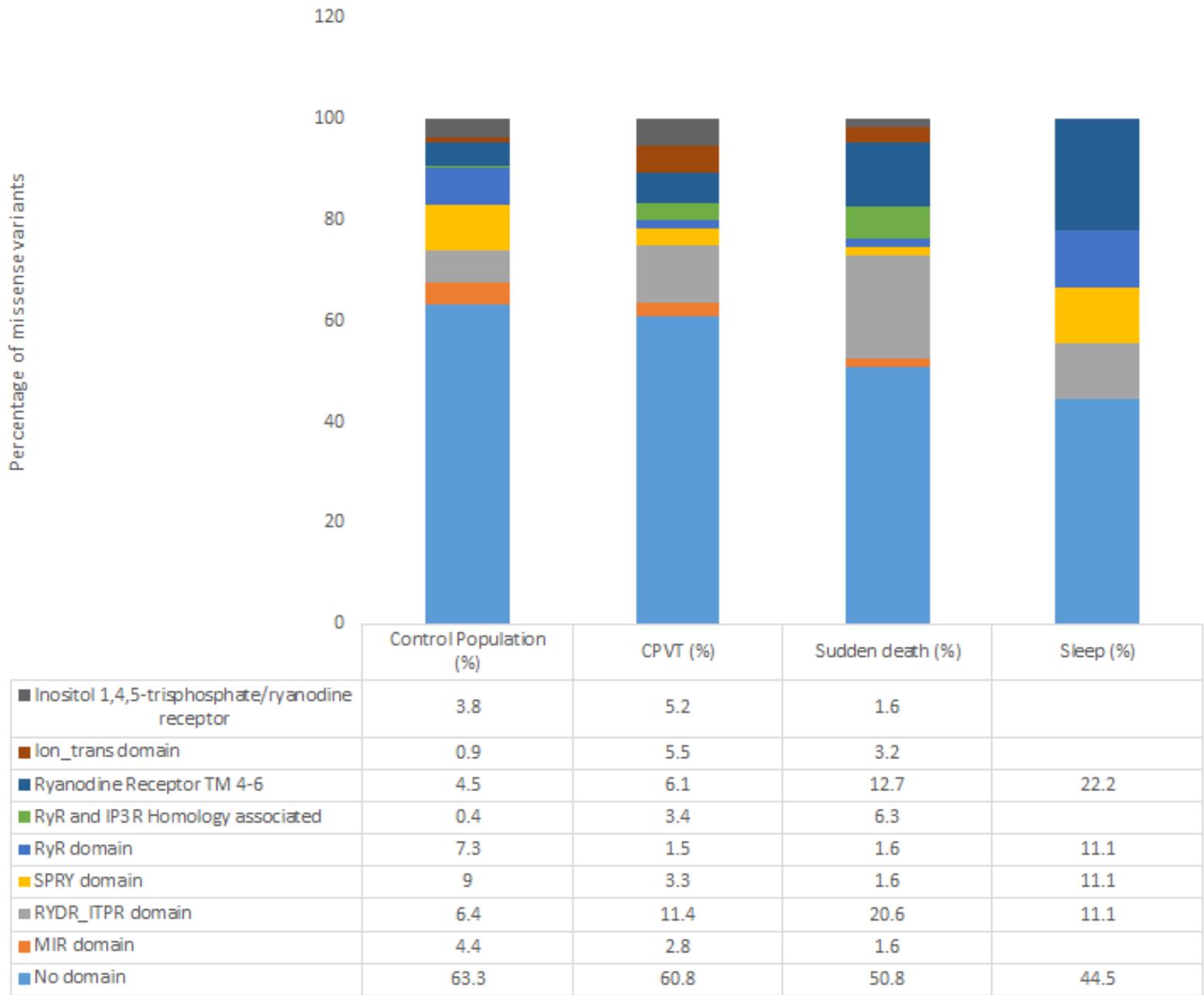
Table 3. *RYR2* variant classification based on the ACMG-AMP guidelines

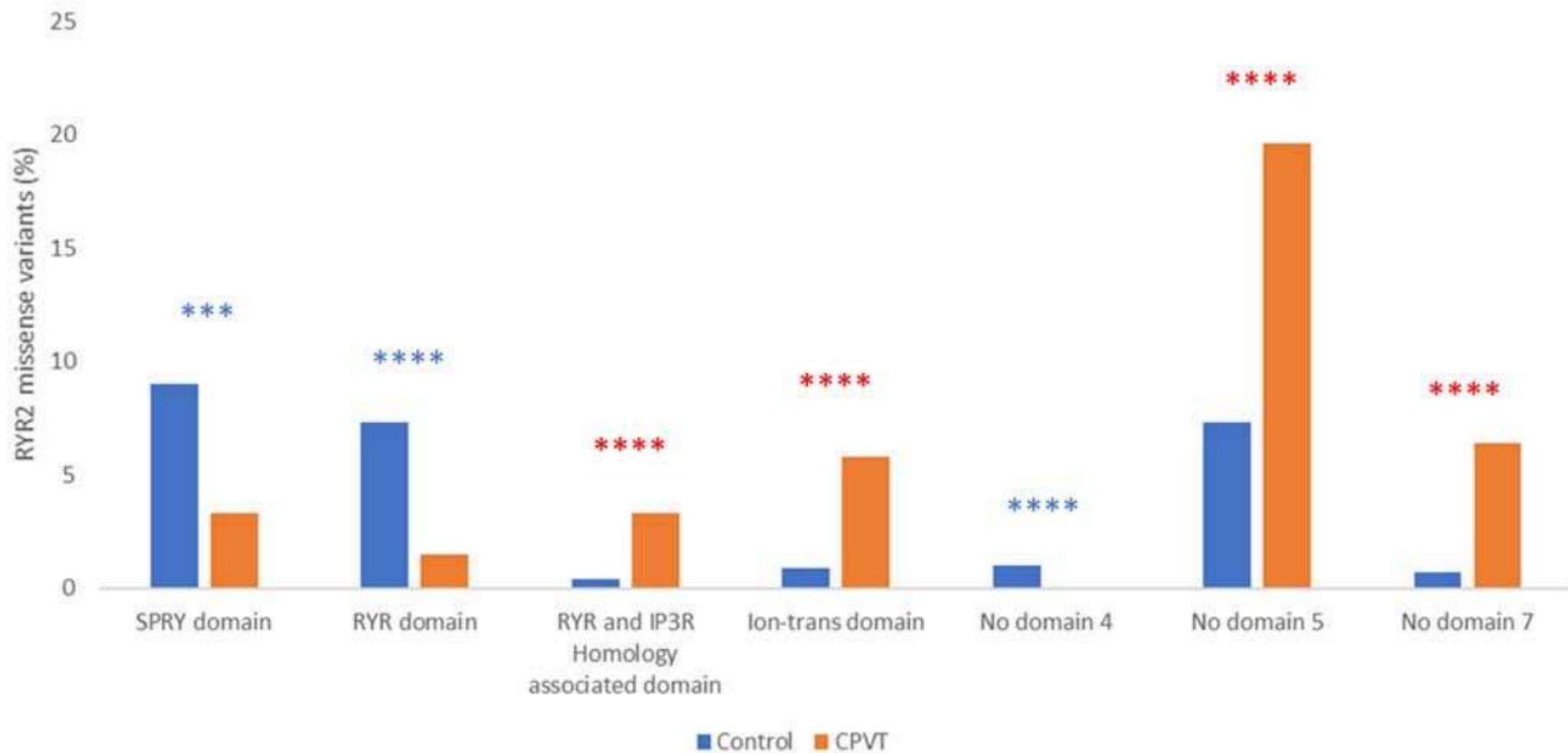
ACMG classification	Number of CPVT associated <i>RYR2</i> variants total = 326 (%)
Benign	55 (16.9)
Variant of uncertain significance	245 (75.6)
Likely pathogenic	14 (4.1)
Pathogenic	12 (3.7)

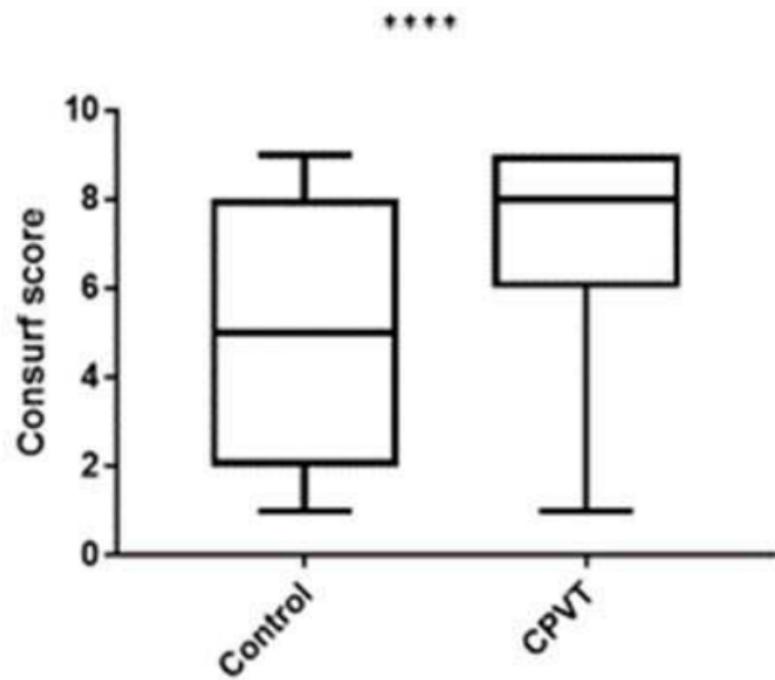
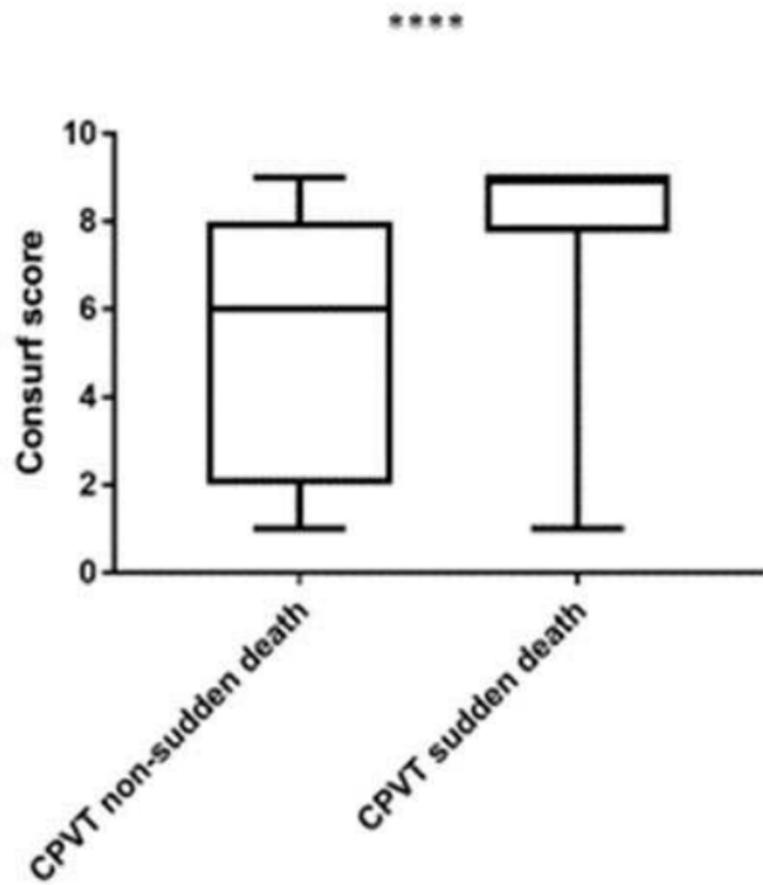
Table 4. Outcome of genetic testing for patients referred for CPVT, arrhythmia and molecular autopsy panels to the Manchester Laboratory (MCGM).

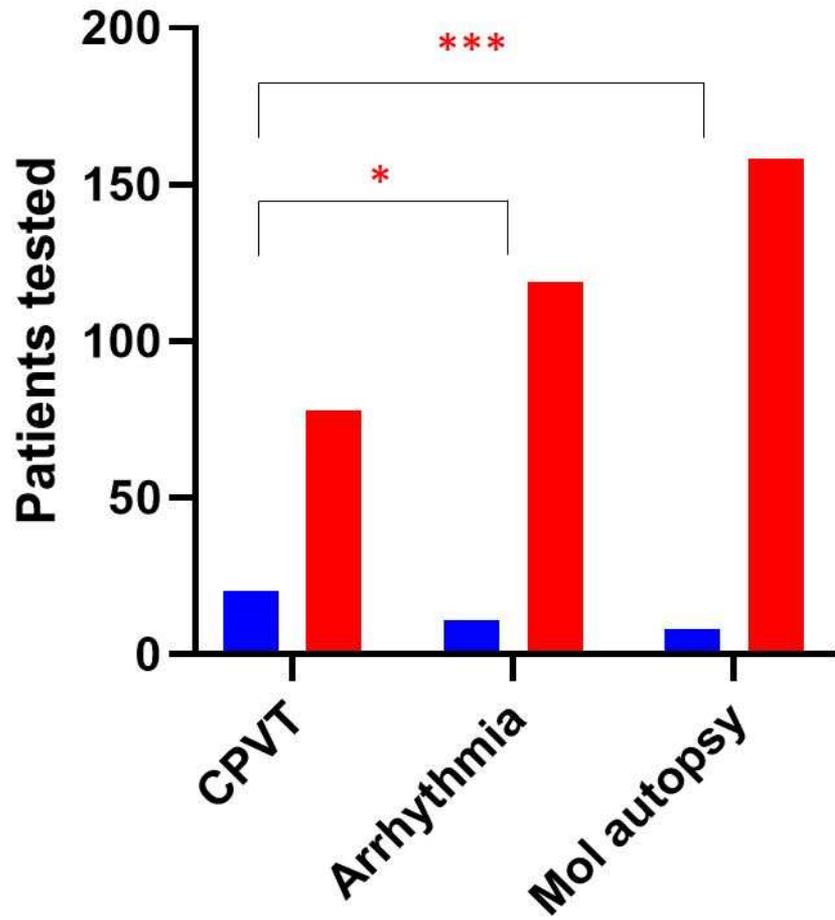
	CPVT panel	Arrhythmia panel	Molecular autopsy panel
Patients tested	98	130	166
Patients with RYR2 variants	20	11	8
Patients with pathogenic RYR2 variants	10	2	1

**A****B****C****D**



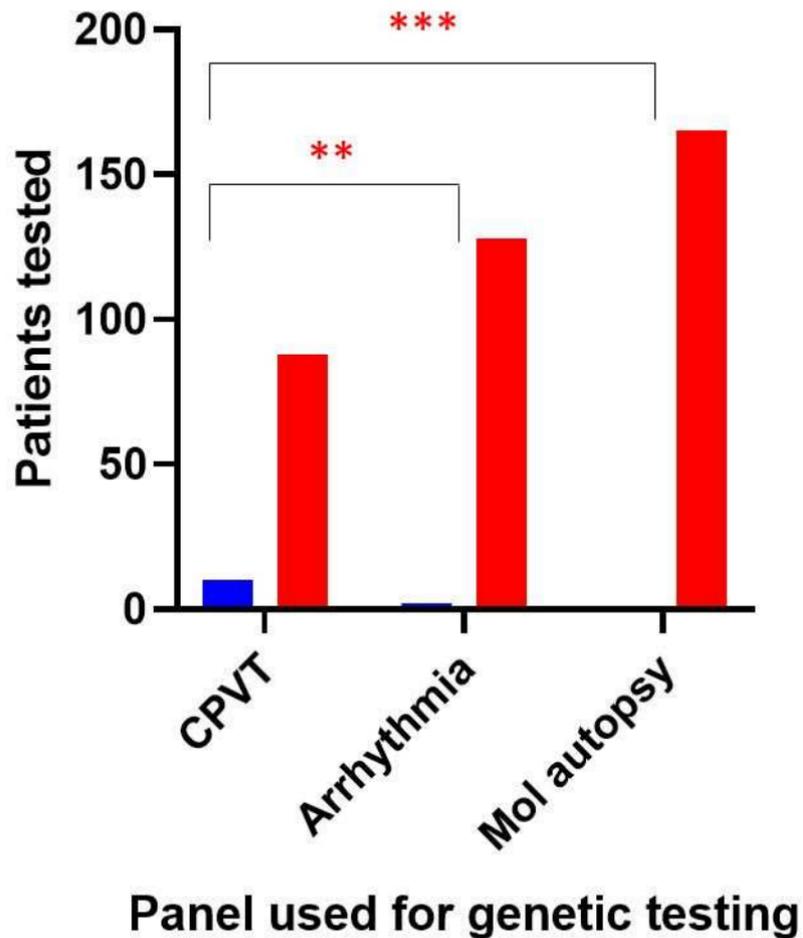


**A****B**



■ Patients with RYR2 variant  
■ Patients without RYR2 variant

Panel used for genetic testing



- Patients with pathogenic RYR2 variant
- Patients without pathogenic RYR2 variant