Clinical Utility of NGS Panel Testing in Patients with a Clinical Suspicion of Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT)

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Introduction

Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is a rare, but potentially fatal channelopathy. Genetic testing may be used to confirm a diagnosis in unclear cases and therefore, is increasingly being performed in a heterogeneous patient population. Another aim was to collect further information of the distribution of the disease causing variants in *RYR2* as 1/113 individuals in gnomAD reference population carries a unique *RYR2* missense variant (not present in anybody else in this cohort) indicating that vast majority unique *RYR2* missense variants are not disease causing.

Methods

Total of 134 patients with clinical suspicion of CPVT send for targeted panel testing either using Blueprint Genetics CPVT panel or Arrhythmia Panel over a 5-year period were analyzed. Majority of the panel orders were Sequence only analysis, whereas only 36 (27%) were ordered as PLUS analyses combining both NGS sequencing analysis and deletion/duplication analysis utilizing NGS data. Blueprint Genetics Deletion/Duplication analysis pipeline can identify both small deletions (1–5 exons) as well as larger copy-number variants.

Results

A total of 134 patients were analyzed between 2013 and 2018. Diagnostic genetic defect was identified in 35 patients (26%; **Figure 1**). These include all pathogenic (P), and likely pathogenic (LP) variants and those variants of uncertain significance (VUS) in *RYR2,* that were absent from gnomAD, fully conserved in evolution and located within four previously described variants clusters [1]. Five such VUS favoring pathogenic *RYR2* variants were identified in this study. Twenty eight patients (80% of diagnoses) had a diagnostic finding in a CPVT-associated gene: 25 in *RYR2*, 2 in *CALM1*, and 1 in *CASQ2* (biallelic). One of these patients had double diagnosis (*RYR2* + *KCNQ1*). Four patients (11.4%) had a P or LP variant in *KCNQ1*, *KCNJ2* or *SCN5A* and three (8.5%) had a P or LP variant in a cardiomyopathy-associated gene (*DSG2*, *DSP* or *PLN*) (**Figure 2**).

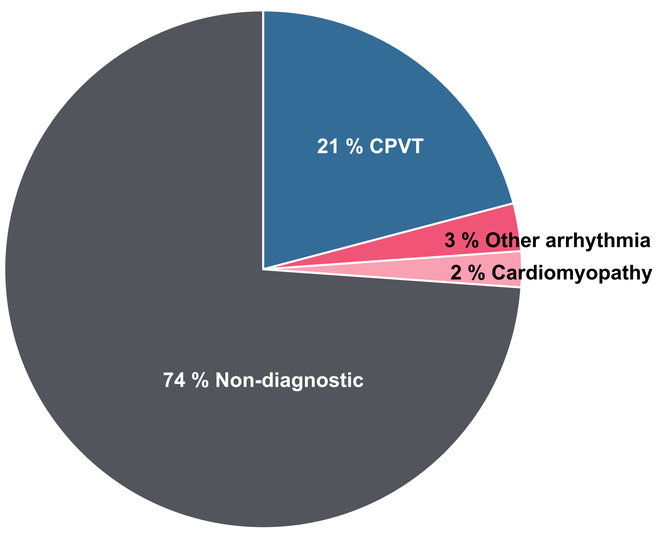

Figure 1. Diagnostic yield in 134 patients with a clinical suspicion of CPVT. 20% (7/34) of diagnosed patients suffered from another genetic heart disease.

All P/LP *RYR2* variants were missense, except a deletion encompassing exon 3 that was identified in two patients. Parental testing was performed in 11/17 cases where P/LP *RYR2* variants were found; 8 (73%) variants were *de novo* (**Figure 2**). The VUS favoring pathogenic *RYR2* variants will be reclassified as likely pathogenic if they are shown to occur as *de novo* in future parental studies.

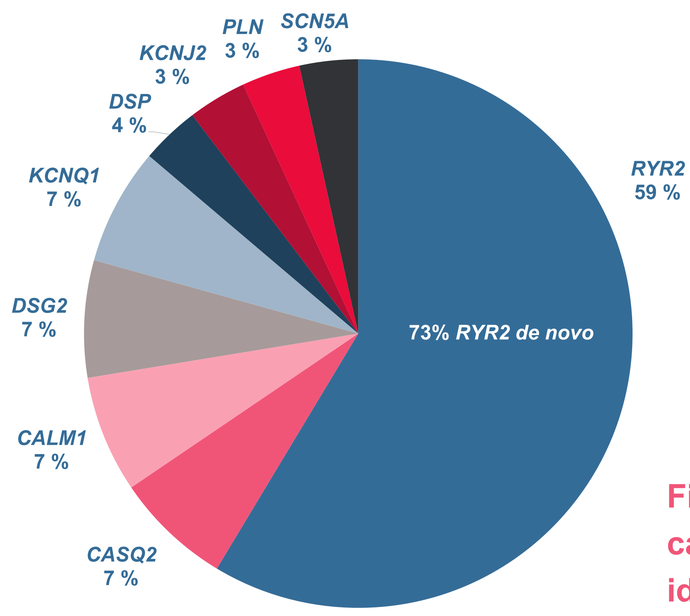

Figure 2. Known disease causing variants were identified in 9 genes.

Table 1. Enrichment of unique *RYR2* missense variants to four previously defined clusters in patients with a suspicion of CPVT (n=134) vs. gnomAD reference population (n=113,148)

Region	BpG total	BpG%	GnomAD	GnomAD%	OR 95% CI
I (aa 44-466)	5	1,87 %	96	0,042 %	45 (18-110)
II (aa 2246-2534)	1	0,37 %	61	0,027 %	14 (1.9-101)
III (aa 3778-4201)	9	3,36 %	56	0,025 %	138 (67-281)
IV (aa 4497-4959)	5	1,87 %	57	0,025 %	75 (30-189)
I-IV	20	**7,46 %**	270	**0,119 %**	**67 (42-108)**
Outside of hot spot	9	3,36 %	730	**0,323 %**	10.8 (5.5-21)
Whole gene	29	10,82 %	1000	0,442 %	27 (18-40)

Enrichment of P/LP *RYR2* variants in the four described regions (OR 67, 95% CI 42-108, P<0.0001) was observed in the cohort compared to gnomAD reference population (**Table 1.**). It is worthwhile to notice, that unique *RYR2* missense variants locating outside these hot spots are common in the general population (730 individuals in gnomAD) suggesting that these are unlikely disease causing.

RYR2 exon 3 deletion

Two patients with clinical suspicion of CPVT were heterozygous for *RYR2* c.(168+1_169-1)_(273+1_274-1)del corresponding exon 3 deletion. One of the patients was previously tested negative for *RYR2* variants in another laboratory. Deletion of *RYR2* exon 3 has been previously reported in at least 25 individuals who presented with CPVT and/or left ventricular non-compaction (LVNC) [2, 3]. In addition to the typical CPVT-related symptoms, patients with a deletion of *RYR2* exon 3 develop additional findings such as atrioventricular block, sinoatrial node dysfunction, atrial fibrillation, and atrial standstill.

Conclusion

- NGS-based panel testing offers good **diagnostic yield** for patients with **clinical suspicion of CPVT** (**26%** in this series).
- 29% (10/35) of patients with a diagnostic test result had a clinically significant variant in a gene other than *RYR2*.
- Known disease causing variants were identified in 9 genes.
- Genetic testing of arrhythmia patient might reveal an underlaying cardiomyopathy if appropriate panel is used.
- Unique *RYR2* missense variants are relatively common in the general population; all of them are not disease causing.
- Large intragenic ***RYR2* deletion** was detected in 2 patients.
- Thus, **del/dup analysis** is recommended in addition to sequencing analysis

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Conflict of interest statement: All authors are employed by Blueprint Genetics.