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Application and Validation of Phenotype-Enhanced Variant Classification in East Asian Patients with Catecholaminergic Polymorphic Ventricular Tachycardia

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Short title: PE-ACMG classification in East Asian CPVT patients

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) has been identified as a notable cause of sudden cardiac death in children and young adults.[1] Mutation of ryanodine receptor 2 (RYR2) is observed in the majority of CPVT patients and is inherited through an autosomal dominant pattern.[2] Genetic tests can help clinicians diagnose CPVT and identify potentially at-risk individuals so that medication can be prescribed before the onset of lethal arrhythmias.[1] The American College of Medical Genetics and Genomics (ACMG) published guidelines for classifying variants as pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), or benign.[3] However, many *RYR2* variants remain classified ambiguously. A previous multicenter study developed a phenotype-enhanced variant classification algorithm for CPVT, integrating the ACMG criteria and clinical data.[1] This CPVT score was developed using Caucasian CPVT cohorts. Whether it is applicable to other ethnicities is yet to be determined.

This was an international, retrospective cohort study of 40 clinically diagnosed Taiwanese CPVT patients and 109 Japanese CPVT patients from multiple tertiary medical centers. Subject data were obtained from the Sudden Arrhythmia Death Syndrome Registry in Taiwan and two Japanese CPVT cohorts described by Shimamoto *et al.* in 2022 and Kawamura *et al.* in 2013.[2, 4] Data sharing agreements and local ethics approval were obtained by each center, and informed consent was obtained from all patients (IRB No. 201305043RINB).

The Taiwanese CPVT patients were screened for CPVT-associated genes using capture-based targeted exon sequencing (Illumina, CA, USA), described in our previous work.[5] The mutations were confirmed by Sanger sequencing. The genetic testing of the 2022 Japanese cohort was performed by combining the conventional Sanger method, multiplex ligation-dependent probe amplification, and next-generation sequencing using MiSeq (Illumina, San Diego, California, USA).[2] The 2013 Japanese cohort received CPVT-related genetic mutation analysis via Sanger sequencing when the study was published.[4] Panel gene analysis was introduced afterwards, and these patients received next-generation sequencing to confirm the results.

The traditional ACMG classification was determined using ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), VarsSome (https://varsome.com) and InterVar (https://wintervar.wglab.org). If discrepancies were noted between these databases during classification, the ACMG classification was determined manually, according to ACMG guidelines and a literature search for previous reports of the variants. Among the 80 different RYR2 variants detected in the largest Asian combined CPVT cohort, 46 (58%) mutations were classified as VUS

while 34 (43%) mutations were classified as P/LP by the ACMG criteria. The CPVT score of the *RYR2* mutation-positive CPVT patients was then calculated according to the scorecard proposed by Giudicessi *et al*.[1] The scorecard includes the patients' symptoms such as exercise-associated cardiac arrest or syncope, exercise stress test or Holter results, genetic test results, and family history.[1] The phenotype-enhanced variant classification reduced the VUS rate to 16% ($p<0.001$) in the combined cohort (Figure 1). Thirty-three (71%) VUS were promoted to P/LP resulting in a total of 67 (84%) P/LP variants. Such a result is consistent with the study by Giudicessi *et al* in which the VUS rate was decreased from 48% to 7% ($p<0.001$) with 65% of VUS promoted to likely pathogenic.[1]

The CPVT score proposed by Giudicessi[1] was generated using Caucasian CPVT cohorts. Our study showed that this score is applicable to the Asian population. The phenotype-enhanced variant classification framework decreased the VUS rate from 58% to 16% ($p<0.001$) in the largest Asian combined cohort. In future practice, this would be a useful tool for interpreting genetic test results that are currently classified as having unknown significance.

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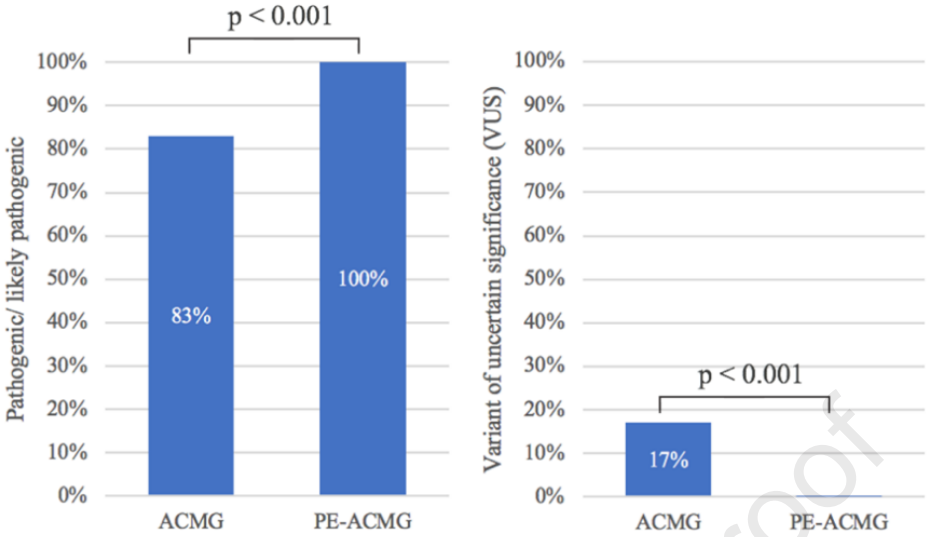
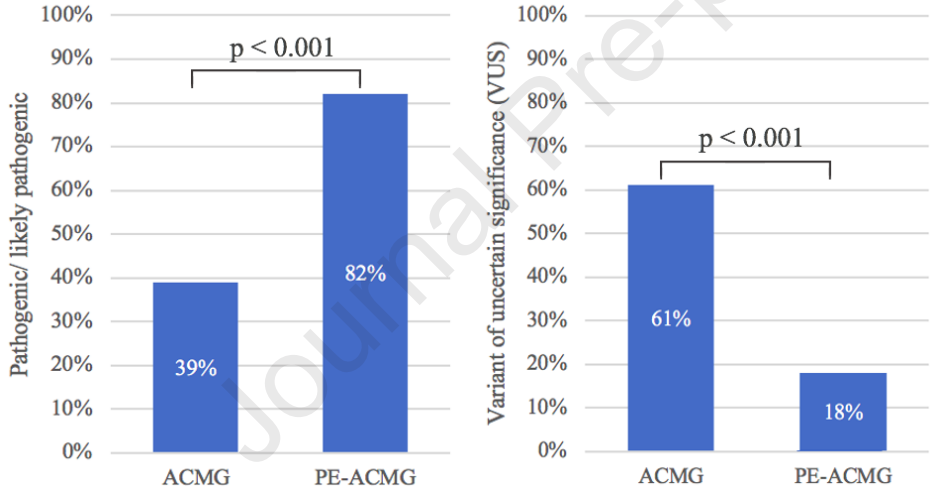
Figure 1. The rate of pathogenic/likely pathogenic and VUS RYR2 variants in Taiwanese (A), Japanese (B), and the largest Asian combined cohort (C). PE-ACMG significantly reduced the VUS rate in all cohorts ($p<0.001$). (ACMG, The American College of Medical Genetics and Genomics variant classification framework; PE-ACMG, Phenotype-enhanced ACMG variant classification framework; RYR2, ryanodine receptor 2; VUS, variant of uncertain significance)

(A) Taiwanese cohort



(B) Japanese cohort



(C) Largest Asian combined cohort