Neurology Asia 2020; 25(3) : 387 – 394

Ryanodine receptor 2 mutation: Not only catecholaminergic polymorphic ventricular tachycardia but also epileptiform discharges in electroencephalogram

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Abstract

Ca^{2+} leak via ryanodine receptor type 2 (RyR2) can cause potentially fatal arrhythmias, and RyR2 mutations have been shown in the aetiology of catecholaminergic polymorphic ventricular tachycardia. We report the case of a patient with catecholaminergic polymorphic ventricular tachycardia resulting from a RYR2 mutation who had not only typical electroencephalogram changes, but also epileptiform discharges in electroencephalogram. We believe the changes were closely related to the RYR2 mutation.

Keywords: Catecholaminergic polymorphic ventricular tachycardia, ryanodine receptor, epilepsy, Purkinje cells, electroencephalogram

INTRODUCTION

Ryanodine receptors (RyR) are huge ion channels that release Ca^{2+} from the endoplasmic reticulum (ER) and sarcoplasmic reticulum (SR). Three different isoforms (RyR1–RyR3) have been found.[1] The RYR2 receptor is mainly expressed in cardiomyocytes, and the RYR2 mutation is well-established in the aetiology of catecholaminergic polymorphic ventricular tachycardia (CPVT).[2] The RYR2 receptor is also expressed in the Purkinje cells of the cerebellum, the cerebral cortex, and the hippocampus.[3] Patients with RYR2 mutations can develop Adams-Stokes syndrome, which can be easily misdiagnosed as epilepsy in the early stage. Many ion channels are also closely related to epilepsy, such as transient calcium channel, long-lasting calcium channel, voltage-gated sodium channels and potassium channels.[4-7] In this case report, we examine whether RYR2 mutation might also be the aetiology of epilepsy.

CASE REPORT

A 9-year-old boy presented with recurrent episodes of unconsciousness for 4 years. Four years before, the boy had presented with palpitations, sweating and blurred vision after activities, followed by unconsciousness, and accompanied by faecal incontinence, upward deviation of the eyes and clonic jerks. After 1 minute, the patient regained consciousness. In the following 4 years, the patient experienced 6 similar attacks, all of which occurred after physical activity. There was no positive family history of sudden cardiac death, seizure, pregnancy loss or neonatal death.

Upon arrival, the patient was conscious, his vital signs were stable, and there were no positive findings confirmed by physical examination. Blood routine, blood electrolytes, blood glucose, blood gas analysis, myocardial troponin, thyroid function, liver function test, creatinine level, chest x-rays and magnetic resonance imaging of the brain were normal. Metabolic screening tests (plasma amino acids, urine organic acids, acylcarnitine profile, a very long-chain fatty acid profile) were also normal. Computed tomography angiography of the chest showed that there were 4 great arteries arising from the aorta's upper convexity, namely the brachiocephalic, left common carotid, left vertebral artery and subclavian arteries. Holter electrocardiogram (ECG) showed that the rhythm of the patient was normal at rest, but frequent polymorphic ventricular premature beats and polymorphic ventricular tachycardia occurred during physical activity, with R on T present (Figure 1). Long-term video electroencephalogram (EEG) showed normal background activity associated with the alpha rhythm of 8 to 9 Hz in the posterior area, but right temporal area showed frequent epileptiform discharges during stage II, stage III and REM sleep (Figure 2).

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Date of submission: 13 January 2020, Date of Acceptance: 29 July 2020

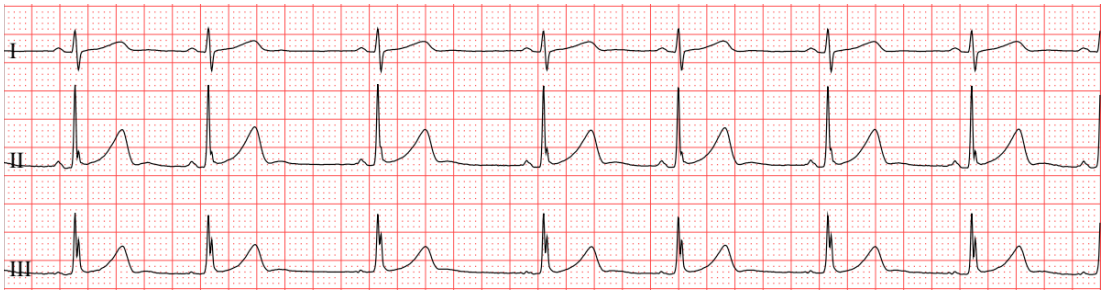
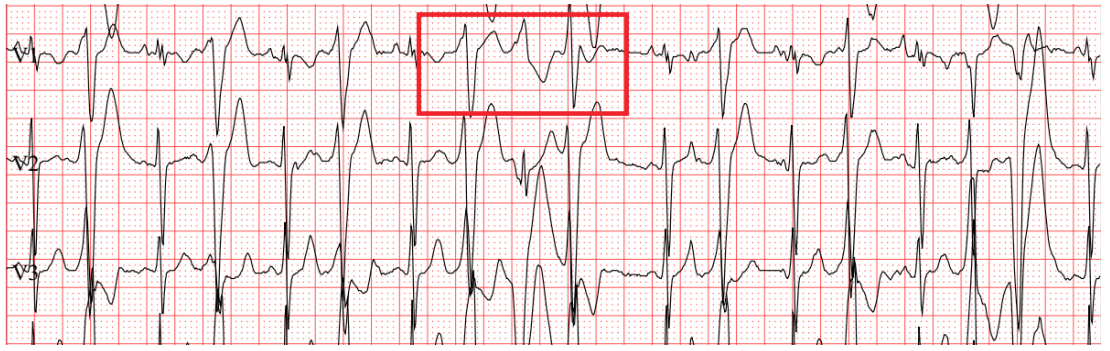
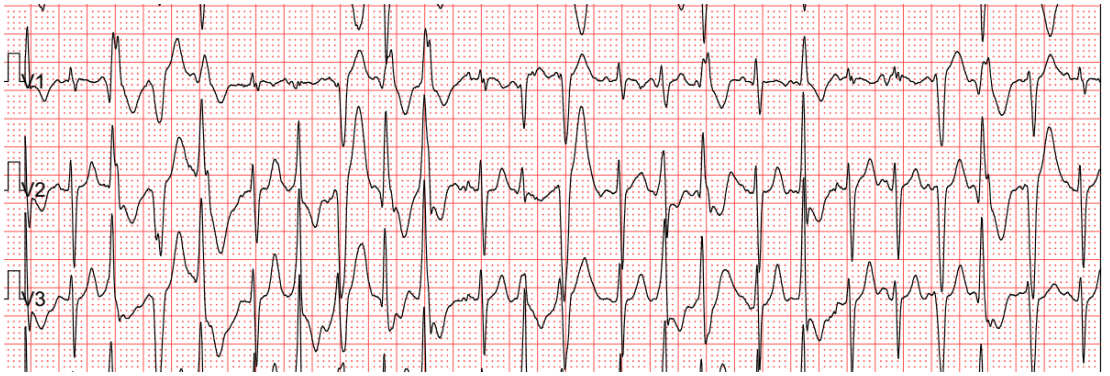


Figure 1. (A) Sinus rhythm.



(B) The red rectangle indicates the non-sustained polymorphic ventricular tachycardia.



(C) Polymorphic ventricular premature beats and non-sustained polymorphic ventricular tachycardia on Holter monitoring were found when the patient was walking fast.

Genetic analysis was performed. At our institution, we analysed the entire exome of genetic disease. We found a heterozygous missense variant in the RYR2 gene (c.6577 G > T/p.V2193L) (Figure 3). The mutation was considered de novo because the genetic analyses of the parents were normal. According to current guidelines[8], the patient was diagnosed with CPVT and was given metoprolol tartrate sustained-release tablets orally. We believe the patient also experiences epilepsy[9-11]; thus, he was also given levetiracetam orally. From the time of treatment until October 10, 2019, the patient had no further attacks at rest or with exercise.

This case report was published with the consent of the patient's guardian.

DISCUSSION

RyRs, RyR1, RyR2, and RyR3, are a family of high conductance cation channels, which release Ca^{2+} from intracellular stores (the ER and SR). RyR2 is expressed mainly in the SR of the mammalian heart.[12] Our patient developed CPVT resulting from the RYR2 mutation.

Patients with RYR2 mutation and CPVT are reported to frequently initially present with seizures. However, in this case, the epilepsy was later thought to be misdiagnosed. Leenhardt[13]

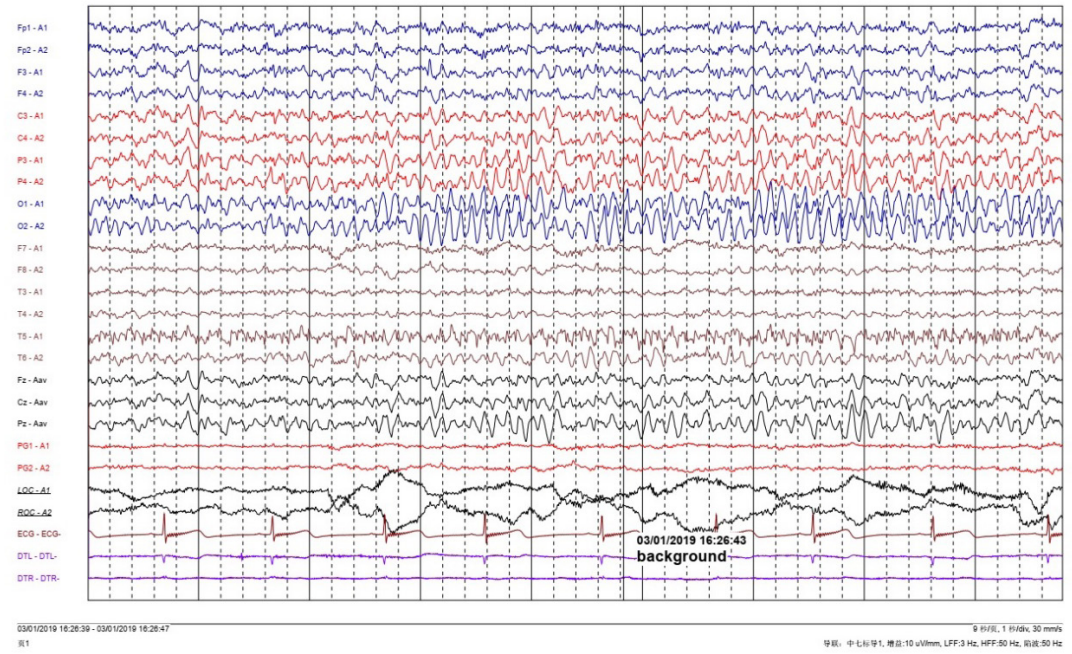
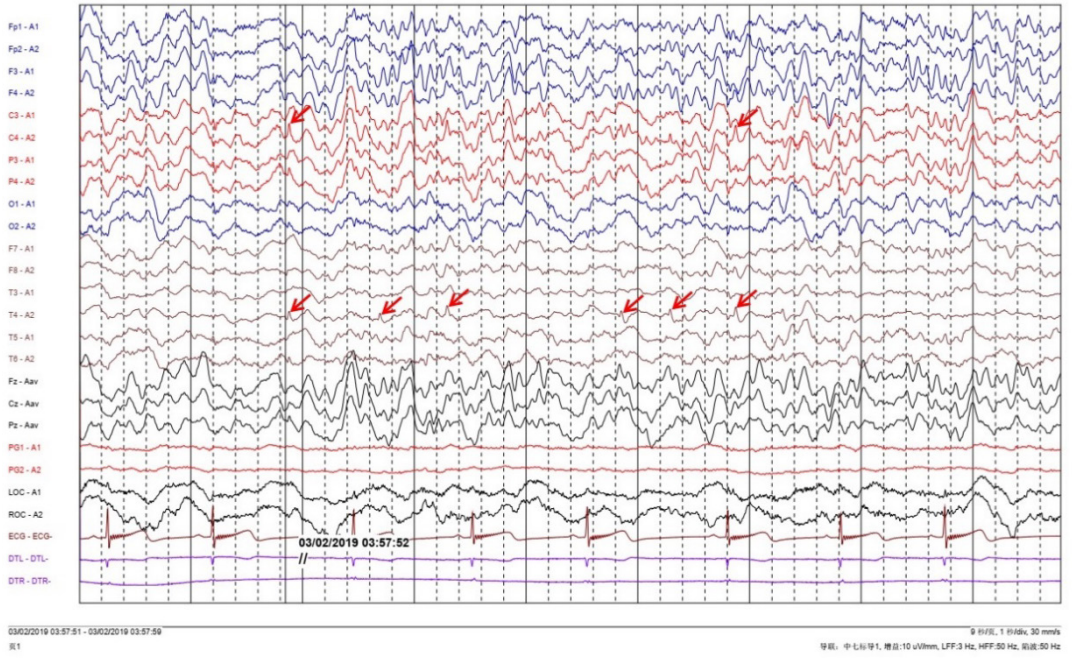
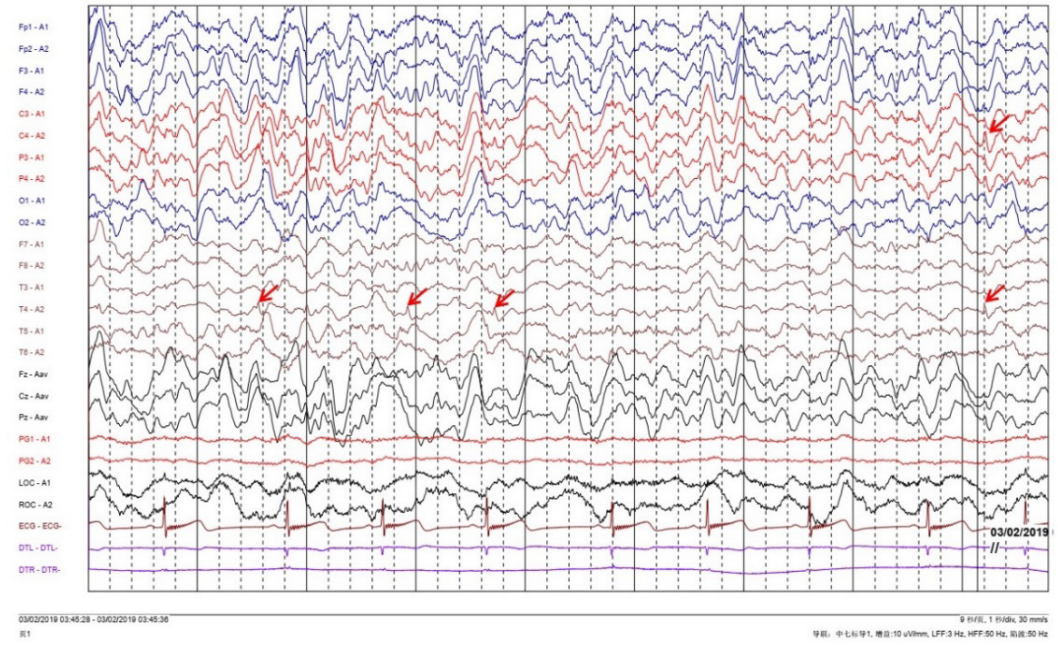


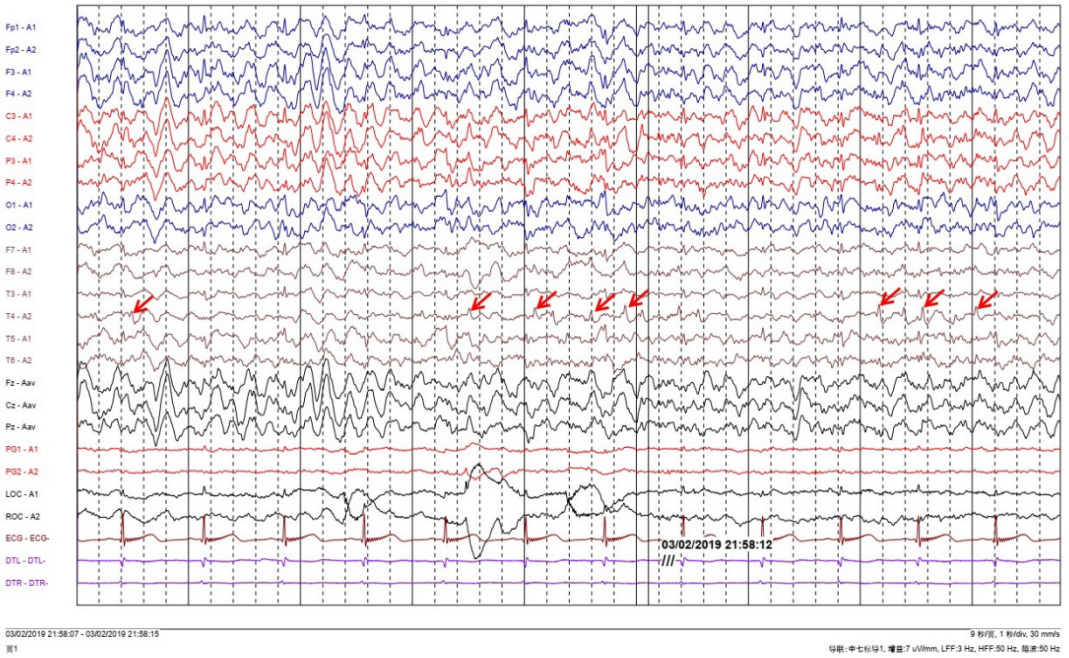
Figure 2. (A) Long-term video- electroencephalogram showed normal background activity associated with alpha rhythm of 8 to 9 Hz in the posterior area.



(B) The red arrows indicate electrodes recording from the right temporal area, which showed frequent epileptiform discharges during non-rapid eye movement II sleep.



(C) The red arrows indicate electrodes recording from the right temporal area, which showed frequent epileptiform discharges during non-rapid eye movement III sleep.



(D) The red arrows indicate electrodes recording from the right temporal area, which showed frequent epileptiform discharges during rapid eye movement sleep.

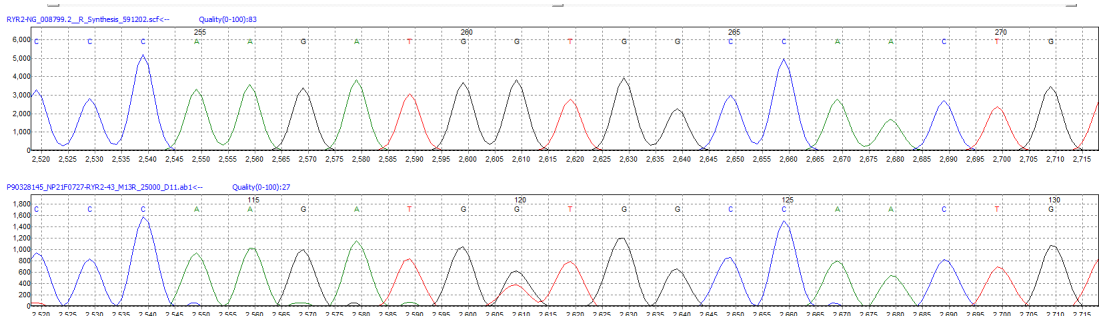


Figure 3. Top, reference sequence. Bottom, patient sequencing diagram. A heterozygous variant (c.6577G>T/p.V2193L) of RYR2 was identified. Sanger sequence analysis of the c.6577G>T detected mutation.

followed 21 patients with CPVT for as many as 7 years, and he believes nearly half of these patients were initially misdiagnosed with epilepsy. Also, approximately 50% of RYR2 mutation carriers (6 of the 12 probands) in a large Dutch cohort presented with convulsive movements resulting from hypoperfusion of the brain. With antiepileptic drugs, syncope was also not relieved.[14]

In previous reports, epilepsy was not excluded as a diagnosis for some patients with RYR2 mutations.[15,16] RyR2 is also expressed at high levels in the Purkinje cells of the cerebellum and cerebral cortex and may play a fundamental role in neuronal Ca^{2+} homeostasis.[3,17] Does the RPR2 mutation cause abnormal electrical activity in the cerebral cortex, leading to seizures?

Aiba[18] found that a leaky human RyR2 mutation, R176Q (RQ), alters the neurotransmitter release probability in mice and reported that rare episodes of spontaneous seizure were detected in RQ/+ mutant mice in vivo. Lehnart[19] proposed that CPVT is a combined neurological and cardiac disease, in which leaky RyR2 channels in the brain trigger epilepsy, whereas the same leaky channels in the heart cause exercise-induced sudden cardiac arrest.

In a family with RYR2 mutation, a female presented with 3 unprovoked generalised seizures over 12 years. EEG showed epileptiform activity, whereas the ECG was normal at the same time. Her brother was diagnosed with CPVT and was found to be heterozygous for a novel mutation in the RYR2 gene. There were no family members with both EEG and ECG changes.[20]

Both polymorphic ventricular tachycardia in ECG and frequent epileptiform discharges in EEG were detected in the present case. Patients with ion channel disease may have focal epileptiform discharges or multifocal epileptiform discharges.[21] The epileptiform activity of our patient appeared in a periodic pattern during wakefulness and sleep and may be the result of genetic factors. Metabolic, structural, immune, infection and other factors were ruled out.

The patient underwent a genetic analysis of the whole exome. Based on the sequencing analysis, a heterozygous mutant in the RYR2 gene at chromosome 1q43, c.6577 G > T/p.V2193L was identified, which is highly conserved across all species (Table 1). The residue was located at the cytoplasmic loop of the RyR2 (Figure 4),

Table 1: The methionine at position 2193 is conserved in RyR2 from humans, mice, rhesus monkeys, dogs, elephants, zebrafish and lampreys

site species	2190	2191	2192	2193	2194	2195	2196
Human	P	K	M	V	A	N	C
Rhesus	P	K	M	V	A	N	C
Mouse	P	K	M	V	A	N	C
Dog	P	K	M	V	A	N	C
Elephant	P	K	M	V	A	N	C
Chicken	P	K	M	V	A	N	C
Zebrafish	P	K	M	V	A	N	C
Lampray	P	K	M	V	A	N	C

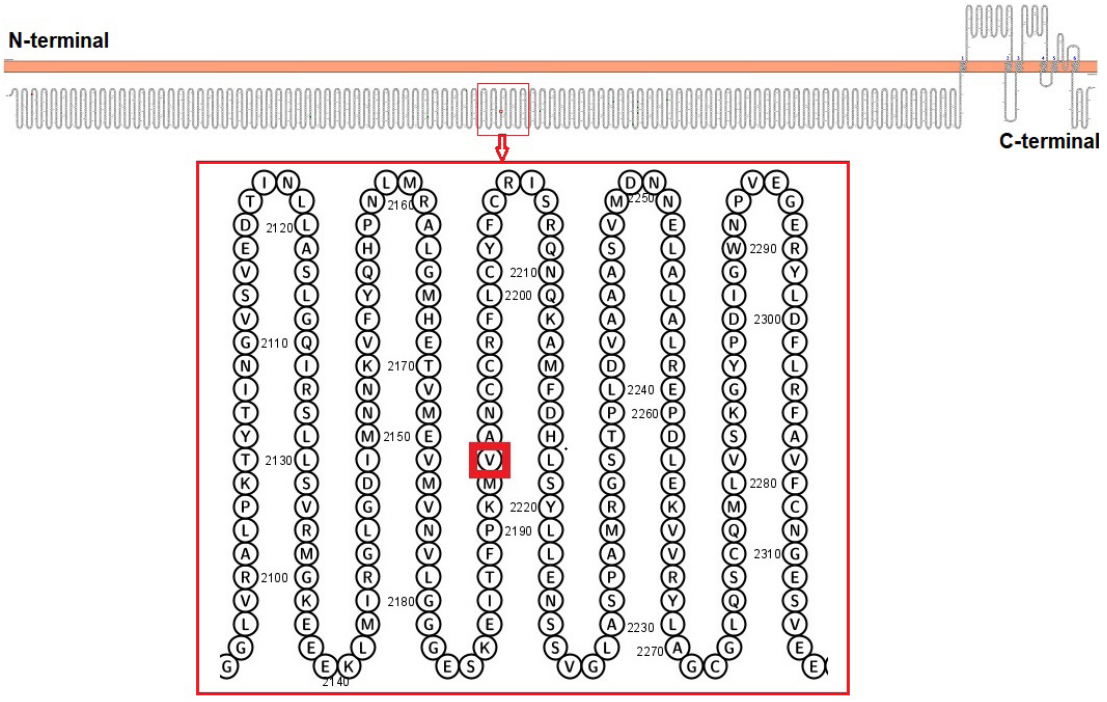


Figure 4. The residue alteration in RYR2 gene was located at the cytoplasmic loop. The red bold square indicates RYR p.V2193L which located at the cytoplasmic loop of the RyR2.

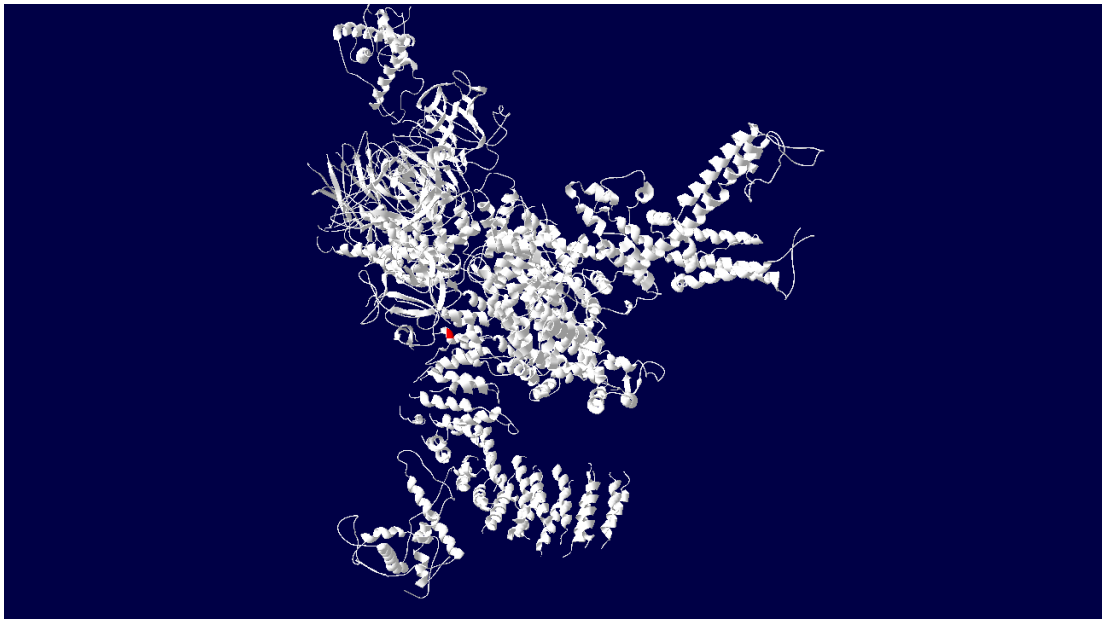
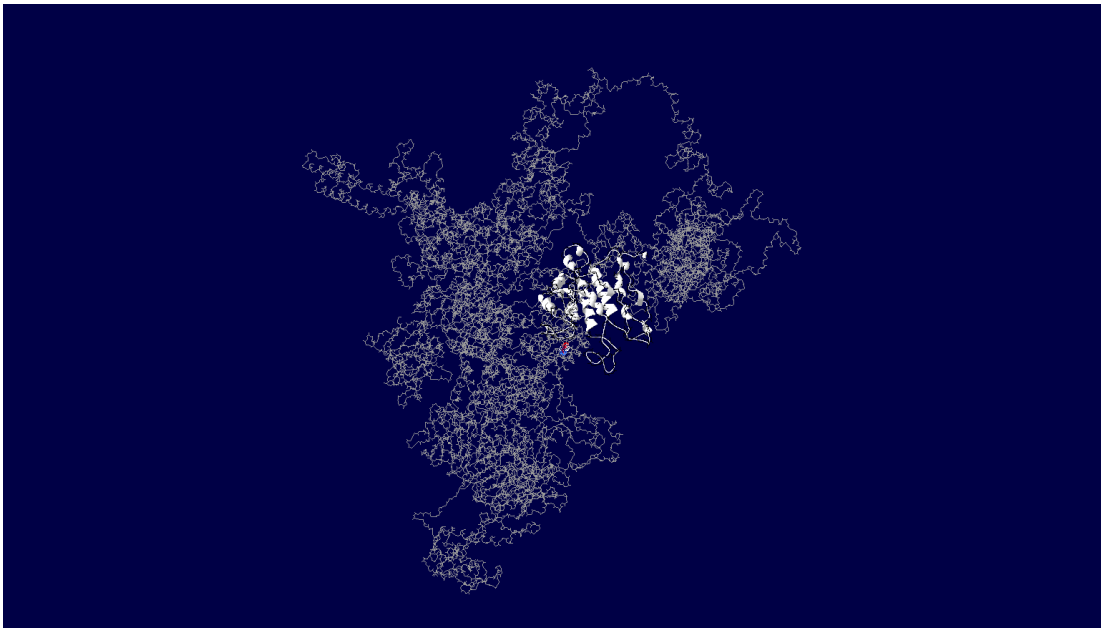


Figure 5. (A) The tertiary structure of the ryr2 protein. The mutation site is identified in red.



(B) The red and blue regions are the mutation sites. The secondary structures displayed as white ribbons are the central domains.

and it was not located at the hot-spot of the previously reported concentrated mutation clusters (Figure 5).[22] This mutation is close to the central domain cluster (2246–2534), which determines whether RyR is in an open or closed state.[23,24] A mutation in the N-terminus construct (p.S2246L) may cause CPVT.[2,25] In addition, RyR2 has been found to have 3 well-defined phosphorylation sites (i.e., S2030, S2809, and S2815), and may have other sites.[26,27]

This mutation site has not been reported previously, and further functional tests are lacking. We used computer software to further analyse the pathogenicity of the mutation at this site. Using polymorphism phenotyping v2, the mutation was predicted to be probably damaging with a score of 1. Therefore, we hypothesised that the mutation leads to a change in the important physiological conformation of RYR2 protein and results in channel dysfunction, which further leads to typical ECG and EEG changes.

CPVT is a genetic disease associated with pathogenic variants of calcium handling genes. Moreover, as the novel variant (c.6577G>T) of RYR2 could result in the alteration of encoded amino acid sequence, we hypothesised that physiologically important altered conformation of RYR2 protein would render the channel more sensitive to stimuli, resulting in channel dysfunction. This new mutant might shed some light on the understanding of RYR2 function and its roles in CPVT if it is further confirmed in more clinical samples and unravelled through structure-function analysis.

DISCLOSURE

Conflict of interest: None

REFERENCES

1. Van Petegem F. Ryanodine receptors: structure and function. J Biol Chem 2012; 287(38): 31624-32.
2. Priori SG, Napolitano C, Tiso N, *et al*., Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001; 103(2): 196-200.
3. Lanner JT, Georgiou DK, Joshi AD, Hamilton SL. Ryanodine receptors: structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol* 2010; **2**(11): a003996.
4. Cueni L, Canepari M, Luján R, *et al*. T-type Ca2+ channels, SK2 channels and SERCAs gate sleep-related oscillations in thalamic dendrites. *Nat Neurosci* 2008; 11(6):683-92.
5. Han DY, Guan BJ, Wang YJ, Hatzoglou M, Mu TW. L-type Calcium channel blockers enhance trafficking and function of epilepsy-associated α1(D219N) subunits of GABA(A) receptors. *ACS Chem Biol* 2015; 10(9): 2135-48.
6. Kaplan DI, Isom LL, Petrou S. Role of sodium channels in epilepsy. *Cold Spring Harb Perspect Med* 2016; 6(6): a022814.
7. Köhling R, Wolfart J. Potassium channels in epilepsy. *Cold Spring Harb Perspect Med* 2016. 6(5): a022871.
8. Pflaumer A, Davis AM. Guidelines for the diagnosis

and management of catecholaminergic polymorphic ventricular tachycardia. *Heart Lung Circ* 2012; 21(2): 96-100.
9. Scheffer IE, Berkovic S, Capovilla G, *et al*.,ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology. *Epilepsia* 2017; 58(4): 512-21.
10. Koutroumanidis M, Arzimanoglou A, Caraballo R, *et al*. The role of EEG in the diagnosis and classification of the epilepsy syndromes: a tool for clinical practice by the ILAE Neurophysiology Task Force (Part 1). *Epileptic Disord* 2017; 19(3): 233-98.
11. Koutroumanidis M, Arzimanoglou A, Caraballo R, *et al*. The role of EEG in the diagnosis and classification of the epilepsy syndromes: a tool for clinical practice by the ILAE Neurophysiology Task Force (Part 2). *Epileptic Disord* 2017; 19(4): 385-437.
12. Inui M, Saito A, Fleischer S. Isolation of the ryanodine receptor from cardiac sarcoplasmic reticulum and identity with the feet structures. *J Biol Chem* 1987; 262(32): 15637-42.
13. Leenhardt A, Lucet V, Denjoy I, Grau F, Ngoc DD, Coumel P. Catecholaminergic polymorphic ventricular tachycardia in children. A 7-year follow-up of 21 patients. *Circulation* 1995; 91(5): 1512-9.
14. Postma AV, Denjoy I, Kamblock J, *et al*. Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients. *J Med Genet* 2005; 42(11): 863-70.
15. Yu TC, Liu APY, Lun KS, Chung BHY, Yung TC. Clinical and genetic profile of catecholaminergic polymorphic ventricular tachycardia in Hong Kong Chinese children. *Hong Kong Med J* 2016; 22(4): 314-9.
16. Hayashi M, Denjoy I, Extramiana F, *et al*. Incidence and risk factors of arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2009; 119(18): 2426-34.
17. Lai FA, Dent M, Wickenden C, *et al*. Expression of a cardiac Ca(2+)-release channel isoform in mammalian brain. *Biochem J* 1992; 288 (Pt 2): 553-64.
18. Aiba I., Wehrens XH, Noebels JL. Leaky RyR2 channels unleash a brainstem spreading depolarization mechanism of sudden cardiac death. *Proc Natl Acad Sci U S A* 2016; 113(33): p. E4895-903.
19. Lehnart SE, Mongillo M, Bellinger A, *et al*. Leaky Ca2+ release channel/ryanodine receptor 2 causes seizures and sudden cardiac death in mice. *J Clin Invest* 2008; 118(6): 2230-45.
20. Yap SM, Smyth S. Ryanodine receptor 2 (RYR2) mutation: A potentially novel neurocardiac calcium channelopathy manifesting as primary generalised epilepsy. *Seizure* 2019; 67: 11-14.
21. Kong YT, Yau K, Hu LY, *et al*. Association between SCN1A and SCN2A mutations and clinical/EEG features in Chinese patients from epilepsy or severe seizures. *Clin Chim Acta* 2018; 483: 14-9.
22. Blayney LM, Lai FA. Ryanodine receptor-mediated arrhythmias and sudden cardiac death. *Pharmacol Ther* 2009; 123(2): 151-77.
23. Wagenknecht T, Radermacher M, Grassucci RA, Berkowitz J, Xin HB, Fleischer S. Locations of calmodulin and FK506-binding protein on the three-dimensional architecture of the skeletal muscle ryanodine receptor. *J Biol Chem* 1997; 272(51): 32463-71.
24. Peng W, Shen HZ, Wu JP, *et al*. Structural basis for the gating mechanism of the type 2 ryanodine receptor RyR2. *Science* 2016; 354(6310): aah5324.
25. Priori SG, Napolitano C, Memmi M, *et al*. Clinical and molecular characterization of patients with catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2002; 106(1): 69-74.
26. Xiao B, Jiang MT, Zhao MC, *et al*. Characterization of a novel PKA phosphorylation site, serine-2030, reveals no PKA hyperphosphorylation of the cardiac ryanodine receptor in canine heart failure. *Circulation Research* 2005; 96(8): 847-55.
27. Rodriguez P, Bhogal MS, Colyer J. Stoichiometric phosphorylation of cardiac ryanodine receptor on serine 2809 by calmodulin-dependent kinase II and protein kinase A. J Biol Chem 2003; 278(40): 38593-600.