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LETTER TO JMG

Catecholaminergic polymorphic ventricular tachycardia: RYR2 mutations, bradycardia, and follow up of the patients

A V Postma, I Denjoy, J Kamblock, M Alders, J-M Lupoglazoff, G Vaxsmann, L Dubosq-Bidot, P Sebillon, M M A M Mannens, P Guicheney, A A M Wilde

J Med Genet 2005;42:863–870. doi: 10.1136/jmg.2004.028993

Background: The aim of the study was to assess underlying genetic cause(s), clinical features, and response to therapy in catecholaminergic polymorphic ventricular tachycardia (CPVT) probands.

Methods and results: We identified 13 missense mutations in the cardiac ryanodine receptor (RYR2) in 12 probands with CPVT. Twelve were new, of which two are de novo mutations. A further 11 patients were silent gene carriers, suggesting that some mutations are associated with low penetrance. A marked resting sinus bradycardia off drugs was observed in all carriers. On β blocker treatment, 98% of the RYR2 mutation carriers remained symptom free with a median follow up of 2 (range: 2–37) years.

Conclusion: CPVT patients with RYR2 mutation have bradycardia regardless of the site of the mutation, which could direct molecular diagnosis in (young) patients without structural heart disease presenting with syncopal events and a slow heart rate but with normal QTc at resting ECG. Treatment with β blockers has been very effective in our CPVT patients during initial or short term follow up. Given the risk of sudden death and the efficacy of β blocker therapy, the identification of large numbers of RYR2 mutations thus calls for genetic screening, early diagnosis, and subsequent preventive strategies.

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare arrhythmogenic disorder characterised by adrenergic induced bidirectional and polymorphic ventricular tachycardia.¹ It occurs in children and adolescents and causes syncope and sudden cardiac death at a young age in the absence of structural heart disease. The resting electrocardiogram (ECG), including the QTc interval, is thought to be normal. The mortality of CPVT is extremely high reaching 30–50% by the age of 30 when untreated.² Furthermore, there is a clear correlation between the age of the first syncope and the severity of the disease, with a worse prognosis in case of early occurrence. β Blockers without sympathomimetic activity are clinically effective in reducing syncope,³ but implantation of an automatic internal defibrillator is occasionally needed in these patients.⁴

The genetic basis of CPVT had been initially elucidated by the establishment of linkage between the disease and chromosomal region 1q42.² Subsequently, two groups independently discovered autosomal dominant missense mutations in the ryanodine type 2 receptor (RYR2) associated with CPVT.^{3–5} The RYR2 gene, located on 1q42, encodes the cardiac ryanodine receptor, which is the major calcium release channel on the sarcoplasmic reticulum (SR) in cardiomyocytes.⁶ More recently, two studies reported consanguineous CPVT families associated with homozygous missense and

nonsense mutations in calsequestrin 2 (CASQ2), a Ca^{2+} binding protein located in the SR, thus describing a recessive form of CPVT.^{7–8} Both RYR2 and CASQ2 play a crucial role in the excitation-contraction coupling, by their involvement in the storage and release of Ca^{2+} from the SR, which subsequently activates cardiomyocyte contraction.

We collected 24 CPVT probands and their family members with documented polymorphic ventricular arrhythmias occurring during physical or emotional stress with a normal heart. The aim of this study was to establish the genetic and phenotypic characterisation of the probands and their family members to allow assessment of the clinical features, response to therapy, and possible genotype-phenotype correlation. We identified 13 RYR2 missense mutations in 12 CPVT probands and report here the family history, genetic, and clinical characterisation of these probands and their family members, and their response to therapy.

METHODS

CPVT families

Families were referred to Paris or Amsterdam, CPVT diagnosis was established in the proband by documenting the occurrence of the characteristic ECG pattern of the disease (mono/polymorphic ventricular premature beats followed by bi-directional ventricular tachycardia and salvos of polymorphic ventricular tachycardia) in the absence of structural heart abnormalities as assessed by clinical examination, blood chemistry, electrocardiography, and echocardiography. When a CPVT diagnosis was established in the proband, 12 lead resting ECG, exercise test, and Holter recording were proposed to family members. Stress induced ventricular beats and couplets were considered sufficient evidence to label additional family members as affected. Patients with a RYR2 mutation labelled as non-penetrant were indistinguishable from healthy individuals. The QTc interval was calculated with the Bazett formula; normal resting heart rates were established with reference to published criteria.⁹ All individuals gave informed consent to the clinical and genetic study, which was approved by the internal ethics committee.

Genotyping of candidate genes

Mutation screening was performed on genomic DNA samples extracted from peripheral blood lymphocytes using standard methods. The genomic sequence of the RYR2 gene (GenBank accession no. NM_001035) was used to design intronic primers for 45 exons, covering areas with known function or mutations, resulting in the amplification of 53 fragments

Abbreviations: CASQ2, calsequestrin 2; CCD, central core disease; CPVT, catecholaminergic polymorphic ventricular tachycardia; ECG, electrocardiogram; ICD, implantable cardioverter defibrillator; PVC, premature ventricular contraction; RYR2, ryanodine type 2 receptor; SA, sino-atrial; SR, sarcoplasmic reticulum

(6365 bp cDNA, exons: 105–90, 75, 73–70, 68–64, 59–56, 51, 49, 47, 44, 40–37, 34, 31, 29–25, 22). Primer sequences are available upon request from the corresponding author. PCR amplified fragments were analysed by uni-directional sequencing with ABI PRISM BigDye Terminator cycle sequencing ready reactions kit (PE Biosystems, Foster City, CA) and run on a 3700 Genetic Analyzer (Applied Biosystems, Foster City, CA). Aberrant sequences were re-amplified and re-sequenced to exclude polymerase errors. A mixed control group of 200 healthy and unrelated subjects from France and The Netherlands was used. In addition to the RYR2 screening, the entire coding regions of cardiac calsequestrin (CASQ2; GenBank accession no. NM_001232), histidine rich binding protein (GenBank accession no. NM_002152), and phospholamban (GenBank accession no. NM_002667) were screened using intronic primers.

Genotyping of parents

Genotypes of parents of de novo probands were determined using the Powerplex 1.2 system (Promega, Madison WI) according to the instructions provided.

Statistical analysis

Data are presented as mean (SEM). RR intervals of resting ECGs were measured and converted to beats per minute, and subsequently compared to age and gender matched control heart rates.⁹ The deviations from these control heart rates were evaluated among different groups. The effect of the genotype, phenotype, and gender on heart rates, as well as the interaction between genotype and gender or phenotype and gender were tested with the Mixed Model Analysis of Variance (SPSS, version 11.0) in which family membership was used as a repeated factor. Statistical significance was accepted at $p < 0.05$.

RESULTS

Genotyping analyses

We identified RYR2 missense mutations in 12 CPVT families or sporadic cases (fig 1). In the probands, the screening of the genes encoding CASQ2, phospholamban, or histidine rich binding protein did not reveal any additional mutation. Among the 13 mutations identified, only one had previously been reported (V4771I⁴) and all others are new. They all occurred in evolutionarily highly conserved positions in the RYR2 protein (fig 2) and were absent from 400 normal chromosomes.

Four mutations were identified in sporadic cases. Two of them, H4108N and H4108Q, occurred de novo in families 8 and 9, in which parental transmission was confirmed. For the two others, F4020L and V4771I, it was impossible to establish inheritance due to unavailability of parental DNA (families 4 and 11).

Among the mutations identified, one, E4076K, was present in two families, and two mutations, G4662S and H4762P, were transmitted in the same family (family 10). For the E4076K mutation, we excluded a familial relationship between the two families by the use of a rare polymorphism located in exon 37 (–136 G/A), which co-segregates with the mutation in family 6 but was absent in family 5. In family 10, the proband carries the two separate mutations, G4662S and H4762P, each inherited from one parent. The two children of the proband both inherited the H4762P mutation (fig 1). Her parents and her two children were clinically unaffected. Interestingly, for three of the identified RYR2 mutations, a corresponding RYR1 mutation has been described. The H4762P RYR2 mutation is equivalent to the H4833Y RYR1 mutation which is associated with central core disease (CCD), a neuromuscular disorder (N Monnier, personal communication). In addition, the RYR2 mutations P4902S

(family 12) and A2394G (family 3) correspond to the RYR1 mutations P4972L and A2428T, respectively, which are associated with malignant hyperthermia.^{10 11}

Clinical evaluation of RYR2 mutation carriers

Of the 12 probands included in the study, nine were referred because of syncopal events and three after rescued cardiac arrest related to life threatening arrhythmias (polymorphic ventricular tachycardia or ventricular fibrillation) occurring during physical or emotional stress (three males, nine females; table 1). Altogether we identified 54 RYR2 mutation carriers including the probands (32 female, 22 male). The median age of onset of the symptoms was 9 years among the probands and 12 years amongst all the carriers (range 4–51 years; tables 1 and 2). Six of the 12 probands presented with seizures during the course of the syncopal events, while no seizure was reported among the other carriers. Previous syncopal events were reported in 26 of the 54 RYR2 mutation carriers (48%), with a median age for the first syncope of 12 years. Moreover, a history of sudden cardiac death was present in seven of 12 families (58%) with a total of 20 lethal events (10 males, 10 females), with a median age at death of 28 years (fig 1, table 2). In all the RYR2 mutation carriers most of the resting ECG parameters were normal including the QTc interval (mean 399 (SD 24); table 1), except for the mean resting heart rate, which was lower than that of age and gender matched groups (see below and table 1). As we did not screen the complete RYR2 gene we can not state for certain that a patient has no RYR2 mutation. As a result, we can not make a sound comparison between the clinical characteristics of the group of patients with a mutation and the group without a mutation.

Genotype-phenotype relation in RYR2 mutation carriers

CPVT diagnosis was confirmed in all the probands by the use of exercise tests, which reproducibly showed the occurrence of characteristic ECG patterns with mono/polymorphic ventricular premature beats followed by bi-directional ventricular tachycardia and salvos of polymorphic ventricular tachycardia. Exercise tests performed in the 38 additional family members carrying a mutation and aged over 5 years were positive for arrhythmic events in only 27 RYR2 carriers. Eleven RYR2 mutation carriers were considered to be phenotypically unaffected (fig 1). Four were too young to perform exercise testing. Therefore, the overall penetrance was 78% (39/50). Three of the eight autosomal dominant families display complete penetrance based on stress tests (families 1, 3, 7; fig 1), while penetrance was incomplete in the other five families (2, 5, 6, 10, and 12) (fig 1, table 1). In family 12, a 24 year old man (II-6) displayed clear cut exercise induced PVT only at the third exercise test, the two first being normal.

Treatment and follow up of RYR2 mutation carriers

CPVT is a life threatening disease as shown by the high number of sudden deaths in the families. Therefore, 50 RYR2 mutation carriers (>5 years old) were treated with β blockers (1–2 mg/kg per day nadolol, 3–4 mg/kg per day propranolol, 50–100 mg per day metoprolol) which were individually titrated until the maximal heart rate was <110 bpm at exercise tests or Holter recordings, in order to prevent polymorphic ventricular salvos. β Blocker treatment had a favourable overall outcome in this group, as 11 of the 12 probands are symptom free with a median follow up period of 6 years (table 1). The remaining proband experienced sudden cardiac death due to non-compliance with the β blocker treatment, after a CPVT diagnosis had been established. Of the 50 treated RYR2 patients, 49 (98%) were

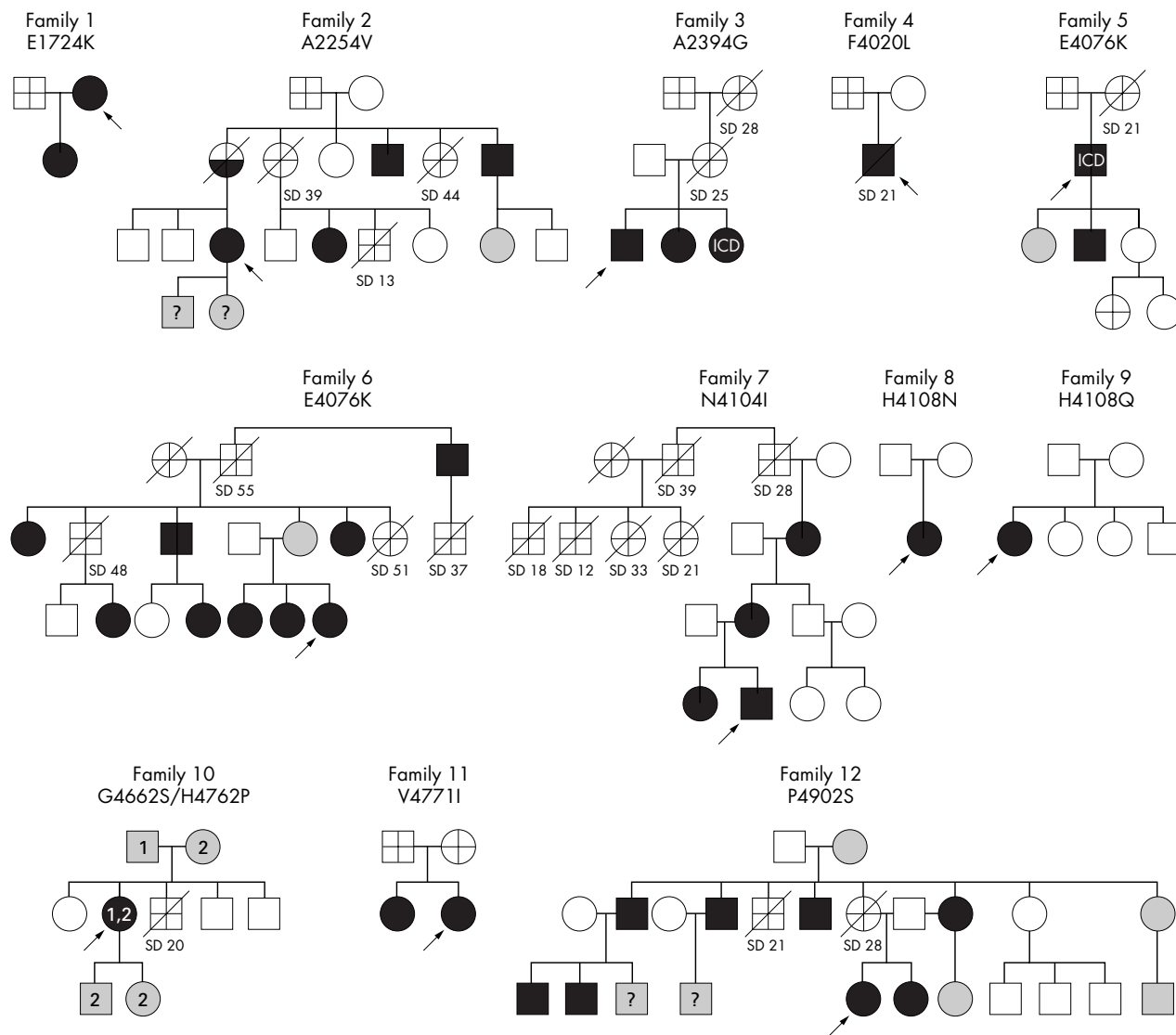


Figure 1 Overview of the 12 CPVT families with identified RYR2 mutations and their clinical phenotypes. Black symbols indicate a CPVT phenotype with a RYR2 mutation, open symbols represent clinically unaffected persons without a RYR2 mutation, grey symbols indicate persons with a RYR2 mutation who are clinically unaffected, and non-genotyped individuals are indicated by a cross. Question marks indicate persons with a RYR2 mutation but undetermined phenotype. Proband of each family is indicated by an arrow, deaths are indicated by a transverse line. CPVT related sudden cardiac deaths are indicated by SD (sudden death) and the age of the death in years. In family 10, number 1 indicates the presence of the G4662S mutation and number 2 the H4762P mutation.

symptom free with a median follow up period of 24 months, although some continued to have isolated premature ventricular contractions (PVCs) at exercise tests (table 2). In two RYR2 positive patients an implantable cardioverter defibrillator (ICD) was implanted. The first ICD was implanted in a man aged 51 (family 5, II-1) because the aetiology of the syncope in a familial context of sudden death had not been known when he was younger, and the second in a symptomatic 18 year old girl because of poor compliance (III-2, family 3). Over a follow up of 1 year, this latter patient received two appropriate shocks to terminate ventricular fibrillation; β blocker treatment was maintained in both patients. In the four youngest RYR2 mutation carriers (<5 years old), we could not establish a definite clinical phenotype as they were too young to perform exercise testing (fig 1). However, as Holter recordings did not show any ventricular arrhythmia or PVCs, β blockers have not been started so far. These children are regularly checked with bi-annual Holter recordings.

Heart rate in RYR2 mutation carriers is significantly lower

Available resting ECGs in the absence of treatment were reviewed in 67 genotyped individuals (40 RYR2 mutation carriers and 27 family members without RYR2 mutation). The resting heart rate of the 12 RYR2 CPVT probands was on average 20 bpm lower than that of age and gender matched controls⁹ (table 1). Likewise, RYR2 carriers had in general a lower heart rate than their family members without a mutation, irrespective of mutation or family, as they deviated significantly more from age and gender matched control heart rates (-12 bpm ν 2 bpm, $p = 0.002$; fig 3). A further analysis of the genotyped population according to gender revealed that male RYR2 carriers tended to deviate more from control heart rates than female RYR2 carriers (-19 bpm ν -7 bpm, $p = 0.067$; fig 3). Interestingly, the heart rate of phenotypically affected RYR2 carriers was also significantly lower than that of their clinically unaffected family members (including seven silent RYR2 mutation carriers) (-12 bpm ν -2 bpm, $p = 0.012$).

	E1724K			A2254V			A2394G			F4020L			E4076K		
RyR2 <i>H sapiens</i>	MNN	E	YIV	NEL	A	LAL	FYS	A	LID	FDM	F	LKL	DEN	E	TLD
RyR2 <i>M musculus</i>	MLS	E	YIV	NEL	A	LAL	FYA	A	LID	FDM	F	LKL	DEN	E	MIN
RyR1 <i>H sapiens</i>	MKN	E	YII	NEL	A	LSL	FYS	A	LID	FDM	F	LKL	DEN	D	MFN
RyR1 <i>S scrofa</i>	MLS	E	YIV	NEL	A	LAL	FYA	A	LID	FDM	F	LKL	DEN	E	MID
RyR1 <i>M nigricans</i>	MNN	E	FIV	NEL	A	LAL	FYA	A	LID	FDM	F	LKL	DEN	E	TLD
RyR3 <i>H sapiens</i>	MKN	E	YII	NEL	A	LGL	FYS	A	LID	FDM	F	LKL	DEN	D	MFN
RyR3 <i>M vison</i>	MNN	E	FII	NEL	A	LAL	FYS	A	LID	FDM	F	LKL	DEN	D	MFN
RyR3 <i>G gallus</i>	TNK	E	FIV	NEL	A	LAL	FYS	A	LID	FDM	F	LKL	DEN	D	MIN
RyR <i>C elegans</i>	MAK	E	YVI	NEL	A	LAL	FYS	S	LVD	SDM	F	LKL	NND	G	KVD
RyR <i>D melano</i>	CKN	E	YIT	TEL	A	LAL	FYC	T	LVD	FDM	F	LKL	NHE	G	KID

	H4108N			N4104I			H4108Q			G4662S			H4762P			V4771I			P4902S		
RyR2 <i>H sapiens</i>	LLT	N	LES	H	MPN	EFY	G	RDR	SVT	H	NGKQLVLT	V	GLL	DTV	P	HGF					
RyR2 <i>M musculus</i>	LLT	N	LES	H	VPH	DIY	G	RER	SVT	H	NGKQLVMT	V	GLL	DTV	P	HGF					
RyR1 <i>H sapiens</i>	LLT	N	LES	H	MPN	DLY	G	AER	SVT	H	NGKQLVLT	V	GLL	DTT	P	HGF					
RyR1 <i>S scrofa</i>	LLT	N	LES	H	VPH	DIY	G	RER	SVT	H	NGKQLVMT	V	GLL	DTT	P	HGF					
RyR1 <i>M nigricans</i>	LLT	N	LES	H	MPN	EFY	G	RDR	SVT	H	NGKQLVLT	V	GLL	DTT	P	HGF					
RyR3 <i>H sapiens</i>	LLT	N	LES	H	MPN	DLY	G	AER	SVT	H	NHKQLVLT	V	GLL	DTT	P	HGF					
RyR3 <i>M vison</i>	LLT	N	LES	H	MPN	DLY	G	AER	SVT	H	NGKQLVLT	V	GLL	DTT	P	HGF					
RyR3 <i>G gallus</i>	LLT	N	LES	H	VPH	DIY	G	RER	SVT	H	NGKQLMMT	V	GLL	DTI	P	HGF					
RyR <i>C elegans</i>	LLV	N	LKE	H	ITN	DQV	D	EET	SVT	H	NLQQLILT	I	MMT	DRM	P	RGF					
RyR <i>D melano</i>	LLT	N	LES	H	MPN	ETY	D	FDS	SVT	H	NGKQLVLT	V	MLL	DIV	P	HGF					

Figure 2 Alignment of RYR protein sequences of multiple species. Amino acid positions at which the various RYR2 mutations occur are indicated by the boxed areas.

DISCUSSION

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a rare arrhythmogenic disorder characterised by syncopal events and sudden cardiac death occurring in young subjects during physical stress or emotion in the absence of structural heart disease. Mutations in calcium handling proteins located in the SR, such as RYR2 and CASQ2, have been associated with CPVT. We report here on the genetic and clinical evaluation of 12 CPVT families with RYR2 mutations.

The phenotypes of the CPVT probands share many similarities: syncopal episodes were usually triggered by exercise or emotion. During exercise testing there was a threshold in the heart rate before the appearance of ventricular arrhythmias, which was individually highly reproducible. The initial resting ECG demonstrated a normal QTc interval in all probands, however, most of them had, compared to control values,⁹ sinus bradycardia as previously reported in non-genotyped populations.¹²⁻¹³ The age of onset of CPVT symptoms in our RYR2 probands (9 years) and the overall age of onset among all our RYR2 patients (12 years) are comparable to previously reported (RYR2) probands.⁴⁻¹⁴ However, some CPVT probands may present a much later age of onset, as one of our RYR2 patients was asymptomatic until 51 years of age. In such cases, which could be misleading, it is crucial to document a positive family history of sudden death. In 50% of our RYR2 probands exercise induced syncopal episodes were accompanied by seizures, which were probably convulsive movements owing to pan-cerebral hypoperfusion. Based on these seizures, the patients were initially referred to a neurologist who recommended

anti-epileptic treatment. However, this did not lead to syncope resolution, and in most cases the diagnosis of CPVT was established only after an exercise ECG or a subsequent syncopal event. Treatment with β blockers had a favourable overall outcome in our group, as 11 of the 12 probands are symptom free with a median follow up period of 6 years. The remaining proband unfortunately experienced sudden cardiac death due to non-compliance after appropriate diagnosis and treatment. In total, 98% of the 50 treated patients of the 12 families are symptom free with a median follow up period of 24 months, although isolated premature ventricular beats were seen at follow up exercise tests. The β blocker doses required to keep the patients symptom free are higher than those used in the long-QT syndrome.¹⁵ Consequently, poor compliance in these patients with spontaneous resting bradycardia could potentially result in serious consequences; however, we were not faced with this in our population. Additionally, we do not rule out the possibility of ICD implantation especially when follow up stress tests or Holter recordings show polymorphic ventricular salvos. In contrast to our group of patients, a recent publication with a comparable follow up time shows that seven of 19 genotyped RYR2 probands had episodes of ventricular tachycardia/ventricular fibrillation while on β blockers.⁴ The discrepancy in efficacy of β blocker treatment between the two studies could reflect a difference in β blocker dosage, a difference in underlying RYR2 mutations, or more mildly affected subjects in our CPVT families compared to the latter study. Obviously, larger groups of genotyped CPVT probands are needed to address the issue of β blocker efficacy in CPVT with longer follow up.

Table 1 Clinical data of CPVT probands

RYR2 mutation	Gender	Age of onset, years	Penetrance, %	Resting ECG pre med, bpm (age, of years)	Median HR age of group ⁹	Deviation, bpm	QTc, ms	Symptoms	Exercise test VT threshold, bpm	Exercise test ECG characteristics	Follow up, years	Origin	
Family 4	F4020L	M	NA	70 (4)	98	28	410	X syncope, seizure	150	BG, PC, PMVT	Sudden death at 20	French	
Family 8	H4108N	F	De novo	50 (14)	73	23	374	X syncope, seizure, CA	90	BG, PC, PMVT	2	Dutch	
Family 9	H4108Q	F	De novo	70 (7)	89	19	400	X syncope	140	MPVB, PMVT	12	French	
Family 7	N4104I	M	100	60 (8)	88	28	400	X syncope, seizure	130	PPVB, SPVT	2	French	
Family 2	A2254V	F	100	66 (5)	84	18	410	CA, exercise VT	135	BG, PC	22	Dutch	
Family 1	E1724K	F	100	43 (12)	78	35	380	X syncope	110	BG, PMVT	15	Dutch	
Family 3	A2394G	F	100	60 (10)	78	18	440	X syncope, seizure, CA	140	MPVB, PMVT	6	French	
Family 6	E4076K	F	66	58 (14)	76	18	405	X syncope	110	BG, PMVT	3	Dutch	
Family 11	V4771I	F	NA	–	–	–	–	X syncope, seizure	130	PPVB, PMVT	7	French	
Family 12	P4902S	F	65	60 (13)	76	16	414	X syncope	120	PPVB, PMVT	6	French Polynesia	
Family 10	H4762P	F	13	–	–	–	410	X syncope	60 W	BG, PMVT	28	French	
Family 5	E4076K	M	51	Com-pound 64	73 (17)	70	–3	351	X syncope, seizure	110	MPVB, PC, PMVT	4	French
			9 (4–51)*			20 (10)†	399 (24)†		124 (18)†		6 (2–28)*		

*Median (range); †mean (SD).

BG, monomorphic bigeminy; CA, cardiac arrest; MPVB, monomorphic premature ventricular beat; NA, not available; PC, polymorphic couplets; PMVT, polymorphic ventricular tachycardia; PPVB, polymorphic premature ventricular beat; SPVT, sustained polymorphic ventricular tachycardia; VT, ventricular tachycardia; X syncope, exercise induced syncope.

Analogous to other inherited arrhythmogenic diseases, RYR2 CPVT has variable expressivity. Eleven out of the 50 RYR2 mutation carriers tested (22%) presented no ventricular arrhythmia during repeated exercise tests. Eight belong to two families (10 and 12) suggesting that three RYR2 mutations, G4662S, H4762P, and P4902S, may represent mutations with partial penetrance. Nevertheless, sudden deaths occurred in both families and seven of these silent gene carriers are female, hinting at a possible gender bias, which is in line with a recent study that suggests that male gender is a risk factor for syncope in genotyped RYR2 patients.⁴

A low heart rate in CPVT patients has been reported in the initial description of the disease¹² before genetic defects were recognised. In this study we observed that RYR2 mutation

carriers have a significantly lower heart rate than their genetically unaffected family members on resting ECGs, irrespective of mutation position or family. Moreover, there is also a noteworthy difference between genders in the deviation in heart rates; male carriers have a lower heart rate than female carriers. This is in concordance with the fact that females have on average a slightly higher heart rate.¹⁶ The fact that CPVT patients with a RYR2 mutation have significant bradycardia could direct molecular diagnosis in (young) patients without structural heart disease presenting with syncopal events and a slow heart rate but with normal QTc at resting ECG. These findings cannot be compared to previous (RYR2) CPVT studies, as the heart rates in these studies were either pooled within a single family or not reported.^{3 4 14} A more recent study of 29 non-genotyped Japanese CPVT patients, with a similar age of onset, also demonstrated sinus bradycardia.¹³ A possible link between ryanodine receptors and heart rate is the presence of RYR2 channels and functional SR in sino-atrial (SA) node cells, which serve as the primary pacemaker of the heart.¹⁷ Moreover, substances that interfere with SR function, such as ryanodine and cyclopiazonic acid, have a negative chronotropic effect.¹⁷ Thus, the bradycardia seen in the RYR2 mutation carriers could be a direct effect of the impaired Ca²⁺ handling of their SA nodal cells. Alternatively, it could represent a feedback mechanism by the vagal system; a low average heart rate reduces the likelihood of reaching the deleterious threshold at which CPVT is induced.

We identified 13 RYR2 missense mutations, of which 12 are novel. These mutations cluster within three functionally important regions of RYR2: the binding site for the FKBP12.6 protein that stabilises the RYR2 channel, the calcium binding site, and the channel forming transmembrane domains as

Table 2 Clinical data of all RYR2 mutation carriers

	RYR2 CPVT
Number of probands	12
Total no. of mutation carriers	54
Families with juvenile sudden death	7/12 (58%)
Age of onset all carriers, years	12 (4–51)*
Clinically affected	39
Phenotype undetermined	4
Silent gene carrier	11
Gender	32F/22M
Exercise related syncope	26/54 (48%)
Patients on β blockers	50/54
Follow up, years	2 (2–37)*
VT/VF on β blockers	1/50

*Median (range).

VT/VF, ventricular tachycardia/ventricular fibrillation.

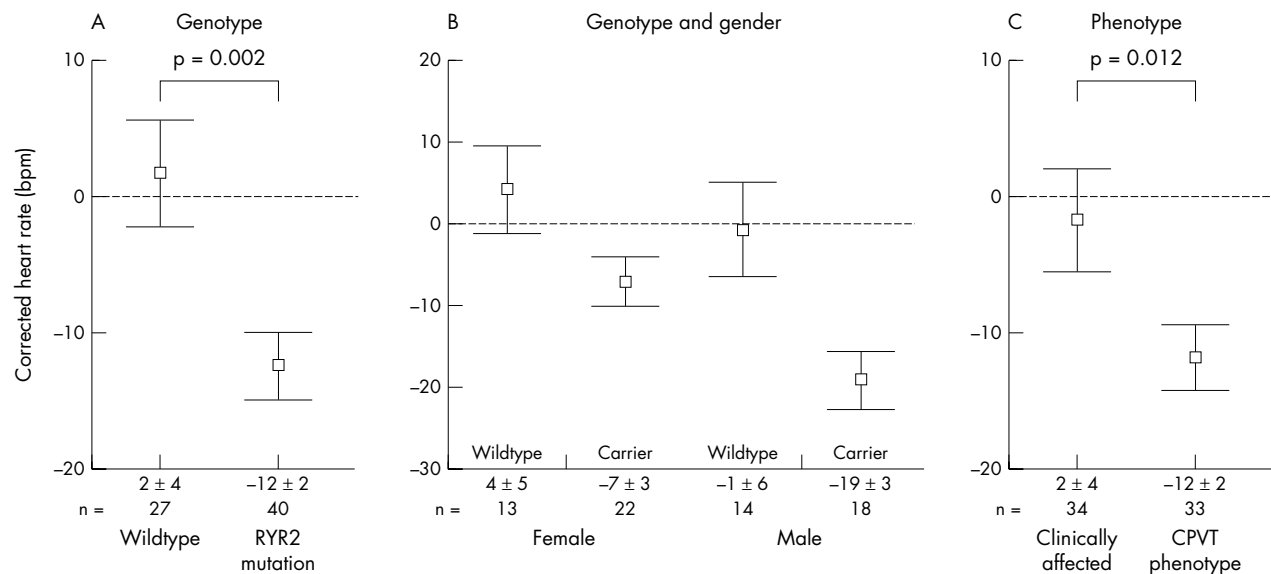


Figure 3 Corrected heart rates, calculated as observed resting heart rate (before medication) minus the heart rate according to age and gender dependent trends,⁹ of 67 genotyped individuals (40 family members with a RYR2 mutation, 27 family members without a RYR2 mutation). Zero means an observed heart rate according to those trends. (A) Wildtype family members v RYR2 mutation carriers, (B) the effect of gender and genotype on the resting heart rate in wildtype family members and RYR2 mutation carriers, and (C) clinically unaffected individuals (27 wildtype family members and seven silent RYR2 mutation carriers) v phenotypically affected CPVT patients (33 mutation carriers).

previously observed^{3 4 14} (fig 4). Moreover, diseases such as malignant hyperthermia and CCD, which are associated with mutations in the ryanodine receptor specific for skeletal muscle, RYR1, also show mutational clustering in the same areas.¹⁸ Interestingly, three of our mutations even occur on positions corresponding to known RYR1 mutations in MD and CCD, suggesting that these areas are of major importance

to the function of ryanodine receptors. Unfortunately, there is no clear correlation among RYR2 probands between the region of mutation and the phenotype of the proband, in contrast to what was reported for RYR1 probands.¹⁸

We identified RYR2 mutations in 50% of the screened CPVT probands. Mutations in RYR2 cluster into the three protein areas described above, but also within discrete mutational

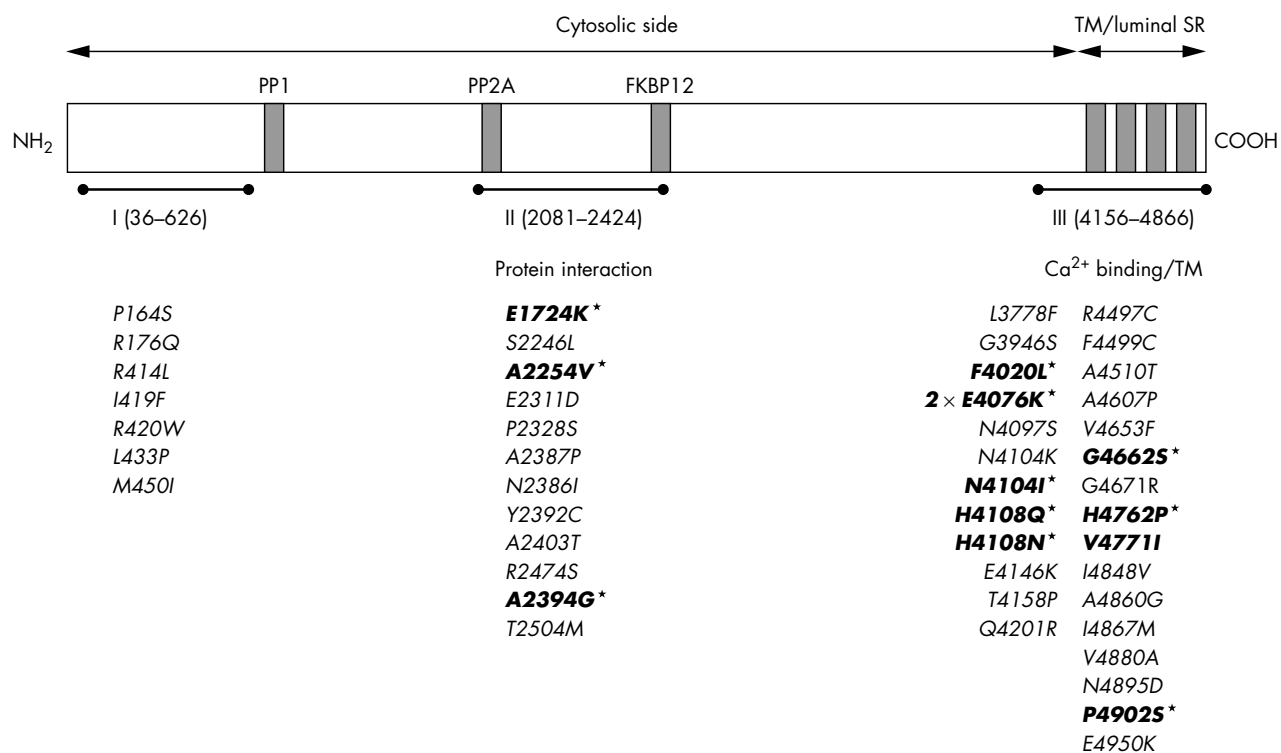


Figure 4 Schematic representation of the RYR2 protein, with an overview of predicted function and topology (segments I-III¹⁹) and the location in amino acids of all the current RYR2 mutations. Indicated in bold are RYR2 mutations identified in this study with novel mutations marked with an asterisk (*); the other mutations have been previously reported.^{3-5 19-22}

hotspots. We found three mutations in close proximity to each other, two at amino acid position 4108 (H4108N, H4108Q) and one at position 4104 (N4104I). Moreover, a recent study also reported a CPVT mutation at position 4104 (N4104K).⁴ Interestingly, both the 4108 mutations and the reported N4104K occurred de novo. Another potential hotspot for RYR2 mutations is amino acid 4076, where we identified the same mutation (E4076K) in two unrelated families (6 and 7), leading to a similar penetrance (70%). Consequently, it appears that clustering and hotspots of RYR2 mutations are relatively common in CPVT. In vitro expression studies of these mutations could provide insight into the exact function of these specific regions.

We report one proband who inherited two independent RYR2 mutations. Analogous to our findings, a recent study described a malignant hyperthermia family in which patients received two unique RYR1 mutations, however, the RYR1 mutations were independently associated with a clinical phenotype.¹⁰ In contrast, family members in our study carrying only one of the RYR2 mutations were symptom free without any arrhythmia triggered by exercise testing, thus raising the possibility of a recessive form of CPVT. Alternatively, the phenotype of this family could be explained by a mutation presenting with reduced penetrance and the presence of a very rare polymorphism. Given the amount of controls screened, the negative exercise tests, and the absence of a clinical history in the single mutation carriers we believe, however, that this could be the first case of a RYR2 recessive form of CPVT.

In summary, genetic and phenotypic characterisation of our CPVT population allowed us to assess clinical features, response to therapy, and genotype-phenotype correlation. We identified 13 mutations in evolutionarily highly conserved residues in the cardiac ryanodine receptor in 12 CPVT families. In addition, we found that RYR2 mutation carriers and phenotypically affected CPVT patients have a significant resting sinus bradycardia. Consequently, CPVT should be considered a possible cause of resting bradycardia in young individuals, especially in the presence of a history of syncope or familial sudden death. Finally, given the risk of juvenile sudden death (50%) and the efficacy of β blocker/ICD therapy for CPVT, the identification of large numbers of RYR2 mutations calls for genetic screening, early diagnosis, and subsequent preventive strategies.

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Authors' affiliations

A V Postma, A A M Wilde, Experimental and Molecular Cardiology Group, Academic Medical Center, Amsterdam, The Netherlands
A V Postma, I Denjoy, J-M Lupoglazoff, P Guicheney, INSERM U582, Institut de Myologie, IFR 14, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
I Denjoy, Service de Cardiologie, Hôpital Lariboisière, Paris, France
J Kamblock, Centre de Cardiologie du Taaoe, Tahiti, French Polynesia
M Alders, M M A M Mannens, Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands
G Vaksman, Service de Cardiologie Pédiatrique, Hôpital Cardiologique, Lille, France
L Duboscq-Bidot, INSERM U621, IFR 14, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
P Sebillon, Laboratoire de génétique et insuffisance cardiaque, Association Claude Bernard, IFR 14, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

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Correspondence to: Alex V Postma, Department of Clinical and Experimental Cardiology, Academic Medical Center, Meibergdreef 9, PO Box 22700 1100 DE, Amsterdam, The Netherlands; a.v.postma@amc.uva.nl

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