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Yield and Pitfalls of Ajmaline Testing in the Evaluation of Unexplained Cardiac Arrest and Sudden Unexplained Death

Single-Center Experience With 482 Families

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ABSTRACT

OBJECTIVES This study evaluated the yield of ajmaline testing and assessed the occurrence of confounding responses in a large cohort of families with unexplained cardiac arrest (UCA) or sudden unexplained death (SUD).

BACKGROUND Ajmaline testing to diagnose Brugada syndrome (BrS) is routinely used in the evaluation of SUD and UCA, but its yield, limitations, and appropriate dosing have not been studied in a large cohort.

METHODS We assessed ajmaline test response and genetic testing results in 637 individuals from 482 families who underwent ajmaline testing for SUD or UCA.

RESULTS Overall, 89 individuals (14%) from 88 families (18%) had a positive ajmaline test result. *SCN5A* mutations were identified in 9 of 86 ajmaline-positive cases (10%). *SCN5A* mutation carriers had positive test results at significantly lower ajmaline doses than noncarriers (0.75 [range: 0.64 to 0.98] mg/kg vs. 1.03 [range: 0.95 to 1.14] mg/kg, respectively; $p < 0.01$). In 7 of 88 families (8%), it was concluded that the positive ajmaline response was a confounder, either in the presence of an alternative genetic diagnosis accounting for UCA/SUD (5 cases) or noncosegregation of positive ajmaline response and arrhythmia (2 cases). The rate of confounding responses was significantly higher in positive ajmaline responses obtained at >1 mg/kg than in those obtained at ≤1 mg/kg (7 of 48 vs. 0 of 41 individuals; Fisher's exact test: $p = 0.014$).

CONCLUSIONS In line with previous, smaller studies, a positive ajmaline response was observed in a large proportion of UCA/SUD families. Importantly, our data emphasize the potential for confounding possibly false-positive ajmaline responses in this population, particularly at high doses, which could possibly lead to a misdiagnosis. Clinicians should consider all alternative causes in UCA/SUD and avoid ajmaline doses >1 mg/kg. (J Am Coll Cardiol EP 2017;3:1400–8)

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Sudden cardiac death (SCD) accounts for 15% to 20% of all deaths (1). Coronary artery disease accounts for a majority of cases at older ages, whereas in SCD cases <65 years of age, approximately 4% are unexplained (2). In cases of sudden unexplained death (SUD) or resuscitated unexplained cardiac arrest (UCA), a heritable cause should be considered (3). Establishing the correct diagnosis is a prerequisite to identifying family members at risk and initiating preventive measures. Despite their rarity, SUD and UCA cases account for a non-negligible portion of referrals for cardiogenetic evaluation, which often results in diagnostic uncertainties often affecting psychosocial well being of family members (4).

Various diagnostic algorithms have been proposed to assess the causes of SUD (3,5,6) and UCA (7,8), which invariably include resting and exercise electrocardiogram (ECG) testing, cardiac imaging, sodium channel blocker testing to unmask Brugada syndrome (BrS), and genetic testing in selected cases. The presence of a type 1 BrS ECG pattern after sodium channel blockade testing in the context of UCA or a family history of SUD is diagnostic for BrS (3,9,10). Intravenous procainamide, flecainide, pilsicainide, and ajmaline are the sodium channel blockers most often used, with a diagnostic yield of 5% to 18% depending on the studied population (SUD vs. UCA) and drug used (higher yield for ajmaline) (5,6,11,12). These yield estimates are based on either a small number of families (n = <50) (5,6,12) or the use of a less potent sodium channel blocker (e.g., procainamide) (11), and most studies do not consider the possibility of false-positive results, which are now well recognized (13).

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In this study, we assessed the yield of ajmaline testing results in cases of UCA and SUD in a large single-center cohort and systematically evaluated confounding results by using genotype-phenotype correlations. The study findings confirm the high yield of ajmaline testing but emphasize the importance of ajmaline doses and highlight the need to always consider alternative diagnoses, even in the presence of a positive ajmaline test result.

METHODS

STUDY DESIGN AND POPULATION. Data were extracted from an ongoing database of patients evaluated in the Academic Medical Centre (Amsterdam, the Netherlands) for suspicion of BrS. From December 2004 to February 2016, 1,799 ajmaline tests were performed. For the present study, we included consecutive patients who underwent ajmaline testing for either a family history of SUD or UCA or resuscitation from UCA with documented ventricular fibrillation (VF). Individuals with ECG documentation of BrS (type 1) prior to ajmaline testing were excluded. Individuals who underwent ajmaline testing because of a known family history of BrS (spontaneous or drug-induced) were also excluded.

Indications for ajmaline testing in the context of UCA or SUD are resuscitation from UCA, defined as documented VF in the absence of coronary artery disease; cardiomyopathy observed on transthoracic echocardiography or an ECG-based diagnosis (e.g., long QT syndrome); a family history of UCA, when the index UCA case is not available for sodium channel blocking drug test or the patient's data are not available; or a family history of SUD <50 years of age or, at an older age, in the context of other features suggestive of an inherited arrhythmia or BrS (e.g., normal autopsy finding, multiple cases, low probability of coronary artery disease, SUD during sleep).

AJMALINE TESTING. Ajmaline testing was performed with continuous ECG monitoring. Intravenous ajmaline was infused at consecutive boluses of 10 mg/min targeting a total dose of 1 mg/kg, rounded up to the next 10-mg step. Electrocardiographic leads were systematically recorded in the third intercostal space cranially to V_1 and V_2, and beginning in 2008, also in the second intercostal space. A positive test result was defined as the appearance of a type 1 BrS pattern: ST-segment elevation >2 mm with a coved morphology in any lead between V_1 and V_2 in the second, third, or fourth intercostal space (3). A negative test result was defined as the absence of a positive response at the maximal dose infused, where the last should be ≥1 mg/kg.

The infusion was stopped when criteria for a positive test were reached. The test was prematurely terminated (<1 mg/kg without a type 1 ECG) for either excessive QRS widening (>140%, by visual inspection) or induction of ventricular couplets, ventricular tachycardia, or recurrent isolated premature ventricular contractions. In cases of premature termination, the test was said to be inconclusive.

In cases where ST-segment elevation with a coved morphology is observed at 1 mg/kg but does not reach 2 mm, more ajmaline is infused beyond the target dose of 1 mg/kg. The rationale for doing this is to avoid "borderline" test results, with the assumption that individuals with suspicious ST-segment abnormalities detected but not fulfilling positivity criteria

ABBREVIATIONS AND ACRONYMS

BrS = Brugada syndrome
ECG = electrocardiogram
SCD = sudden cardiac death
SUD = sudden unexplained cardiac death
UCA = unexplained cardiac arrest
VF = ventricular fibrillation

TABLE 1 Clinical Characteristics of Study Population*

	All Indications		UCA Probands		FH UCA/SUD	
	All (N = 637)	**Aj+ (n = 89, 14.0%)**	**All (N = 54)**	**Aj+ (n = 11, 20.4%)**	**All (N = 583)**	**Aj+ (n = 78, 13.4%)**
Age, yrs	43.5 ± 13.9	45.5 ± 12.7	41.8 ± 13.8	42.4 ± 14.9	43.7 ± 13.9	45.9 ± 12.4
Males	52.6	51.7	63.0	72.7	51.6	48.7
Ajmaline, mg/kg	1.02 (1.00-1.06)	1.03 (0.91-1.11)	1.00 (1.00-1.04)	1.00 (0.76-1.03)	1.03 (1.00-1.06)	1.03 (0.93-1.13)
Weight, kg	78.4 ± 14.9	76.5 ± 13.5	79.3 ± 15.3	74.2 ± 12.9	78.4 ± 14.9	76.8 ± 13.6

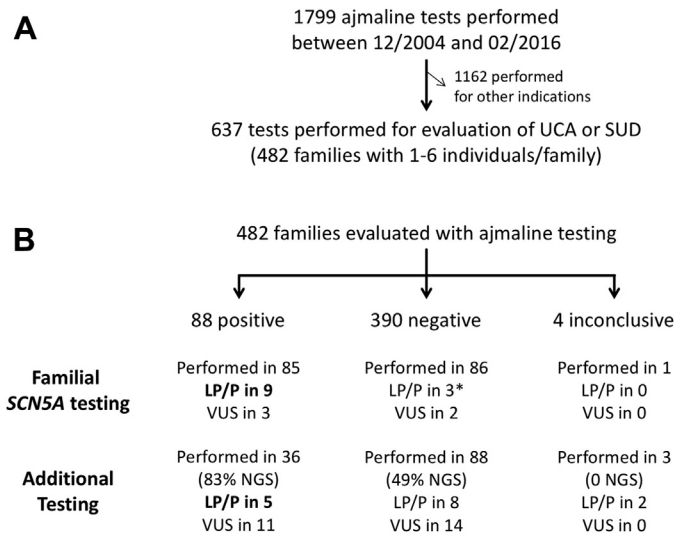
Values are mean ± SD, %, or median (interquartile range). *Effects of age and sex and indications on ajmaline response: all insignificant in univariate regression. No significant 2-variable interaction (sex-age, sex-indication, and age-indication) on ajmaline response. No statistically significant difference of weight or administered dose between Aj+ and Aj− groups in total cohort and in UCA and FH UCA/SUD subgroups.

Aj+ = positive ajmaline; FH = family history; SUD = sudden unexplained death; UCA = unexplained cardiac arrest.

at <1 mg/kg may have BrS but require higher doses to unmask it. Systematic studies to establish the optimal dose for ajmaline drug testing have not been conducted. The validity of this approach will be qualitatively assessed as described below. The absence of a true gold standard to diagnose BrS prevents us from precisely calculating sensitivity and specificity (14).

CLINICAL GENETIC TESTING. Sequencing of *SCN5A* was performed in the first individual of a family in whom an ajmaline test was positive. Sequencing of other genes, including screening for the idiopathic VF *DPP6* risk haplotype (15) was performed at the discretion of the treating physician. In SUD cases, post-mortem genetic testing was performed if DNA was available. Genetic variant classification was performed by experienced laboratory specialists (16). Variants are divided into 5 classes: 1 = certainly not pathogenic; class 2 = unlikely pathogenic mutations; class 3 = unknown pathogenicity; class 4 = likely pathogenic; class 5 = pathogenic. Only variants in classes 3 to 5 are reported.

FIGURE 1 Study Population



(A) Flowchart of the study population. **(B)** Ajmaline response and genotypic correlations. *Likely pathogenic and pathogenic *SCN5A* variants in ajmaline-negative families were identified in post-mortem testing or in family members that did not undergo ajmaline testing. None of the ajmaline-negative individuals was shown to carry a pathogenic *SCN5A* variant. LP/P = likely pathogenic or pathogenic variant (class 4 or 5); no individual had more than one LP/P (class 4 or 5) variant; NGS = next-generation sequencing; SUD = sudden unexplained death; UCA = unexplained cardiac arrest; VUS = variant of unknown significance (class 3).

CONFOUNDING AJMALINE RESPONSES. Because the specificity of ajmaline testing is currently being questioned (13), we systematically assessed all families with a positive ajmaline test for confounding results. These were defined as either a positive ajmaline test result and the presence of a familial pathogenic mutation accounting for the UCA/SUD case(s) but not causing BrS or an ajmaline response not cosegregating with the arrhythmia phenotype (VF). In these cases, the positive ajmaline response is considered a confounder or incidental finding (i.e., UCA/SUD is likely *not* explained by BrS).

STATISTICAL ANALYSES. For continuous variables, deviation from normal distribution was assessed using the Shapiro-Wilk test of normality. Normally distributed variables are presented as mean ± SD and were compared using Student *t* test. Other continuous variables are presented as median (interquartile range) and were compared using the Wilcoxon rank sum test. Fisher's exact test was used to compare categorical variables within 2 groups. Statistical analyses concern individuals from different families; units of analysis are thus independent. All statistical analyses were performed using R version 3.3.1 software (R Core Team, Vienna, Austria). A p value of <0.05 was considered significant.

RESULTS

STUDY POPULATION. A total of 637 ajmaline tests were performed for UCA or SUD (Table 1). Of these,

TABLE 2 Identified Variants in the *SCN5A* Gene in Ajmaline-Positive Cases

Class	Patient Type	Ajmaline Dose, mg/kg	cDNA Change*	Predicted Protein Change	ClinVar (ID#)†	ExAC Global AF	Comments
5	FH-SUD	0.27	c.4978A>G	p.(Ile1660Val)	LP/P (67947)	0.00002	NA
4	FH-SUD	0.64	c.3695G>A	p.(Arg1232Gln)	LP/P (67814)	0.00002	NA
5	FH-SUD	0.75	c.934+1G>A	splicing	NR	0	NA
5	FH-SUD	0.93	c.4086delG	p.(Arg1362fs*12)	NR	0	NA
4	FH-SUD	0.98	c.1502A>G	p.(Asp501Gly)	P (67664)	0	NA
4	FH-SUD	1.00	c.2678G>A	p.(Arg893His)	P (67749)	0	NA
5	FH-UCA	0.71	c.2582_2583delTT	p.(Phe861fs*90)	P (201561)	0	Recurrent Dutch mutation
4	FH-UCA	1.03	c.3956G>T	p.(Gly1319Val)	LP/P (67838)	0.00001	NA
4	UCA	0.53	c.2635T>C	p.(Trp879Arg)	NR	0	NA
3	FH-UCA	0.58	c.4140_4142delCAA	p.(Asn1380del)	VUS (201570)	0	Classified as VUS, found in another Dutch UCA survivor. Absent from public databases. Variant p.Asn1380Lys reported in BrS (ClinVar ID 67863)
3	FH-SUD	1.06	c.-27_-15del	NA (no demonstrated effect on mRNA)	NR	0	Failed cosegregation analysis (2 noncarriers from same family have a positive ajmaline test)
3	UCA	1.60	c.5689C>T	p.(Arg1897Trp)	VUS (68003)	0.00010	Patient also carries the *DPP6* risk haplotype

*cDNA and protein changes refer to *SCN5A* RefSeq transcript NM_198056.2 and related protein NP_932173. †ClinVar accessed on November 8, 2016.

AF = allele frequency; BrS = Brugada syndrome; ExAC = Exome Aggregation Consortium (version 0.3.1); LP = likely pathogenic (class 4); NA = not applicable; NR = not reported; P = pathogenic (class 5); VUS = variant of unknown significance (class 3); other abbreviations as in Table 1.

583 tests were relatives of SUD or UCA cases, and 54 were UCA probands. Altogether, 482 families were evaluated (Figure 1A): 77% with only 1 individual, 17% with 2 individuals, and the remainder with 3 to 6 individuals.

YIELD AND SAFETY OF AJMALINE TESTING. Of the 637 tests, 89 had positive results (14.0%), and 543 had negative results (85.2%) for BrS. Five tests were prematurely terminated because of ventricular ectopy (3) or excessive QRS prolongation (2). The test result was positive in 13.4% of UCA/SUD family members and in 20.4% of UCA probands. As expected, UCA probands were more likely to have a positive ajmaline test result than the 66 healthy controls from a recently published cohort (20.4% vs. 4.5% respectively; $p = 0.009$) (17), confirming that an ajmaline-induced BrS pattern is associated with UCA.

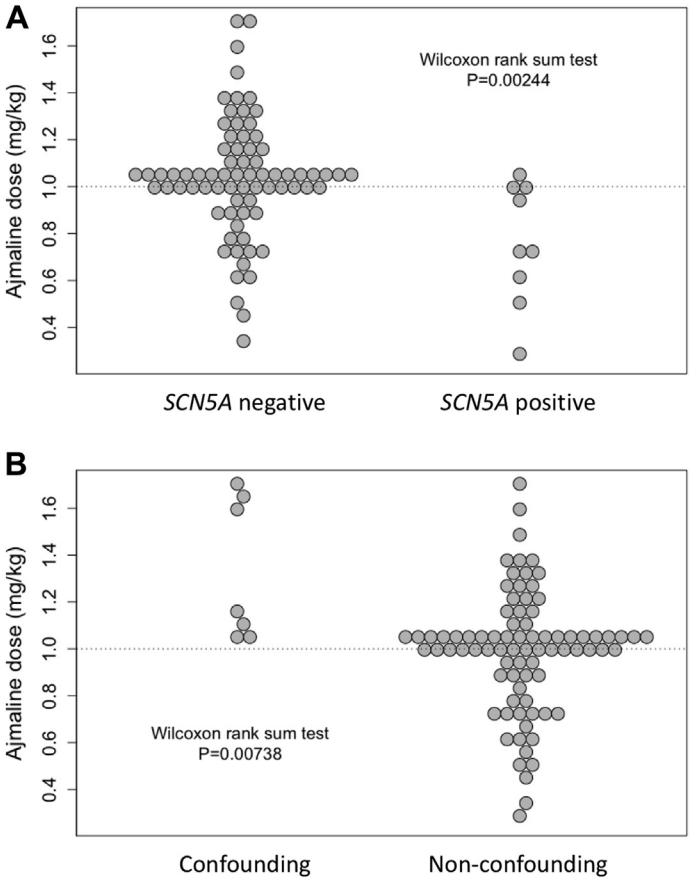
Of the 482 evaluated families, 88 had at least 1 individual with a positive ajmaline test result (18.3%; 95% confidence interval [CI]: 14.9% to 21.8%). Of interest, both parents of a 28-year-old man with SUD in his sleep had a positive ajmaline test result, explaining the discordance between the number of cases ($n = 89$) and families ($n = 88$) with a positive test result.

No major adverse events requiring or prolonging hospitalization were observed. Nonsustained ventricular tachycardia was induced in 2 patients (1 with positive and 1 with inconclusive test results).

GENETIC TESTING OF *SCN5A*. Sequencing of *SCN5A* was performed in 86 of the 89 ajmaline-positive cases (85 of 88 families). A pathogenic (class 5) or likely pathogenic (class 4) mutation in *SCN5A* was identified in 9 of the 86 cases (10.5%), and a variant of unknown significance was identified in 3 additional cases (Table 2, Figure 1B). The dose at which the ajmaline test result was positive was significantly lower in *SCN5A* mutation carriers (class 4 or 5) than in noncarriers (0.75 mg/kg [0.64 to 0.98 mg/kg] vs. 1.03 mg/kg [0.95 to 1.14 mg/kg], respectively; $p < 0.01$) (Figure 2A). These dose differences remained statistically significant ($p < 0.05$) after we excluded cases with a positive test result at >1.1 mg/kg.

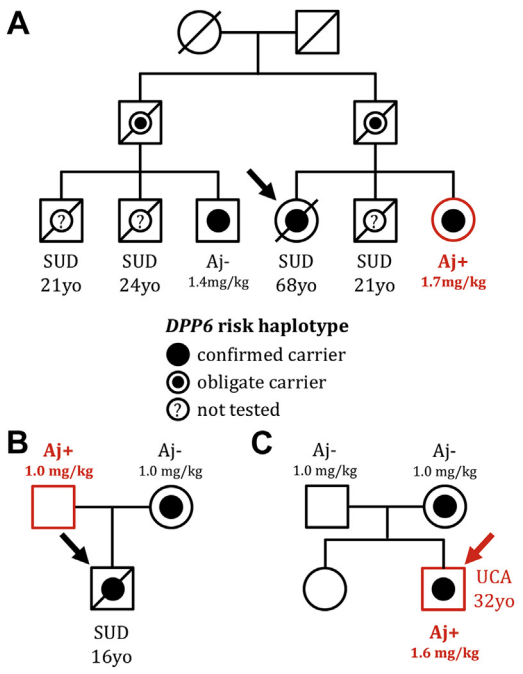
CONFOUNDING AJMALINE RESULTS. We systematically assessed for positive ajmaline responses that were unlikely to account for SUD/UCA in the family. Of the 88 families with a positive ajmaline test result, 36 underwent genetic testing of other genes in addition to *SCN5A* (Figure 1B). A pathogenic (class 5) mutation not causing BrS was identified in 5 of 36 families (14%): 3 with the idiopathic VF-associated *DPP6* risk haplotype (ClinVar ID 16794) (Figure 3), 1 with the *MYBPC3*:c.2373dupG (NM_000256.3; ClinVar ID 42619) (Figure 4), and 1 with the *RYR2*:p.Arg420Trp (NM_001035.2; ClinVar ID 201215) (Figure 5). Clinical details about these families appear in the legend of their respective

FIGURE 2 Ajmaline Dosages



(A) Ajmaline dose required to induce a type 1 BrS pattern in *SCN5A* mutation carriers was significantly lower than that in nonmutation carriers. Individuals not tested for mutations in *SCN5A* and those with variants of unknown significance were excluded. **(B)** Drug-induced type 1 BrS pattern in confounding cases was obtained at significantly higher doses (all >1 mg/kg) than that of other confounding ones. BrS = Brugada syndrome.

FIGURE 3 3 Families With the *DPP6* Risk Haplotype and a Positive Ajmaline Test Result

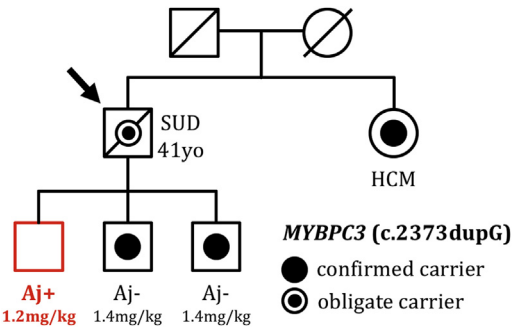


(A) Family investigated for recurrent SUD including a recent case of a 68-year-old member with normal autopsy findings (index, **arrow**). A positive ajmaline response at a high dose was observed in an asymptomatic sibling of the index case. Genetic testing identified the presence of a *DPP6* risk haplotype in multiple family members, including the index. **(B)** Family investigated for SUD of a 16-year-old male (index, **arrow**) showing a positive ajmaline response in the father. Familial genetic testing demonstrated that the deceased index case carried the *DPP6* risk haplotype, inherited from his mother. **(C)** Family investigated for resuscitated UCA in a 32-year-old male (index, **arrow**). Ajmaline testing was positive for BrS at a high dose in the index case but negative in both parents. The proband carried the *DPP6* risk haplotype inherited from his mother. Individuals with a positive ajmaline test are marked in **red**. Aj+ = positive ajmaline testing; Aj− = negative ajmaline testing; BrS = Brugada syndrome; SUD = sudden unexplained death; UCA = unexplained cardiac arrest.

pedigrees (Figures 3 to 5). None of these 5 families had a class 4 or 5 variant in *SCN5A*. In all these families, the treating physicians concluded that the most likely cause of cardiac arrest was not BrS, despite a positive ajmaline test in the proband or first-degree relative.

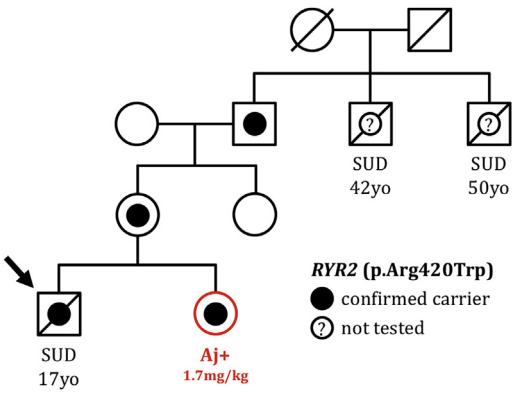
In 2 families with a positive ajmaline responses, the BrS pattern did not cosegregate with VF. The first case consisted of a young male with Klinefelter syndrome. At 16 years of age, he was resuscitated from VF, which occurred while he was walking after consumption of cannabis. The cause of VF remains unclear; cardiac magnetic resonance imaging, coronary computed tomography, epinephrine testing, and ajmaline testing (1.25 mg/kg) results were normal. An ajmaline test performed in his asymptomatic father showed a positive response at 1.08 mg/kg. Both the index and his father had negative results on genetic testing (47 arrhythmia gene panel). The second case was that of a 31-year-old man who was also resuscitated from VF that occurred while he was showering. Results of diagnostic evaluation, including a flecainide test at the referring hospital, were negative. Because he was not available initially for an ajmaline test, his 33-year-old asymptomatic sister underwent an ajmaline test, which had positive results at a dose

FIGURE 4 Family With a *MYBPC3* Mutation and a Positive Ajmaline Test



Pedigree of a family investigated for SUD in a 41-year-old male (index, **arrow**), where no autopsy was performed. One of his sons has a positive ajmaline response. A sister of the index was later diagnosed with hypertrophic cardiomyopathy, associated with the presence of an unequivocally pathogenic mutation in *MYBPC3* (c.2372dup). Two of three children of the index case carried the variant, making the index case an obligate carrier, likely accounting for the SUD. Abbreviations as in Figure 3.

FIGURE 5 Family Carrying a *RYR2* Mutation and a Positive Ajmaline Test Result



Pedigree of a family evaluated for recurrent SUD, including 1 in a 17-year-old boy during swimming (**arrow**, index case). Cardiac autopsy finding was normal, and initial sequencing of the *RYR2* mutation hotspots did not reveal a pathogenic mutation. His father (not shown) had a normal cardiac echocardiogram but had exercise-induced ventricular ectopy and a pathogenic *LMNA* mutation (p.Arg331Gln), which was absent in both his children. Clinical evaluations of the mother and sister of the index, including a baseline ECG and exercise testing, were normal, and both were discharged from clinical care. Nearly 10 years later, the sister of the proband, then 24 years old, had syncope during mild exercise that resulted in traumatic brain injury. Given the family history of SUD, ajmaline testing was performed and the result was positive at a high dose (1.7 mg/kg). The patient then received a diagnosis of BrS and received an implantable cardioverter-defibrillator. A few months later, she received an appropriate shock for fast ventricular tachycardia during exercise. Exercise testing revealed exercise-induced ectopy, and sequencing of all *RYR2* exons revealed a pathogenic mutation (p.Arg420Trp) inherited from the mother and which was also shown to be present in the index SUD case. Abbreviations as in Figure 3.

of 1.03 mg/kg. The index later also underwent ajmaline testing, and results turned out to be negative (1.01 mg/kg). Results of genetic testing in the index case were negative (43 arrhythmia gene panel), but genetic testing was not performed in his sister. In these 2 families, it remains unclear whether BrS was the cause of UCA (i.e., false-negative test in the index) or whether the ajmaline-induced BrS pattern in the first-degree relative was an incidental finding.

In summary, ajmaline testing was confounding in 7 of 88 families with a positive test result. Interestingly, positive responses in these cases occurred at higher doses than in other cases (1.18 mg/kg [1.06 to 1.64 mg/kg] vs. 1.01 mg/kg [0.88 to 1.08 mg/kg], respectively; $p < 0.01$) (Figure 2B). Concomitantly, the rate of confounding responses was significantly higher in positive ajmaline responses >1 mg/kg than in those ≤1 mg/kg (7 of 48 vs. 0 of 41, respectively; Fisher's exact test: $p = 0.014$). The fact that all these confounding positive responses occurred at a dose of >1 mg/kg (range: 1.03 to 1.69 mg/kg) arguably raises the possibility of dose-dependent false-positive responses.

DISCUSSION

Major study findings can be summarized as follows: 1) ajmaline testing induces a type 1 BrS pattern in 14% of individuals (18% of families); 2) an *SCN5A* mutation is identified in only 11% (9 of 86) of cases with a positive test; 3) confounding positive ajmaline tests results are observed in 7 of 88 of families where BrS is arguably not the primary cause of SUD/UCA, despite a positive ajmaline test result; and 4) confounding ajmaline responses occurred at doses >1 mg/kg (range: 1.03 to 1.69 mg/kg).

YIELD OF SODIUM CHANNEL BLOCKADE TESTING RESULTS IN UCA AND SUD. Testing for BrS by using a sodium channel blocker is recommended for the diagnostic evaluation of UCA and SUD (3). In SUD, the diagnostic yield is estimated to be 5% to 18% depending on cohort and drug used (highest for ajmaline; lowest for procainamide) (5,6,11,12), with <50 families included in each of these studies (Table 3). Regarding UCA, in a study (11) from the CASPER (Cardiac Arrest Survivors With Preserved

TABLE 3 Comparison of Studies of the Yield of Sodium Channel Blocker Testing

Country (Ref. #) Year	Population	n Tested (Unrelated)	Drug Used (Dose)	Positive, n (%)*	FP†	*SCN5A* Mutation/Tested (%)
Amsterdam (5) 2005	SUD	NR (43)	Flecainide	2 (5)	NR	1/2
United Kingdom (6) 2008	SUD + structurally normal heart on autopsy	50 (NR)	Ajmaline	6 (12)	NR	NR
Amsterdam (12) 2010	UCA	25 (25)	Flecainide or Ajmaline	3 (12)	NR	NR
	SUD	NR (39)		7 (18)		
United Kingdom (28) 2011	SUD	NR (NR)	Ajmaline (1 mg/kg)	49 (NR)	NR	5/28 (18)
Canada (11) 2014	UCA	115 (115)	Procainamide	7 (6)	2	1/7 (14)
	SUD	40 (NR)		2 (5)	0	0/1
Current study	UCA	54 (54)	Ajmaline	11 (20)	7	10/86 (12)
	SUD‡	583 (434)		78 (18)		

*For SUD = reported % refer to %families with at least 1 positive test, whenever available. †FP = confounding cases where BrS probably does not account for the UCA/SUD despite a positive test ("false-positive"). ‡For simplicity and to facilitate comparisons, family members of UCA cases are included together with SUD family members, as in Table 1.

Abbreviations as in Tables 1 and 2.

Ejection Fraction Registry) study, which included 115 UCA probands, procainamide challenge resulted in a positive response in 6.1% of cases.

In the present large cohort of 482 families, a positive ajmaline test was observed in 18% (95% CI: 15% to 22%) overall and in 20% (95% CI: 9% to 31%) of UCA survivors. These rates of positive testing are higher than those in previous cohorts (**Table 3**), likely because of systematic recording in high intercostal spaces as well as the use of more potent sodium channel blockade: systematic use of ajmaline and perhaps higher doses, the latter not being systematically reported in previous studies. In what proportion this reflects increased sensitivity versus decreased specificity is difficult to assess in the absence of a gold standard, as discussed below. Nevertheless, this large real-life study highlights the high yield of sodium-blocker drug testing in this population.

REVISITING THE UTILITY OF SODIUM CHANNEL BLOCKER TESTS IN UCA/SUD. The limited studies assessing the sensitivity and specificity of sodium channel blocker tests were reviewed in a recent editorial (13). In previous studies using *SCN5A* mutation status as the gold standard, the reported sensitivity was 80% (18,19), whereas a sensitivity of 100% was reported when the presence of an intermittent spontaneous type 1 pattern was used as the gold standard (20).

Although initial data for the specificity of sodium channel blocker testing were encouraging (20), accumulating data are conflicting. In a study from our group using *SCN5A* mutation status as the gold standard, the specificity of a flecainide test was estimated to be 80% (19). Interestingly, response to ajmaline does not seem to be specific to BrS. The induction of a type 1 BrS ECG pattern with sodium channel blockade is also seen in other conditions, such as 27% of patients with atrioventricular nodal re-entrant tachycardia (n = 96) (17), 18% of patients with myotonic dystrophy (n = 44) (21), and 16% of patients with arrhythmogenic right ventricular cardiomyopathy (n = 55) (22). Although these scientifically fascinating findings could make us long reflect about possible mechanistic links between these conditions and BrS (23,24), such high positive test rates are intriguing because BrS is classically considered to be a rare disease that is currently diagnosed solely by the presence of the ECG pattern (3). Of even more concern is the recent observation that 5% of healthy controls (N = 66) showed a positive response to ajmaline (13,17). These cases would be diagnosed with BrS according to the 2013 expert consensus statement (3) but not using previous diagnostic criteria (9,10) or the newly proposed criteria and Shanghai BrS score (25), all of which require additional clinical evidence of BrS.

In the current UCA/SUD study, considering the above-mentioned limited data for the questionable specificity of ajmaline testing, it is likely that some positive ajmaline responses are "false-positive" ones, suggesting that the actual proportion of SUD/UCA explained by BrS is lower than the high rate of positive ajmaline test results (18% in this cohort). Strictly speaking, in the absence of a gold standard, one cannot ascertain beyond doubt whether a positive test result is true-positive or false-positive. From a practical clinical standpoint, the objective of diagnostic evaluation of SUD/UCA cases is to identify family members at risk of SCD, and the prerequisite to do so is to correctly identify the cause of SUD/UCA in

the index case. In the 7 of 88 presented confounding cases, the cause of SUD/UCA is thought not to be BrS despite a positive ajmaline test in the proband or first degree relative. In such cases, the positive ajmaline response is a likely confounder when it comes to family screening. Importantly, a misdiagnosis in the evaluation of UCA/SUD could have important implications for family screening if the correct cause of arrest is overlooked. As such, alternative diagnoses should always be considered and excluded. This is particularly important when using potent sodium channel blockade (e.g., ajmaline at high doses). Further supporting the likelihood of false-positive ajmaline responses in some cases is the observation that *SCN5A* mutation rate among ajmaline positive cases is significantly lower than in a previously published international study (26), including 2,111 BrS probands (9 of 86 vs. 438 of 2,111, respectively; $p < 0.05$). Nonetheless, sodium channel blocker testing appropriately identifies cases with unequivocal BrS (e.g., those with an associated *SCN5A* mutation) and thus remains indicated in the diagnostic evaluation of UCA/SUD. Whether genetic testing alone could adequately identify family members at risk of SCD and what is the net clinical value of cascade sodium channel testing in predicting arrhythmic events are questions that remain unanswered. Large multicenter studies specifically addressing these issues are undeniably needed.

STUDY LIMITATIONS. Although the study included all consecutive ajmaline tests performed for UCA/SUD in a real-life setting, it suffers from some limitations due to its retrospective design. Detailed clinical information for SUD cases is not readily available, and autopsy was not performed or available in all cases. In addition, this design does not allow for the precise assessment of false positive rates in the context of UCA/SUD which would require systematic ajmaline testing in families with an alternative diagnosis.

Only ajmaline was used in this study. Applicability of results to less potent sodium channel blockers used to diagnose BrS is uncertain. Whether confounding responses could also be observed at doses <1 mg/kg is to be determined in larger cohorts. In our cohort, all confounding positive responses occurred at >1 mg/kg. Others have also observed such responses with the less potent drug procainamide (11).

It is not impossible that in some of the 5 families with an alternative genetic diagnosis, a BrS phenotype partly contributed to SUD/UCA in addition to the primary genetic disease. For instance, it is possible that BrS and *DPP6*-related idiopathic VF share some arrhythmogenesis mechanisms and may coexist (27). Nonetheless, only screening for BrS in members of these families would be inappropriate, since the risk of SCD is thought to be predominantly associated with the primary genetic defect (e.g., **Figure 5**, mutation in *RYR2*). In the 2 cases with ajmaline response-arrhythmia noncosegregation, it is not possible to determine whether the positive test is a false-positive one or the index case has a false-negative test. Given the mounting data on ajmaline's limited specificity (13), we suspect that false-positive tests are more likely to be seen.

CONCLUSIONS

We conclude that, in concordance with previous smaller studies, ajmaline testing in the diagnostic evaluation of families with UCA/SUD has a high yield for establishing a diagnosis of BrS, according to current criteria (3). However, confounding responses are not uncommon and may result in misdiagnoses, potentially hindering the identification of family member at risk of SCD. Given these results, clinicians are encouraged to consider all possible diagnoses in families with UCA/SUD and are strongly discouraged from using ajmaline at doses exceeding 1 mg/kg. Large outcome studies are needed to assess the clinical gain of ajmaline testing for the evaluation of UCA/SUD and in cascade screening.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: BrS is frequently diagnosed with ajmaline testing in families with SUD/UCA. A positive ajmaline test could be observed in cases where BrS is arguably not the cause of SUD/UCA, especially when using high doses. Other diagnoses should be considered and systematically excluded with appropriate tests in families with SUD/UCA.

TRANSLATIONAL OUTLOOK: Large multicenter studies addressing the net clinical benefit of ajmaline testing in SUD/UCA and cascade screening are needed.

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