Cardiac voltage-gated sodium channel mutations associated with left atrial dysfunction and stroke in children

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Aims	Cardiac atrial arrhythmias are the most common type of heart rhythm disorders. Its genetic elucidation remains challenging with poor understanding of cellular and molecular processes. These arrhythmias usually affect elderly population but in rare cases, young children may also suffer from such electrical diseases. Severe complications, including stroke, are commonly age related. This study aims to identify a genetic link between electro-mechanic atrial dysfunction and stroke in children.
Methods and results	In two unrelated boys of 11 and 14 years with both stroke and atrial arrhythmias, the clinical phenotype was determined through a complete physical examination, electrocardiogram (ECG), Holter ECG, and computed tomography. The genetic testing was performed on a large 95 genes panel implicated in myocardial electrical imbalance, using the next generation sequencing method. The panel also includes the genes usually associated with the development of cardiomyopathies. In one child, a left atrial dilation was observed. The 2nd boy suffered from atrial standstill. Both suffered from atrial bradycardia, flutter, and fibrillation. The complete genetic testing revealed the *SCN5A* c.3823G>A (p.D1275N) mutation in the first family, c.1141-2A>G and c.3157G>A (p.E1053K) mutations in the second family.
Conclusion	Our results strengthen the association between Na$_v$1.5 mutations and the occurrence of stroke in young patients. It emphasizes the need to look for atrial myopathy in the decision process for anticoagulation in young patients with atrial arrhythmic events.
Keywords	Atrial arrhythmias • Children • Stroke • Voltage-gated sodium channels • Fibrosis

Introduction

Near 50% of sudden death can be attributed to atrial dysfunctions.[1] Genetic of cardiac atrial-specific arrhythmias remains challenging with only poor understanding of cellular and molecular processes. Increasing knowledge about genetics of atrial arrhythmias is obtained through several studies focused on large genetics approaches based on important patients cohorts. Ion channels constitute a group of multiple proteins where mutations are associated with increased risks of atrial arrhythmias.[2]

The heart contraction responsible for the appropriate blood circulation is governed by a specific electrical activity called action potentials (AP). Voltage-gated sodium channels (Na$_v$) are large transmembrane proteins responsible for the initiation of AP. Ten different subtypes are known in humans (Na$_v$1.1–1.9 and Na$_x$). In the heart, Na$_v$1.5 channels, encoded by *SCN5A*, mediate the upstroke of cardiac AP through the rapid entry of Na$^+$ ions form outside the cells.[3] Na$_v$1.5 channels are 2016 amino acids proteins composed of 24 transmembrane segments (TM) organized in 4 homologous domains (DI–DIV) each containing 6 TM.[4] The assembly of S5–S6 segments of the four domains forms the

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What's new?

- Severe atrial dysfunction is an increased risk factor for stroke in CHADS$_2$-VASc score 0 children.
- Specific SCN5A mutations may be associated to an increased risk for stroke.

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physiological pore of the channel, also called the pore domain (PD) ensuring the flow of Na$^+$. The voltage sensitivity is given by the S1–S4 TM of each domain that form the voltage sensitive domain (VSD). The structure of VSDs is modulated by changes in the membrane potential and these re-arrangements open the PD.[5] Due to the crucial physiological role of Na$_v$1.5 channels, their dysfunctions are known to be associated with a large spectrum of cardiac disorders. Na$_v$1.5 dysfunctions are primarily known to cause electrical disturbances such as Brugada syndrome (BrS), Type 3 long QT syndrome (LQT3), progressive cardiac conduction defect (PCCD), or also sick sinus syndrome (SSS).[3,6–8] SCN5A mutations have also been associated with atypical clinical phenotype combining mixed arrhythmias and cardiac dilatation.[4,9–11]

Atrial arrhythmias include hyperexcitability (tachycardia, fibrillation, and flutter) and/or hypoexcitability (bradycardia, standstill). Atrial arrhythmias, mainly atrial fibrillation (AF), usually affect elderly population. In contrast, Na$_v$1.5 mutations associated with such rhythm disturbances are usually described in younger patients.[9,12]

In this study, we report two unrelated patients carrying SCN5A mutations and suffering from atrial arrhythmias associated with stroke before the age of 15.

Methods

Clinical investigation

The local ethics committee approved the study protocol. The study was conducted according to the principle of the declaration of Helsinki. Informed consents were obtained for all cases. After their identification in the rhythm disorders hospital department, patients were oriented towards the hereditary heart rhythm disturbances reference centre ('Centre de référence des troubles du rythme cardiaque héréditaires, CERA'). The clinical investigation included a medical history review, physical examination, 2D echocardiography, 12-lead electrocardiography (ECG), and 24 h Holter ambulatory ECG. When required, computed tomography (CT-scan) was also performed.

Genetic testing

Next Generation Sequencing sequencing, by a custom design and a strategy based on SeqCap EZ Solution-Based Enrichment strategy (Roche NimbleGen, Madison, WI, USA) was used to test genomic DNAs. Forty-seven cardiomyopathy causing genes and 48 arrhythmias syndrome causing genes (Supplementary material online, Table S1) were tested as previously reported.[13,14]

Results

Genetic screening

A genetic evaluation was performed on two young unrelated patients from two families (F1 and F2) suffering from atrial arrhythmias and stroke (Figure 1). To get better insights into their genetic profile and the potential multi-genic origin of their cardiac dysfunction, the sequencing of a large gene panel involved in sudden cardiac death was performed. Genes tested are recapitulated in Supplementary material online, Table S1 and include 95 genes usually associated with multiple cardiac defect from morphological defects to rhythm disturbances.

In the first patient, the genetic testing revealed a SCN5A heterozygous missense mutation (NM_198056.2: c.3828G>A, D1275N) (Figure 1). This mutation affects an amino acid located in the third voltage sensor of the protein (Figure 1).

In the second patient (F2), compound SCN5A mutations has been found (Figure 1). The first results in a heterozygous mutation (NM_198056.2: c.1141-2A>G) affecting the intron 9 acceptor splice site (Figure 1). The second SCN5A mutation in F2 consists in a homozygous missense mutation (NM_198056.2: c. 3157G>A, E1053K) (Figure 1). This mutation affects an amino acid located in the intracellular loop linking the second and third domain of Na$_v$1.5. Other genetic variations have been detected but classified as non-pathological or as variant of unknown significance, according to the American College of Medical Genetics and Genomics and Association of Medical Pathologists guidelines[14] (Supplementary material online, Table S2).

Clinical evaluation

An eight-year-old male patient (F1—III.4) presented in 2013 with asymptomatic junctional bradycardia (57 bpm) and an incomplete right bundle branch block. Sinus bradycardia and paroxysmal atrial flutter were also present (Figure 2). The clinical characteristics are recapitulated in Table 1. The bradycardia was confirmed through a 24 h Holter recording. His echocardiography was normal. He was admitted in 2015 for pre-syncope ascribed to bouts of atrial flutter (Figure 2). A right atrial flutter ablation was performed. One year later, the patient experienced an acute stroke with right-sided hemiplegia and aphasia. The cardiac CT-scan identified left atrial dilatation characteristic of atrial myopathy (Figure 3). No blood coagulation system defects were detected, including the absence of Leiden mutation in gene coding for factor V and mutation FII g.20210G>A in genes coding for prothrombin.

His mother (F1—II.5) was asymptomatic and did not carry the mutation, his father (F1—II.4) was an asymptomatic carrier. His ECG displays a slight PQ segment depression (Figure 2).

The 2nd proband, a 9-year-old male patient (F2—III.1) presented in 2003 with bradycardia (47 bpm) associated with atrial premature beats and a 2:1 sinoatrial bloc. The clinical characteristics are shown in Table 1. Junctional rhythm and long sinus pauses (>7 s) were detected in 2005. The bradycardia was confirmed through a 24 h Holter recording. The same year, he was admitted for a pre-syncope, preceded by palpitations attributed to sinoatrial node dysfunction. Early 2006, a pacemaker was implanted because of an exercise poor rhythm adaptation (Figure 4). Six months after the implantation, the patient complains of palpitations. Twenty-four hours Holter ECG monitoring recorded atrial flutter episodes. Two years later, he was admitted for acute stroke while the transthoracic echocardiography showed a normal heart without patent foramen ovale. No blood coagulation system defects were detected. The next year, because a 24 h Holter ECG revealed more than 200 AF episodes, fluindione
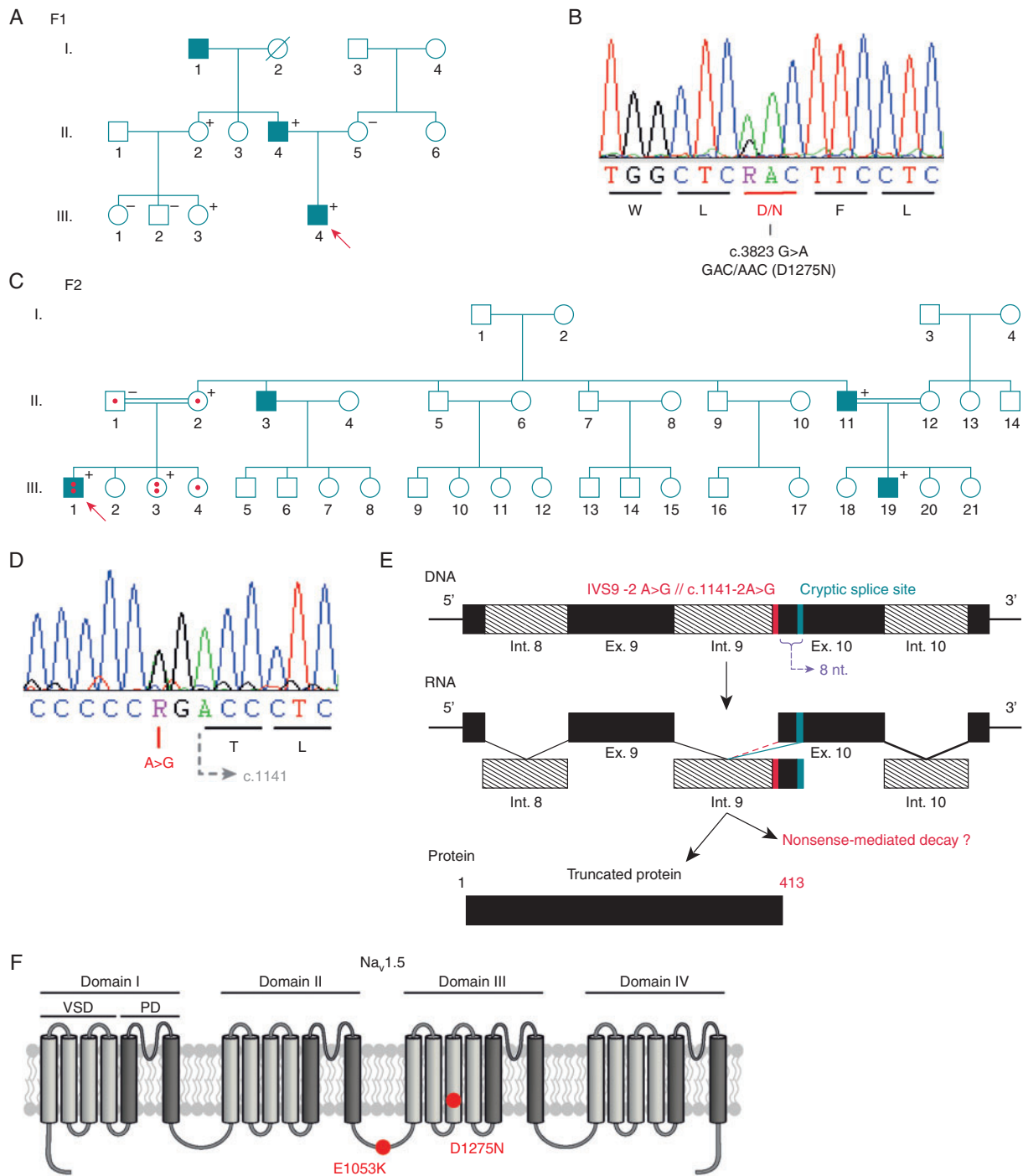
Figure 1 Family pedigrees and genetic analysis. For family pedigrees, females are represented by circles and males by squares. When known, a '+' indicates a mutation carrier while a '−' indicates a non-mutation carrier. Affected patients are represented in black while non-affected or unknown status are depicted in white. (*A*) Partial pedigree of family 1, '+' indicates c.3823G>A *SCN5A* mutation. (*B*) The genetic analysis revealed a *SCN5A* heterozygous missense mutation (NM_198056.2: c.3823G>A). (*C*) Partial pedigree of family 2, '+' indicate c.1141-2A>G *SCN5A* mutation, single and double red circles indicate, respectively heterozygous and homozygous c.3157G>A *SCN5A* mutation. (*D*) *SCN5A* heterozygous mutation in F2 affecting the intron 9 acceptor splice site (NM_198056.2: c.1141-2A>G). (*E*) Schematic 2D representation of the DNA and RNA showing the location of the mutation. Potential consequences on the protein are also proposed. (*F*) Schematic 2D representation of the 24-TM of Na$_v$1.5 organized in four homologous domains (DI–DIV), each containing 6 TM. The S1–S4 segments of each domain form the VSD (light grey) while the assembly of the S5–S6 segments (dark grey) form the pore of the channel. The locations of the D1275N and E1053K mutations are indicated by a red circle. PD, pore domain; TM, transmembrane segments; VSD, voltage sensitive domain.
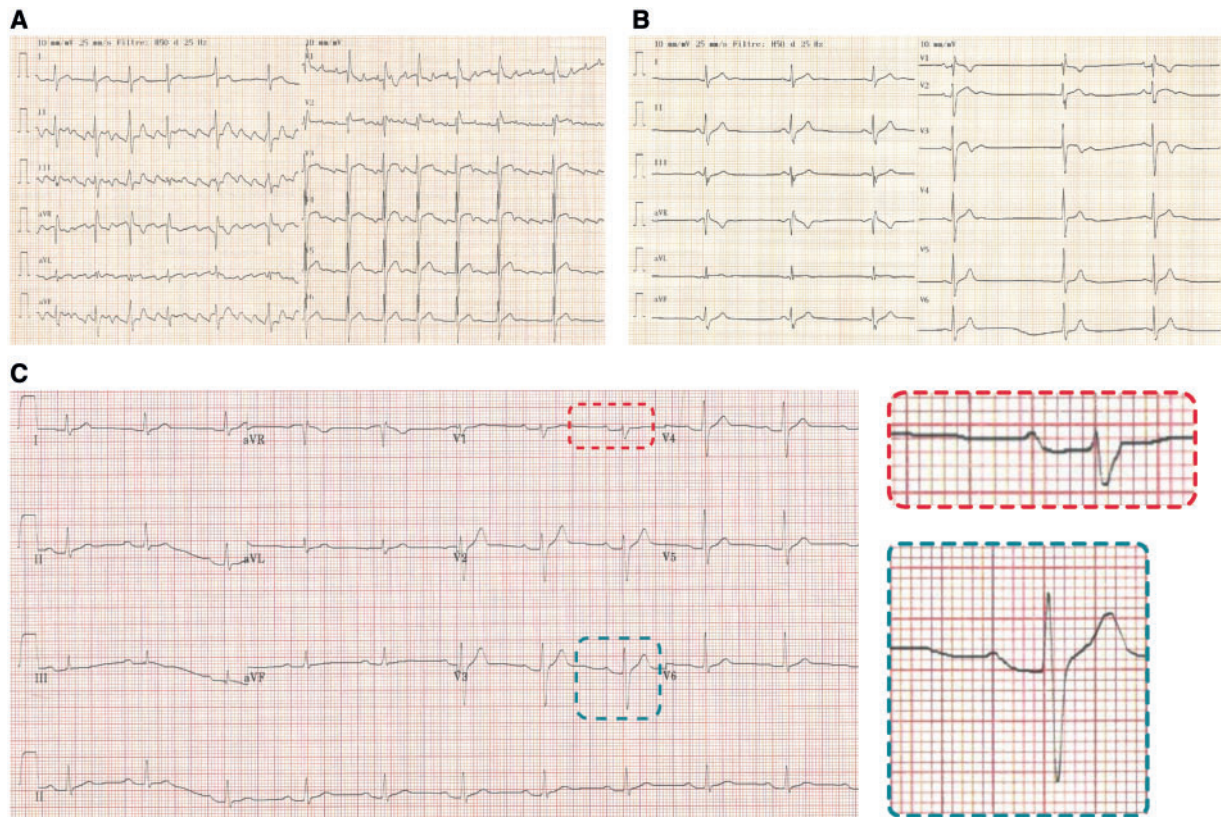
Figure 2 Electrocardiogram characteristics of F1—III.4 patient. 12-lead ECG recorded at rest for the proband F1—III.4 showing flutter episodes (A) and slow junctional rhythm associated with incomplete right bundle branch block (B). (C) 12-lead ECG recorded at rest for the F1—II.4 (proband's father). Dashed red and blue lines depict magnification of clear PQ depression. ECG, electrocardiogram.

Table 1   Clinical characteristics

Proband	Age at first presentation (years)	Age at stroke (years)	HR (bpm)	P-wave duration (ms)	Flutter episodes	AF episodes	Pauses > 5 s	Heart block	APB	PM	*SCN5A* mutations
F1—III.4	8	11	38	150	+	−	+	RBBBi, BBB	+	−	c. 3823G>A (D1275N)
F2—III.1	9	14/16	40	160	+	+	+	AVB	+	+	c. 1141-2 A>G and c. 3157 G>A

AF, atrial fibrillation; APB, atrial premature beats; AVB, atrio-ventricular block; BBB, bundle branch block; HR, slower heart rhythm; PM, pacemaker; RBBBi, incomplete right bundle branch block.

and acebutolol were prescribed. During the pacemaker replacement the same year, the atria could not be stimulated. Until its last examination in 2013, several AF and flutter episodes were recorded and the patient became pacemaker dependent.

The proband (F2—III.1) was part of a large family, but most of them refused clinical or genetic diagnosis. The proband has three clinically unaffected sisters, as same as his father. Both parents carry the heterozygous *SCN5A* c.3157G>A (E1053K) mutation. One of his sister (F2—III.3) presents a genotype identical to his affected brother. Additionally to the heterozygous *SCN5A* c. 3157G>A (E1053K) mutation, his mother (F2—II.2) also carries the *SCN5A* c.1141-2A>G mutation and is known until her 16 to be affected by sinus node

dysfunction. She received a pacemaker in 2005 at the age of 37. A proband's uncle (one brother of his mother, F2—II.11) was known since he is 15 to suffer from bradycardia with alternating sinus and junctional rhythm. Five years later, Holter ECG revealed more than 2000 pauses >4 s and atrial tachycardia. He refused clinical examination. His son (F2—III.19) is also a c.1141-2A>G mutation carrier.

Discussion

Na$_v$1.5 channels encoded by *SCN5A* gene play a critical role in the excitation process underlying the heart contraction.[3] Dysfunctions of
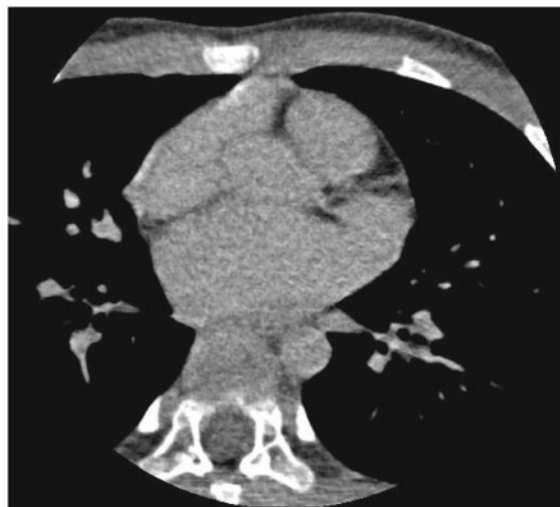
Figure 3 Heart morphology of F1—III.4 patient. Computed tomography-scan without contrast injection. Frank dilation of the left atria corresponding to an atrial myopathy. The patient was in sinus rhythm at the time of the imaging.

these channels is well known to cause heart defects, mainly characterized by rhythm disturbances.[3,8] Here, we report two unrelated families suffering from severe atrial rhythm disturbances. Both probands (F1—III.4 and F2—III.1) suffered from stroke and both carry *SCN5A* mutations. The D1275N mutation has been identified in the F1. This mutation has previously been described in several families and studied in diverse expression or animal systems including *Xenopus* oocytes, mammalian cells, transgenic zebrafish, and mice.[9,12,15–17] The clinical phenotype associated with this mutation concern several heart dysfunctions: atrial dysfunctions (including sick sinus syndrome, atrial standstill, and atrial arrhythmias), conduction system disorders, and dilated cardiomyopathy. Functionally, the results concerning the biophysical defect due to the D1275N mutation are still controversial and seem to largely depend on the evaluation system.[16,17] The first characterization in *Xenopus* oocytes revealed that the biophysical properties of D1275N mutant channels were not affected (in presence of the 1 subunit). In a comparative study between expression in *Xenopus* oocytes or HEK 293 cells, Gui *et al.*[16] described different biophysical defects according to the expression system. Furthermore, they also reported a drastic current decrease and activation/inactivation shifts for D1275N mutant channels. Such defects were less pronounced in HEK cells.[16] To get further insights in the biophysical characteristics of D1275N channels, Watanabe *et al.*[17] generated a Na$_v$1.5/D1275N transgenic mouse and compared their results with channels expressed in Chinese Hamster Ovary (CHO) or tsA201 cells. They reported similar biophysical properties of wild type and D1275N channels when expressed in CHO cells, whereas mice cardiomyocytes displayed a drastic decrease in Na$^+$ current density, an increase in persistent Na$^+$ current and defects in their voltage dependence.[17] The S1–S4 segments of each domain form a VSD, responsible for the voltage-sensing process.[4,5] The voltage-sensing process is ensured by the movement of positives charges (arginine and/or lysine) located on the S4 segment. The gating charge

transfer centre (GCTC) located on the surrounding S1–S3 segment constitutes a specific structure dedicated to the stabilization of these positives charges on the S4 segment.[4,5] The D1275 is part of the GCTC and this particular location in the VSD also raises the possibility that the D1275N mutation could open a gating pore by disrupting the interactions between the S4 segment and the surrounding GCTC. However, while the creation of a gating pore has been described with other Na$_v$1.5 mutation causing dilated cardiomyopathy, such hypothesis has never been tested with this specific D1275N mutation.[10,11,18] Since dilated cardiomyopathy is a phenotype previously associated with the Na$_v$1.5 D1275N mutation, a close follow-up of this family is a pre-requisite.

The genetic analysis of F2 revealed an atypical mutation on the *SCN5A* gene (c.1141-2A>G or IVS9-2A>G). This mutation, never previously described, does not affect the *SCN5A* coding sequence but disrupt an acceptor splicing site before exon 10 (*Figure 1*). This disruption would most probably be balanced by the activation of a cryptic splice site eight nucleotides later, leading to a frameshift. However, even after considering this cryptic splice, a stop codon occurs rapidly, resulting in the best case to a truncated protein of 413 amino acids (*Figure 1*). This would most probably result in either a non-functional truncated Na$_v$1.5 protein or in the activation of the non-sense mediated decay and thus the RNA degradation. This would finally lead to haploinsufficiency or to even lower Na$_v$1.5 levels if dominant negative process occurs as shown for other Na$_v$1.5 mutations.[19] However, such channel defect is usually associated with the development of Brugada syndrome (BrS) where decreased Na$^+$ current also causes ventricular arrhythmias.[3,6] A second homozygote *SCN5A* mutation has also been identified (c.3157G>A, p.E1053K). The E1053K mutation has previously been described, and its molecular consequences have been studied.[20] The initial study of molecular consequences described a destabilized interaction between Na$_v$1.5 channels and ankyrin G proteins.[20] This would result in both drastic current density reduction and in modification of gating properties. A recent study describes a large family with patients carrying the heterozygous or homozygous E1053K mutation. Several of them are completely free of cardiac symptoms (including the patient with the homozygous mutation). This observation strongly questions the pathological classification of this *SCN5A* variation. Furthermore, this is in accordance with our study showing that most of E1053K carriers do not present clinical symptoms.

It is also important to note the incomplete penetrance in both families since all genetic carriers do not express the pathological phenotype. Although this phenomenon is already described for many *SCN5A* mutations, further studies are clearly required to understand this process.

Atrial arrhythmias have rarely been associated with Na$_v$1.5 mutations, and this link remains highly intriguing. Na$_v$1.5 channels are expressed in both atria and ventricles, and it is hard to explain that a defect would cause rhythm disturbances only at the atrial level. Other mutations targeting atrial-specific genes could explain this susceptibility while protective ventricular-specific variations could also explain this phenomenon. Large gene panels as tested in our study revealed no other pathogen or likely pathogen variations. This consequently precludes any effects of other genes currently known as potentially detrimental. However, this does not eliminate that other genes so far not associated with cardiac dysfunctions could
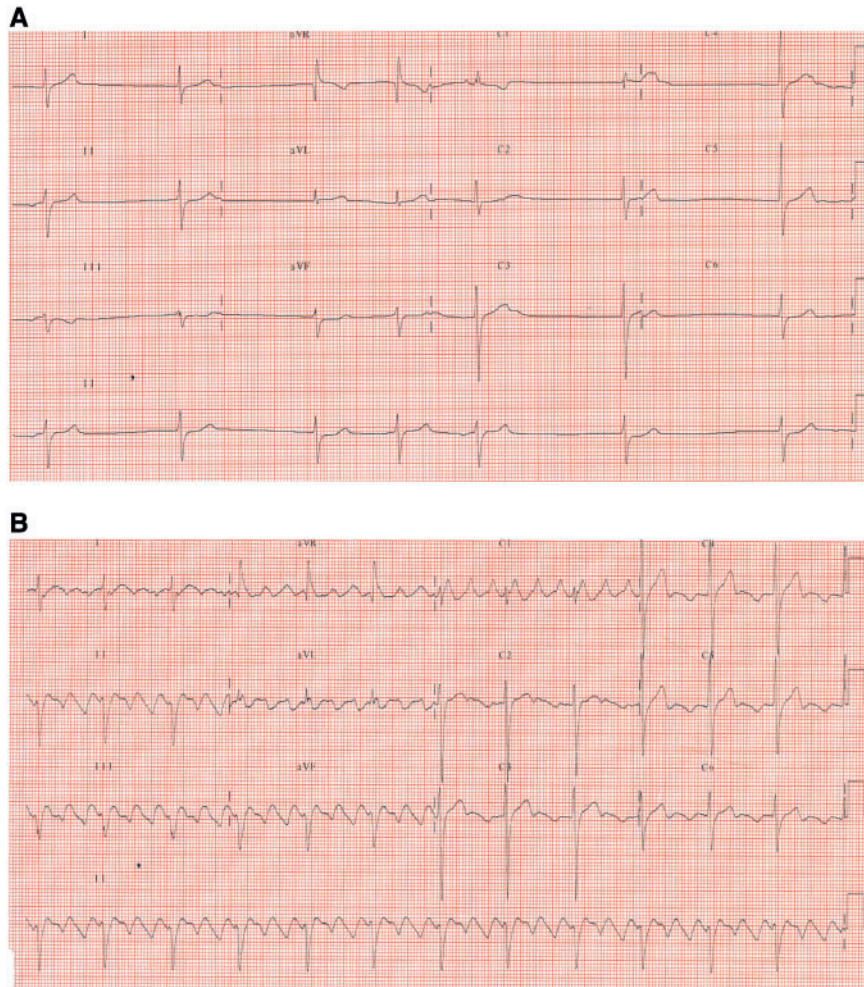
Figure 4 Electrocardiogram characteristics of F1—II.4 patient. Twelve-lead ECG recorded at rest for the proband F2—III.1 showing a slow junctional rhythm (*A*) and a flutter episode (*B*). ECG, electrocardiogram.

participate to the pathological phenotype. Furthermore, genetic polymorphisms without direct pathological consequences when considered alone could also modulate and favour the disease expression when combined to other mutations. As genotyping techniques evolve, more and more genes can be routinely evaluated potentially leading to future identification of protective, pathogenic, or modifier genetic variants.

In our two patients, there were evidence for atrial myopathy. The CT-scan identified fibrosis in one (F1—III.4) and in the second patient (F2—III.1) electrical capture of atrial muscle was impossible. Atrial fibrosis isolating the myocardium could potentially explain the impossible atrial capture. In both cases, the atrial endothelium could thus have lost its antithrombotic properties, favouring atrial thrombi formation. A third clue that favours a link between the variant and the atrial myopathy may come from the F1—II.4 patient who is asymptomatic while his ECG displays a PQ segment depression (*Figure 2*). Such depression would rely on atrial repolarization defect. In another study, a young patient, carrying a *SCN5A* mutation, was reported with similar atrial electrical disturbances but without stroke.

Interestingly, he has no functional or morphological evidence for atrial myopathy.

This study thus highlights the occurrence of stroke in very young patients carrying Na$_v$1.5 mutations. Even if more than 300 Na$_v$1.5 mutations have been identified only few of them are associated with stroke at this very young age.[9,12,15] Alternating atrial bradycardia and tachycardia may be a risk factor for stroke, but it is still debated. Many other Na$_v$1.5 mutation leading to similar atrial arrhythmias are not associated with cerebrovascular accidents.[15] Our study thus widens the spectrum of Na$_v$1.5 mutations identified in young patients with strokes, with the description of the novel *SCN5A* mutation (c.1141-2A>G or IVS9-2A>G). Besides the few Na$_v$1.5 mutations associated with stroke, this study is the third report of Na$_v$1.5/D1275N associated with stroke.[9,12] This intriguing association, as pointed out by Laitinen-Forsblom *et al.*,[12] requires further mechanistic studies. Such dramatic events should be carefully monitored, and our results raise the possibility to carefully consider preventive anticoagulation strategy in very young patients displaying these specific *SCN5A* mutations.

Conclusion

We describe two French families in which two young Na$_v$1.5 mutation carriers suffering from similar electrical atrial dysfunctions and severe stroke. Our report widens the phenotypic spectrum of Na$_v$1.5 mutations, highlighting that stroke can be associated with such mutations. Furthermore, the association of specific Na$_v$1.5 mutation with the occurrence of a stroke is even more suggested since this study constitutes the third report of a patient carrying the Na$_v$1.5 D1275N mutation with stroke.

Supplementary material

Supplementary material is available at *Europace* online.

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Conflict of interest: none declared.

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