**MOGI**: A New Susceptibility Gene for Brugada Syndrome

**Running title**: Kattyynarath et al., Dominant negative MOGI mutation Brugada Syndrome

Darouna Kattyynarath, PharmD, Svetlana Maugrené, BS, Nathalie Neyroux, PhD, Elise Balse, PhD, Carole Ichai, MD, Isabelle Denjoy, MD, PhD, Gilles Dilanian, BS, Raphaël P. Martins, MD, Véronique Fressart, MD, PhD, Myriam Berthet, BS, Jean Jacques Schott, PhD, Antoine Leenhardt, MD, Vincent Probst, MD, PhD, Hervé Le Marec, MD, PhD, Bernard Hainque, PharmD, PhD, Alain Coulombe, PhD, Stéphane N. Hatem, MD, PhD, Pascale Guicheney, PharmD, PhD.

1 INSERM, UMRS 956, Paris; 2 Université Pierre et Marie Curie, Paris; 3 AP-HP, Groupe Hospitalier Pitié-Salpêtrière, Service de Biochimie Métabolique, UF Cardiogénétique et Myogénétique Moléculaire et Cellulaire, Paris; 4 Hôpital Saint Roch, Service de Réanimation Médico-Chirurgicale, Nice; AP-HP, Hôpital Lariboisière, Service de Cardiologie, Université Denis Diderot, Paris; 5 INSERM, UMRS 915, Nantes; Université de Nantes, Nantes; 6 Centre Hospitalier Universitaire Paul Brousse, APHP, Centre Hospitalier Universitaire de Paris, Paris; 7 Université de Nantes, Nantes; 8 CHU Nantes, Institut du thorax, Service de cardiology, Nantes; Université Paris René Descartes, UFR des Sciences Pharmaceutiques et Biologiques, Paris, France.

**Corresponding Author:**
Dr Pascale Guicheney
UMRS-956, Faculté de Médecine Pierre-Marie Curie
91 boulevard de l’Hôpital
75013 Paris, FRANCE.
Tel: 33-1-40 77-96-49
Fax: 33-1-40 77-96-45
E-mail: pascale.guicheney@upmc.fr

Abstract:

**Background** - Brugada syndrome (BrS) is caused mainly by mutations in the SCN5A gene, which encodes the α-subunit of the cardiac sodium channel, Na\textsubscript{v}1.5. However, approximately 20% of probands have SCN5A mutations, suggesting the implication of other genes. MOG1 was recently described as a new partner of Na\textsubscript{v}1.5, playing a potential role in the regulation of its expression and trafficking. We investigated if mutations in MOG1 could cause BrS.

**Methods and Results** - MOG1 was screened by direct sequencing in BrS and Idiopathic Ventricular Fibrillation (IVF) patients. A missense mutation p.Glu83Asp (E83D) was detected in a symptomatic female with a type-1 BrS ECG, but not in 281 controls. Wild type (WT) and mutant E83D MOG1 were expressed in HEK Na\textsubscript{v}1.5 stable cells and studied using patch-clamp. Over-expression of WT MOG1 alone doubled sodium current (I\textsubscript{Na}) density compared to control conditions (p<0.01). In contrast, over-expression of mutant E83D alone, or E83D+WT, failed to increase I\textsubscript{Na} (p>0.05), demonstrating the dominant negative effect of the mutant. Microscopy revealed that Na\textsubscript{v}1.5 channels failed to properly traffic to the cell membrane in the presence of the mutant. Silencing endogenous MOG1 demonstrated a 54% decrease in I\textsubscript{Na} density.

**Conclusions** - Our results support the hypothesis that dominant negative mutations in MOG1 can impair the trafficking of Na\textsubscript{v}1.5 to the membrane, leading to I\textsubscript{Na} reduction and clinical manifestation of BrS. Moreover, silencing MOG1 reduced I\textsubscript{Na}, demonstrating that MOG1 is likely to be important in the surface expression of Na\textsubscript{v}1.5 channels. Altogether, our data support MOG1 as a new susceptibility gene for BrS.

**Key words:** Brugada syndrome, sudden cardiac death, arrhythmia, MOG1, SCN5A, Sodium channel, protein trafficking
Introduction

Brugada syndrome (BrS) is a rare autosomal dominant clinical entity (OMIM 601144) characterized by ST-segment elevation in right precordial leads \(^1\text{–}^3\) but without structural heart disease. Patients may develop syncope, ventricular arrhythmia, and sudden cardiac death (SCD) resulting from episodes of ventricular fibrillation. In 1998, Chen et al. reported the first BrS mutations in SCN5A, the gene encoding the \(\alpha\) subunit of the cardiac sodium channel \(\text{Na}_\text{v}1.5\) \(^4\). However, these mutations occur in approximately only 20% of affected individuals \(^3\text{,}^5\), suggesting involvement of other genes. The \(\text{Na}_\text{v}1.5\) \(\alpha\) subunit interacts with several proteins such as the \(\beta\) \(1\) sodium channel subunit, ankyrin G, caveolin 3, or syntrophin \(^6\) which are all implicated in channel function and localization. Mutations or some of these partners have already been linked to BrS. One recent example has been reported by Watanabe and co-workers, who described mutations in the \(\text{SCN1B}\) gene encoding the \(\beta\) 1 subunit, which affected the trafficking of \(\text{Na}_\text{v}1.5\) to the cell surface, and which co-segregated with BrS and atrio-ventricular conduction abnormalities \(^7\).

Human MOG1 (Multicopy suppressor Of Gsp1 \(^8\)) is a small ubiquitous 28 kDa protein, encoded by the \(\text{MOG1}\) gene \(^9\). In yeast, MOG1 was characterized as a nuclear protein that regulates nuclear import and export by binding to the RanGTP complex and stimulating GTP release, and thereby mediating the Ran GTP gradient from cytoplasm to nucleus \(^8\text{,}^10\text{,}^11\). Recently, MOG1 was described as a new partner of \(\text{Na}_\text{v}1.5\). MOG1 interacts with \(\text{Na}_\text{v}1.5\) via its intracellular loop between domains II and III, and co-localizes with the channel at intercalated disks in mouse ventricular myocytes. Moreover, SCN5A and MOG1 co-expression results in an increase in the sodium current without changes in biophysical properties of the channel, suggesting that MOG1 regulates the surface expression of \(\text{Na}_\text{v}1.5\) \(^12\).
In this study, we tested whether MOG1 could be a relevant candidate gene within a BrS SCN5A negative cohort, and we report that a missense mutation was associated with a pro-arrhythmic phenotype in a symptomatic BrS patient. Furthermore, we investigated whether this missense mutation could affect Na,1.5 activity, and provide molecular and clinical evidence that the MOG1 loss of function mutation is linked to BrS physiopathology.

Materials and methods

Patients

Patients were diagnosed with having either BrS or idiopathic ventricular fibrillation (IVF), based on the established criteria. Diagnosis was based on 12-lead ECG analysis, personal history of syncope or resuscitated SCD, and family history of SCD or arrhythmic events. BrS patients (n=246) displayed a type-1 ECG pattern (ST segment elevation ≥ 2 mm in one or more right precordial leads) either spontaneously or following a sodium blocker challenge test (ajmaline). Patients with no clear established BrS phenotype but displaying ventricular fibrillation with or without documented resuscitated SCD were categorized as IVF patients (n=24). Structural heart disease was excluded by echocardiography. Related family members were known to be affected in 11 percent of the cases, suggestive of familial disease. All patients had previously given informed consent for genetic testing and were negative for SCN5A and SCN1B mutations.

MOG1 Mutational analysis

DNA was extracted from peripheral blood leukocytes according to standard procedures. Screening for mutations in the MOG1 gene (GenBank accession number NM_016492) was performed by genomic DNA amplification of all exons and splice junctions (Primers and Polymerase Chain Reaction conditions are available on request). PCR products were directly sequenced with the Big Dye Terminator v.3.1 kit (Applied Biosystems®). Sequencing was
performed on the ABI PRISM 3730 automatic DNA sequencer (Applied Biosystems®). Variants and mutations were identified by visual inspection of the sequence with Seqscape® software (Applied Biosystems®). We also genotyped MOG1 in 281 healthy Caucasian subjects as a control population, and 48 BrS patients harboring SCN5A mutations.

**MOG1 cloning and mutagenesis**

The pcDNA3.1C-MOG1 plasmid was provided by Q. Wang from the Cleveland Clinic Foundation. MOG1-Enhanced Green Fluorescent Protein (EGFP) fusion constructs (in the pEGFP-C3 vector, BD Biosciences Clontech®) were designed to trace the trafficking of MOG1 in cardiomyocytes by microscopy. Constructs with the pIRES bi-cistronic vector and EGFP (pIRES2-EGFP, BD Biosciences Clontech®) were generated for patch clamp assays. The mutant construct E83D MOG1 was prepared using the QuickChange IIXL Site-Directed Mutagenesis Kit (Stratagene®) according to the manufacturer’s instructions. Inserts were subsequently sequenced to ensure that there were no other mutations.

**Cell preparation and transfection of HEK293 Na,1.5 stable cell line**

The Human Embryonic Kidney (HEK) 293 cell line stably expressing human Na,1.5 was cultured in Dulbecco’s modified Eagle’s medium (Gibco, Invitrogen®) supplemented with 10% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 25 μg/ml Zeocin (Invitrogen®) at 37°C in a 5% CO2 incubator. For over-expression assays, cells were transiently transfected at 80% confluence using a liposomal approach (Lipofectamine 2000, Invitrogen®). The amounts of each constructs used for transfection of a 6-well plate well were: 1 μg of wild type (WT) MOG1, 1μg of mutant E83D, or 1 μg of empty vector pIRES-EGFP as a negative control. To simulate allelic heterozygosity, cells were transfected with an equimolar mix of 0.5 μg WT / 0.5 μg mutant E83D. For silencing assays, cells were also co-transfected at 80% confluence with either 12 nM scrambled siRNA (siSCR) Cy3™ tagged as negative control, or a mix of 10 nM anti human MOG1 siRNA (siMOG1) / 2nM of Cy3™.
tagged scrambled siRNA, both purchased from Ambion®/Applied biosystems® (siMOG1 sequence reference: ID# s195167). Western blotting confirmed the efficacy of silencing.

**Electrophysiological measurements**

Forty-eight to 72h after transfection, cells displaying green fluorescence (GFP) for expression assays, or red fluorescence (Cy3™) for silencing experiments were selected for electrophysiological measurements. Cells were bathed in an extracellular Tyrode solution containing (in mM): 135 NaCl, 4 KCl, 1 NaH2PO4, 1 CaCl2, 2 MgCl2, 10 HEPES, and 20 glucose, adjusted to pH 7.4 with NaOH. Patch pipettes were filled with an internal solution containing (in mM): 5 NaCl, 130 CsF, 4 MgATP, 2 MgCl2, 15 EGTA, and 10 HEPES, adjusted to pH 7.4 with CsOH. During patch clamp recordings, cells were perfused with an external solution with reduced sodium concentration containing (in mM): 80 NaCl, 50 CsCl, 2 CaCl2, 2.5 MgCl2, 10 HEPES, and 10 glucose, also adjusted to pH 7.4 with CsOH. Sodium currents were recorded at room temperature (20°C) in the whole-cell configuration with a patch-clamp amplifier (Axopatch 200B, Molecular Devices®). Patch pipettes (Corning® Kovar Sealing code 7052, WPI) had resistances of 1 to 2 MΩ. Currents were low-pass filtered at 10 kHz (-3db), digitized with a DigiData 1200 (Molecular Devices®). Data were acquired and analyzed with Acquis-1® software (G.Sadoc, CNRS, Gif/Yvette, France). Patch clamp recordings for peak I\textsubscript{Na} amplitude, activation, and steady-state inactivation, were assessed with standards protocols, as described previously by Pinet et al.\textsuperscript{13} The current amplitudes were normalized by cell capacitances (current densities, pA/pF). The activation-potential and steady-state inactivation curves were all fitted with a single Boltzmann function: \[ y = \frac{1}{1 + \exp - \left[ \frac{\left( V_m - V_{1/2} \right)}{k} \right]} \] (V\textsubscript{1/2} half-activation potential or half-inactivation potential (i.e. half-availability), V\textsubscript{m} test voltage, k slope factor).

**Adult rat cardiac myocyte isolation and transfection**
Myocytes were isolated from the atrium of adult Wistar rats, as previously described by Abi-Char et al.\textsuperscript{14} Following dissociation, cardiomyocytes were cultured overnight in a 1% CO\textsubscript{2} incubator before transfection with 1 μg of GFP fused construct of WT or mutant E83D MOG1 using a liposomal approach (Lipofectamine 2000, Invitrogen®). Three days after transfection, cardiomyocytes were fixed for immunocytochemistry.

**Immunocytochemistry**

Indirect immunofluorescence was performed on adult rat cardiomyocytes primary culture fixed with 4% paraformaldehyde (PFA) solution for 10 min at room temperature. Cells were then incubated for one hour at room temperature with permeabilizing/blocking buffer (0.1% Triton X-100 in PBS, 1% BSA, 10% Normal Goat Serum (NGS) and 10% Chicken Serum (ChS)), and double-labeled overnight by incubation at 4°C with a mixture of primary antibodies diluted in blocking buffer (PBS containing 1% BSA, 3% NGS and 3% ChS): mouse-anti-GFP (1:300; Roche®) to detect MOG1-GFP and rabbit anti-Na\textsubscript{v}1.5 (1:50; ASC 005, Alomone®). Detection was performed the following day by a one hour-incubation with secondary antibodies: chicken anti-mouse Alexa® Fluor 488, goat anti-rabbit Alexa® Fluor 594 (1:500, Molecular Probes®) and the nuclear dye DAPI (1:500, Sigma®) also diluted in the blocking buffer. Control experiments were performed by omitting the primary antibodies.

**Imaging and quantitative fluorescence**

Labeled cardiomyocytes were observed with an Olympus® epifluorescent microscope (60X). Images were acquired with a CoolSnap camera (Ropper scientific®) and analyzed with Metamorph® software (Molecular devices®) equipped with a 3D-deconvolution module. For each sample, series of consecutive plans (stack of images) were acquired (sectioning step: 0.2 μm). To quantify the proportion of Na\textsubscript{v}1.5 at the membrane, five random regions of similar length were delineated at the membrane cell periphery, and fluorescence for Na\textsubscript{v}1.5 antibody was quantified in each of these regions with ImageJ® software. For each cell, a mean of
fluorescent signal was normalized to the length to generate a value in arbitrary units (AU).

For each transfection condition, 21 cells from three different cultures (seven cells per culture and per condition) were quantified.

Statistical analysis
Data are expressed as means ± S.E.M. (Standard Error of the Mean). Statistical significance was estimated with a Student’s t test or a Mann-Whitney Rank Sum Test, as appropriate. P values of less than 0.05 were considered significant. Statistical analyses were performed with SigmaPlot® software.

Results
Identification of a MOG1 mutation in a patient with a BrS ECG pattern
Mutational analysis of the MOG1 gene revealed a novel mutation, potentially causing BrS, in one of 270 (0.3%) Caucasian subjects with BrS or IVF phenotypes. DNA sequencing revealed a c.249G>C transversion in exon 3 (Fig1A, black arrow), predicting a glutamic-to-aspartic acid substitution at amino acid 83 (p.Glu83Asp, E83D). This missense mutation was absent in 281 ethnically matched unrelated controls and in 48 BrS patients with SCN5A mutation. Alignment of the MOG1 amino acid sequence demonstrated that the glutamic acid in position 83 is conserved in several species, suggesting the importance of a glutamic acid at this position (Fig1B). The patient was a symptomatic 41-year old female with a medical history of syncope who experienced a cardiac arrest event. She required defibrillation followed by adrenaline injection to recover a sinus rhythm. The ECG recorded following the rescue maneuver showed a sinus tachycardia with an elevation of ST segment in precordial leads (V1, V2), an atypical right bundle branch block (RBBB) and a prolonged PR interval, evoking a type-1 BrS pattern (Fig1C). Blood tests showed metabolic acidosis and hyperlactemia but no traces of cocaine or other drugs of abuse. Due to the abnormal ECG
pattern, echocardiography and coronary angiography were performed showing an absence of structural cardiac abnormalities, healthy coronary arteries, and normal left ventricular function. Twenty-four hours later, the ST elevation had regressed but the atypical RBBB persisted, suggestive, but not diagnostic, of BrS (Fig 1D). Electroencephalogram showed post-anoxic encephalopathy and the patient died four days later from sepsis while in a comatose state. None of her family members have been available for clinical or genetic testing.

**WT MOG1 increases sodium current density but not mutant E83D MOG1**

We next tested if the E83D-MOG1 mutation had a pro-arrhythmic cellular phenotype. To characterize the functional consequences on sodium current, we performed whole-cell patch-clamp recordings in HEK293 cells stably expressing Nav1.5 (Fig 2). Cells transfected with WT MOG1 alone almost doubled their sodium current density compared to control conditions (at -20mV, -171.0 ± 19.0 pA/pF (n=20) for WT MOG1 versus -91.5 ± 11.6 pA/pF (n=21) for empty vector; p<0.01, Fig 2B). In contrast, mutant E83D failed to increase I$_{Na}$ density (-89.5 ± 28.4 pA/pF (n=12) versus empty vector; p NS). Allelic heterozygosity was simulated by transfecting an equimolar ratio of WT and mutant E83D-MOG1. This co-expression also failed to increase current density (-106.7 ± 6.1 pA/pF (n=12) versus empty vector; p NS). These effects on current density were not associated with any change in voltage dependence of the activation and steady state inactivation (Table 1).

**MOG1 silencing reduces sodium current density**

To further characterize the consequences of the loss of MOG1 on sodium current, we studied the effects of endogenous MOG1 silencing using siRNA in HEK293 cells stably expressing Na$_{v}$1.5, transfected with a siRNA directed against human MOG1 (siMOG1) or a scrambled siRNA (siSCR). Firstly, we confirmed that siMOG1 suppressed endogenous MOG1 by Western blot as shown in Figure 3A. Secondly, whole-cell patch clamp recordings in cells transfected with siMOG1 showed that the I$_{Na}$ density was reduced by 54% compared to cells
transfected with siSCR (at -20mV -51.6 ± 6.7 pA/pF (n=12) for siMOG1 versus -114.6 ± 12.5 pA/pF (n=12) for siSCR; p<0.001) (Figures 3B and 3C). The decrease in peak current by siMOG1 was not associated with changes in current biophysical properties (Table 1).

E83D-MOG1 mutation causes a defect in Na,1.5 intracellular trafficking to the membrane in isolated adult rat cardiomyocytes

Subsequently, we studied cellular localization of MOG1 and Na,1.5 in isolated atrial Adult Rat Cardiomyocytes (ARC) transfected with GFP tagged MOG1 constructs (WT and mutant E83D) using 3-dimensional microscopy. Figure 3 shows 3D-deconvolution images of cardiomyocytes double stained with anti-GFP and anti-Na,1.5 antibodies. MOG1 was widely distributed throughout the cardiomyocytes except in the nucleus. WT and mutant E83D-MOG1 displayed a similar sub-cellular distribution. In contrast, Na,1.5 channel distribution was clearly different. In cells transfected with WT-MOG1 (Figure 4A), channels were mostly localized at the membrane of the cells (yellow arrows) and around the nuclei (white arrows). In contrast, Na,1.5 accumulated in the perinuclear regions in cardiomyocytes transfected with mutant E83D, while a low fluorescent signal was detected at the cell periphery (Figure 4B). This observation was confirmed by quantification of the fluorescence intensity in random regions delineated at the cell membrane periphery (Figure 4C). The fluorescent signal for the sodium channel Na,1.5 was drastically reduced by 58% in mutant E83D transfected myocytes compared to WT MOG1 (normalized membrane fluorescence intensity with anti Na,1.5 antibody, expressed in Arbitrary Units (AU), for WT MOG1-GFP transfected 1224 ± 160 AU vs E83D MOG1-GFP transfected 507 ± 17 AU; p<0.001).

Discussion

The first mutations to be identified in BrS patients were in SCN5A, resulting in a loss of channel function \(^4,15\). They reduce sodium current by altering Na,1.5 cell surface
expression and/or modify its gating properties, and are the major cause of this disorder.\textsuperscript{4,16,17} However, SCN5A mutations only account for approximately 20% of patients\textsuperscript{5}, and the frequency of mutations in other implicated genes (GDP1\textsuperscript{18}, CACNA1C\textsuperscript{19}, CACNB2b\textsuperscript{19}, SCN1B\textsuperscript{7}, SCN3B\textsuperscript{20}, and KCNE3\textsuperscript{21}) appears to be rare\textsuperscript{15}. Thus, almost 75% of BrS cases remain without a genetic cause. In the heart, sodium channels are not only composed of pore-forming α subunits encoded by SCN5A, but also by multiple interacting partners to form macromolecular complexes (the “channelosome”). Some of them have already been implicated in the pathogenesis of arrhythmias and SCD\textsuperscript{6}.

In this study, we provide for the first time molecular and clinical evidence implicating a novel partner of Na\textsubscript{v}1.5, MOG1, in BrS, by reporting a patient in which the observed disease phenotype may result from a mutation in the MOG1 gene. Genetic screening identified the MOG1 missense mutation E83D in one symptomatic patient presenting a type-1 BrS ECG out of 270 affected individuals (1/270=0.3%), and which was absent in 562 healthy ethnically-matched control alleles. Using targeted mutagenesis, heterologous expression in an HEK Na\textsubscript{v}1.5 stable cell line, and a functional approach by patch clamp, we demonstrated that the E83D mutation failed to increase \(I_{\text{Na}}\) density in contrast to WT-MOG1, in homozygous and heterozygous conditions. We thus assumed that the mutant exerted a dominant negative effect on WT MOG1. Therefore, the presence of a mutation in one affected individual, its absence in a healthy control population, and the clear effect of the mutation on sodium current, support E83D-MOG1 as a BrS-susceptibility mutation rather than as a polymorphism.

Mechanisms underlying the effects of MOG1 on Na\textsubscript{v}1.5 remain unclear. A recently published study reported MOG1 as a new partner of Na\textsubscript{v}1.5. The two proteins co-localize at the level of the intercalated disks in mouse ventricular myocytes and moreover, MOG1 increased sodium current when co-expressed with Na\textsubscript{v}1.5 in a heterologous system and in...
neonatal cardiomyocytes \(^{12}\). Here we provide further evidence for the role of MOG1 as a partner of Na\(_v\)1.5 channels by demonstrating that the missense E83D mutant had no effect on I\(_{Na}\) and that silencing endogenous MOG1 dramatically reduced sodium current in a heterologous expression system. As MOG1 shows no effect on the biophysical and gating properties of the sodium current, this partner could regulate the surface expression of channels rather than their intrinsic properties. This is supported also by the observation that in cardiomyocytes transfected with mutant E83D, Na\(_v\)1.5 channels remained trapped in the perinuclear region while their localization at the membrane periphery was reduced, suggesting a defect in channel trafficking. Several mutations in SCN5A which affect trafficking have already been described as causing a BrS phenotype \(^{18}\). Indeed, a reduced channel density at the membrane has been observed for the GDP1L mutation associated with a BrS family \(^{18}\), as well as for a SCN5B mutation in one BrS affected individual \(^{20}\). Therefore, in accordance with these previous studies, E83D-MOG1 mutation may cause BrS by reducing Na\(_v\)1.5 channel trafficking to the cell surface. This observation is also consistent with the role of MOG1 in other cell types which is to regulate protein trafficking by mediating the Ran GTP gradient from nucleus to cytoplasm \(^{26}\).

Others Na\(_v\)1.5 partners are known to regulate membrane expression of the channel. For instance, ankyrin G associates with the cardiac sodium channel at intercalated disks and T-tubules and silencing of ankyrin G reduces both the surface expression of Na\(_v\)1.5, and I\(_{Na}\) density \(^{27,28}\). Interestingly, MOG1 and ankyrin G \(^{27}\) co-localize with Na\(_v\)1.5 at the level of the intercalated disks, and both partners interact with intracellular loop between domains II and III of the channel \(^{12}\), suggesting that this loop is involved in the surface expression of Na\(_v\)1.5 channel. In our study, silencing endogenous MOG1 resulted in a reduction of sodium current, as also observed after ankyrin G silencing. Taken together, these observations
suggest that MOG1 and ankyrin G may belong to the same protein complex that regulates the expression of sodium channels to the plasma membrane of cardiac myocytes.

BrS patients with SCN5A haploinsufficiency mutations display variable phenotypic penetrance, even between individuals carrying the same mutation. In fact, a mouse model with a heterozygous targeted disruption of Scn5a+/− show a similar pattern of phenotypic heterogeneity. Ultimately, phenotypic severity was correlated with levels of wild type Na,1.5 protein expression. Considering that MOG1 regulates the sodium channel expression at the membrane, it is clear that MOG1 could also be a potential BrS modifier gene, and could explain some of the variable penetrance of the disease.

Limitations of the study

The genotype-phenotype correlation between the MOG1 mutation and the BrS phenotype is hampered by the absence of a sodium channel blockade test to confirm the diagnosis of BrS. However, the presence of a type-1 BrS ECG a few hours after the resuscitated SCD and the persistence of atypical RBBB for 24 hours in a patient with a personal history of syncope are in favor of a true BrS diagnosis. Linkage analysis was also impossible as genetic material from other family members was not available. Thus, we could not provide co-segregation data to determine if this mutation is familial or sporadic. In such patients with an incomplete clinical and genetic exploration, it is difficult to prove whether the observed phenotype is due only to the presence of the MOG1 mutation. Genetic screening of other BrS populations would indeed confirm the role of MOG1.

Another limitation is the difficulty in extrapolating data obtained in a heterologous system to cardiac electrophysiology. We cannot eliminate the possibility that in situ, some other mechanisms could compensate for the MOG1 loss of function. However, there is no other example of a mutation causing more than a 50% decrease in I Na without it affecting cardiac function. For instance, an extensive review of SCN5A mutations showed that every
mutation associated with BrS and/or a conduction defect, invariably lead to a reduced peak of sodium current in heterologous systems 31. In addition, mutations in known partners such as GDP1L 18, SCN1B 7 and recently SCN3B 20 which displayed drastic decreases of $I_{Na}$ in vitro, were also found in patients with BrS and/or conduction defects. Thus, we can speculate that the in vitro cellular characteristics of the E83D-MOG1 mutation is in accordance with the BrS phenotype observed in our patient, and which, in combination with our genetic data, provide evidence of a relationship between the mutation and the disease.

**Conclusion**

In summary, we propose MOG1 as a novel susceptibility gene for BrS. Moreover, we have established that MOG1 is likely to be an important partner for the normal surface expression of Na, 1.5 channels. Our findings are not only relevant for BrS, but also for other “channelopathies”, as they underline the presence of a multi protein machinery specialized in sodium channel membrane targeting. Thus, understanding the dynamics of cardiac channel surface expression has become even more essential. Beyond revealing precise explanations of the physiopathology of acquired and inherited channelopathies, it may provide new clues in improving clinical management by generating new targets for anti-arrhythmic drugs.

**Acknowledgments:** We are grateful to Dr Rachel Peat for careful reading of the manuscript.

**Funding Sources:** This work was supported by Institut National de la Santé et de la Recherche Médicale (INSERM), the Université Pierre and Marie Curie, the Agence Nationale de la Recherche (ANR) (ANR-09-GENO-003-CaRNaC, ANR-08-GENO-006-CHAT), and the Leducq Foundation.

**Conflict of Interest Disclosures:** None.
References:


### Table 1. Effect of MOG1 expression on kinetic parameters of the sodium current

<table>
<thead>
<tr>
<th></th>
<th>Steady State V&lt;sub&gt;1/2&lt;/sub&gt; activation (mV)</th>
<th>Steady State V&lt;sub&gt;1/2&lt;/sub&gt; inactivation (mV)</th>
<th>Slope factor k activation (mV)</th>
<th>Slope factor k inactivation (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>empty vector</td>
<td>-50.0 ± 3.4 (n=10)</td>
<td>-80.7 ± 1.5 (n=8)</td>
<td>3.4 ± 1.0</td>
<td>-7.4 ± 1.6</td>
</tr>
<tr>
<td>WT MOG1</td>
<td>-52.9 ± 3.9 (n=11)</td>
<td>-80.6 ± 2.5 (n=8)</td>
<td>3.7 ± 0.6</td>
<td>-7.7 ± 1.1</td>
</tr>
<tr>
<td>E83D MOG1</td>
<td>-50.8 ± 2.7 (n=9)</td>
<td>-80.9 ± 1.8 (n=8)</td>
<td>3.6 ± 0.5</td>
<td>-6.1 ± 0.9</td>
</tr>
<tr>
<td>siSCR</td>
<td>-48.1 ± 3.5 (n=7)</td>
<td>-79.7 ± 1.8 (n=8)</td>
<td>3.5 ± 0.9</td>
<td>-5.7 ± 0.4</td>
</tr>
<tr>
<td>siMOG1</td>
<td>-49.2 ± 3.8 (n=7)</td>
<td>-78.9 ± 1.9 (n=8)</td>
<td>4.5 ± 0.5</td>
<td>-6.5 ± 0.9</td>
</tr>
</tbody>
</table>

In HEK293 cell line stably expressing Na(v)1.5 transiently transfected with an empty vector (control cells), wild type MOG1 (WT MOG1), mutant E83D MOG1, siRNA scrambled (siSCR) or siRNA anti human MOG1 (siMOG1). For I<sub>Na</sub> activation and inactivation curves, data points from n individuals cells were averaged and reported as means ± SEM. Parameters for inactivation and activation curves were fitted with Boltzmann relationship.

* p non significant vs empty vector
† p non significant vs MOG1 WT
‡ p non significant vs siSCR
Figure Legends:

Figure 1. MOG1 mutation identified in a patient with Brugada ECG pattern. (A) Mutation c.249G>C resulting in p.Glu83Asp (E83D). (B) Alignment of MOG1 protein sequence showing the conservation of Glutamic acid at position 83 (E83) across species. (C) First ECG recorded in the carrier, a few hours after the resuscitated sudden cardiac death, showing ST segment elevation in precordial leads (V1, V2) with an atypical right bundle branch block (RBBB) and a prolonged PR interval, evoking type-1 BrS pattern, (D) Second ECG recorded the day after, where the ST elevation regressed but the atypical RBBB persisted.

Figure 2. Electrophysiological characteristics of the mutant E83D MOG1 (A) Representative traces of sodium current recorded in HEK cell line stably expressing Na1.5 transfected with either WT MOG1 (1 μg), mutant E83D MOG1 (1 μg), an equimolar mix of WT (0.5 μg) and mutant (0.5 μg), or with empty vector (negative control). (B) Dot plot of sodium current densities at −20 mV. Each dot represents the current density for one cell; lines represent the means for each condition. Means ± SEM: empty vector (INa -91.5 ± 11.6 pA/pF n=21), WT MOG1 (INa -171.0 ± 19.0 pA/pF n=20), mutant E83D MOG1 (INa -89.5 ± 28.4 pA/pF n=12), and WT + mutant E83D MOG1 (INa -106.7 ± 6.1 pA/pF n=12). NS = Not Significant, * = p<0.05, ** = p<0.01.

Figure 3. Effect of silencing endogenous MOG1. (A) Western blot of total protein lysates of HEK293 cells stably expressing Na1.5 alone or transiently transfected with scrambled siRNA (siSCR) or siRNA against human MOG1 (siMOG1). (B) Representative traces of sodium current demonstrating a reduced sodium current in MOG1 silenced HEK cells. (C)
Dot plot of sodium current densities at –20 mV in HEK 293 cells stably expressing Na1.5 transiently transfected with siRNA. Each dot represents the current density for one cell, lines represent the means. Means ± SEM: siSCR (I\text{Na} -114.6 ± 12.5 pA/pF; n=12) or siMOG1 (I\text{Na} -51.6 ± 6.7 pA/pF; n=12). **= p<0.001.

**Figure 4.** MOG1 and Na1.5 localization in isolated adult rat atrial cardiomyocytes. Three-dimensional deconvolution images of MOG1-GFP (green) and Na1.5 (red) in myocytes transfected with WT MOG1-GFP construct (A) or with mutant E83D MOG1-GFP construct (B). Note the localization of Na1.5 around the nucleus (white arrows) and at the cell membrane (yellow arrows) in WT MOG1 transfected myocytes and the lack of Na1.5 at the cell membrane in mutant E83D MOG1 transfected myocytes. Scale white bar, 10 μm. (C) Dot plot of normalized membrane fluorescence intensities with anti Na1.5 antibody, expressed in Arbitrary Units (AU) in cells transfected with WT MOG1 or mutant E83D MOG1. Each dot represents the normalized membrane fluorescence intensity for one cell, lines represent the means for each condition. Means ± SEM: WT MOG1 (1224 ± 160 AU, n=21), mutant E83D MOG1 (507 ± 17 AU n=21), **= p<0.001.
Control
WT MOG1
G T G G A G T C T G T
Val Glu

Patient
c.249G>C p.Glu83Asp (E83D)
G T G A C T C T G T
Val Asp

Homo_sapiens
Pan_troglodytes
Macaca_mulatta
Loxodonta_africana
Bos_taurus
Canis_familiaris
Felis_catus
Myotis_lucifugus
Echinops_telfairi
Monodelphis_domestica
Mus_musculus
GVQGARAVHVESVQPLSLENLA
GVQGARAVHVESVQPLSLENLA
GVQGARAVHVESVQPLSLENLA
GVQGARAAQVEAVQPLPLENA
GVQGARAVHVESVQPLSLENLA
GMQEARALQVDSVQPLSLENLA
GAQEARAVQETVQPLFLENA
GVPQGARTVQVEAVQPLLENLG
GVQGARGVHVESVQPLPLENA
GVQGSGDEQVEAVQPLSLQNLG
RVQGARAVHVESVQPLCLENLS

Circulation
Cardiovascular Genetics
Journal of the American Heart Association
**Figure**

**A**
- Empty vector
- WT MOG1
- E83D MOG1
- WT MOG1 / E83D MOG1

**B**
- Current density pA/pF
- 50 pA/pF
- 5 ms

---

**Legend**
- WT MOG1
- E83D MOG1
- WT MOG1 / E83D MOG1

---

**Note**
- The figure shows the comparison of current density pA/pF for different conditions: Empty vector, WT MOG1, E83D MOG1, and WT MOG1 / E83D MOG1. The graph indicates a significant difference (*) and a highly significant difference (**) in the current density between the conditions.
A  Adult Rat Cardiomyocytes WT MOG1-GFP transfected

B  Adult Rat Cardiomyocytes E83D MOG1-GFP transfected

C  Normalized membrane fluorescence intensity with anti Nav1.5 antibody (AU)
MOGI: A New Susceptibility Gene for Brugada Syndrome

Darouna Kattygnarath, Svetlana Maugenre, Nathalie Neyroud, Elise Balse, Carole Ichai, Isabelle Denjoy, Gilles Dilanian, Raphaël P. Martins, Véronique Fressart, Myriam Berthet, Jean Jacques Schott, Antoine Leenhardt, Vincent Probst, Hervé Le Marec, Bernard Hainque, Alain Coulombe, Stéphane N. Hatem and Pascale Guicheney

Circ Cardiovasc Genet. published online March 29, 2011;
Circulation: Cardiovascular Genetics is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 1942-325X. Online ISSN: 1942-3268

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circgenetics.ahajournals.org/content/early/2011/03/29/CIRCGENETICS.110.959130

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Genetics can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Genetics is online at:
http://circgenetics.ahajournals.org//subscriptions/