Mechanistic insights into the interaction of the MOG1 protein with the cardiac sodium channel Na$_v$1.5 clarify the molecular basis of Brugada syndrome

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Na$_v$1.5 is the $\alpha$-subunit of the cardiac sodium channel complex. Abnormal expression of Na$_v$1.5 on the cell surface because of mutations that disrupt Na$_v$1.5 trafficking causes Brugada syndrome (BrS), sick sinus syndrome (SSS), cardiac conduction disease, dilated cardiomyopathy, and sudden infant death syndrome. We and others previously reported that Ran-binding protein MOG1 (MOG1), a small protein that interacts with Na$_v$1.5, promotes Na$_v$1.5 intracellular trafficking to plasma membranes and that a substitution in MOG1, E83D, causes BrS. However, the molecular basis for the MOG1/Nav1.5 interaction and how the E83D substitution causes BrS remains unknown. Here, we assessed the effects of defined MOG1 deletions and alanine-scanning substitutions on MOG1’s interaction with Na$_v$1.5. Large deletion analysis mapped the MOG1 domain required for the interaction with Na$_v$1.5 to the region spanning amino acids 146–174, and a refined deletion analysis further narrowed this domain to amino acids 146–155. Site-directed mutagenesis further revealed that Asp-148, Arg-150, and Ser-151 cluster in a peptide loop essential for binding to Na$_v$1.5. GST pulldown and electrophysiological analyses disclosed that the substitutions E83D, D148Q, R150Q, and S151Q disrupt MOG1’s interaction with Na$_v$1.5 and significantly reduce its trafficking to the cell surface. Examination of MOG1’s 3D structure revealed that Glu-83 and the loop containing Asp-148, Arg-150, and Ser-151 are spatially proximal, suggesting that these residues form a critical binding site for Na$_v$1.5. In conclusion, our findings identify the structural elements in MOG1 that are crucial for its interaction with Na$_v$1.5 and improve our understanding of how the E83D substitution causes BrS.

Cardiac arrhythmias cause more than 300,000 sudden deaths each year in the United States alone (1). Mutations in the SCN5A gene cause inherited cardiac arrhythmias, including Brugada syndrome (BrS)$^3$, sick sinus syndrome (SSS), cardiac conduction disease, atrial fibrillation, and dilated cardiomyopathy (2–5). Abnormalities of cardiac sodium current were also observed in common cardiovascular diseases such as myocardial ischemia and heart failure, which are also associated with sudden deaths (6).

The cardiac voltage-gated sodium channel is responsible for the generation and conduction of cardiac action potential (7, 8). The cardiac sodium channel is a multiprotein complex consisting of an $\alpha$-subunit of Na$_v$1.5 encoded by the SCN5A gene, $\beta$-subunits (B1–B4), and other accessory interacting proteins such as the chaperon MOG1 identified by our group using yeast two-hybrid screen (9). MOG1 was initially identified as a Ran-binding protein and a guanine nucleotide release factor of Ran (10, 11). The interaction between MOG1 and Ran was shown to be required for efficient nuclear protein import (12). The X-ray crystal structure of yeast MOG1 was determined at 1.9 Å resolution (13). MOG1 has an interesting structure with a backbone formed by six-stranded antiparallel $\beta$-sheets flanked on both sides by helices (14). The solution structure of human MOG1 was determined recently by conventional NMR spectroscopy and has a sandwich-like structure with nine $\beta$-strands and four helices (15). The $\beta$3 to $\beta$9 form the central antiparallel $\beta$-sheets, which were packed by helices $\alpha$2 and $\alpha$4 on either side (15). The Asp-25, Arg-30, Asp-70, and Glu-53 residues of MOG1 play an important role in Ran binding and GTP release in mice (16). A recent study suggested that the Asp-27, Glu-50, Asp-70, and Glu-53 residues of human MOG1 were also involved in the MOG1–Ran interaction (15).

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The abbreviations used are: BrS, Brugada syndrome; SSS, sick sinus syndrome; aa, amino acid; GST–Nav1.5–LII, GST–Nav1.5–loop II.
We reported previously that MOG1 is a key component of the cardiac sodium channel complex and is required for the full function of Na\textsubscript{1.5}. MOG1 was identified as a protein that interacts with the cytoplasmic loop II (between domains DII and DIII) of Na\textsubscript{1.5} by a yeast two-hybrid screen. MOG1 increases the density of the cardiac sodium current \(I_{\text{Na}}\) by promoting the intracellular trafficking of Na\textsubscript{1.5} from the endoplasmic reticulum to the plasma membrane (9, 17). Na\textsubscript{1.5} failed to be transported to cell surface and accumulated in the endoplasmic reticulum when MOG1 was knocked down in Na\textsubscript{1.5}/HEK cells, the HEK293 cells with stable overexpression of Na\textsubscript{1.5}. Overexpression of MOG1 successfully rescued the defective trafficking and reduced \(I_{\text{Na}}\) density associated with Na\textsubscript{1.5} mutation D1275N causing SSS, atrial fibrillation, and dilated cardiomyopathy, and mutation G1743R causing BrS (17). Therefore, MOG1 is a potential therapy for carriers with mutant Na\textsubscript{1.5} having defects in cell surface trafficking of Na\textsubscript{1.5}. However, the underlying molecular mechanism of how MOG1 interacts with Na\textsubscript{1.5} is unknown. Interestingly, a mutation in MOG1, E83D, was identified in a patient with BrS (18). MOG1 mutation E83D was reported to be a dominant negative mutation that significantly reduced the density of \(I_{\text{Na}}\) associated with Na\textsubscript{1.5} mutation E83D, was reported to be a dominant negative mutation that significantly reduced the density of \(I_{\text{Na}}\) (18). However, the molecular mechanism of how mutation E83D results in BrS is poorly understood.

In this study, we investigated the mechanism by which MOG1 interacts with Na\textsubscript{1.5}. Our data demonstrate that amino acid residues Glu-83, Asp-148, Arg-150, and Ser-151 are essential for the binding of MOG1 to Na\textsubscript{1.5} to generate full-scale \(I_{\text{Na}}\). Furthermore, amino acids Leu-138 and Leu-139 of MOG1 are required for the integrity of MOG1’s structure. Moreover, we found that MOG1 mutation E83D disrupted the interaction between MOG1 and Na\textsubscript{1.5}, and significantly reduced intracellular trafficking of Na\textsubscript{1.5} to the plasma membranes, thereby providing a novel molecular mechanism for the pathogenesis of BrS.

**Results**

Large deletion analysis defines the MOG1 functional domain for interaction with Na\textsubscript{1.5} between amino acid residues 146 and 174

MOG1 is an evolutionarily conserved protein from yeast to humans (19). Yeast two-hybrid screening and follow-up GST pulldown and co-immunoprecipitation studies showed that MOG1 interacts with cardiac sodium channel Na\textsubscript{1.5} (9). Overexpression of MOG1 in HEK293 and tsA201 cells or mouse cardiomyocytes facilitates the cell surface expression of Na\textsubscript{1.5} and enhances sodium current density (9). To determine the functional domain(s) of MOG1 in enhancing Na\textsubscript{1.5} cell surface expression, and thus increasing the sodium current density, we carried out a systematic deletion analysis. We created serial deletions of MOG1 from the C terminus, including deletion 1–174 aa, 1–146 aa, and 1–101 aa (Fig. 1A). The deletion mutants were then co-transfected into Na\textsubscript{1.5}/HEK cells with constant expression of Na\textsubscript{1.5} together with pmaxEGFP. Whole-cell patch clamp recordings of selected green fluorescent cells with successful transfection of MOG1 plasmids were performed to determine the effects of MOG1 deletions on Na\textsubscript{1.5} channel function. Like the full-length WT MOG1, the first C-terminal deletion of MOG1 (1–174 aa) increased sodium current density. However, MOG1 deletions MOG1 (1–146 aa) and MOG1 (1–101 aa) failed to increase sodium current density (Fig. 1, B–D). These data suggest that amino acid residues between 146 and 174 aa are required for MOG1 to increase sodium current density.

MOG1 interacts with the cytoplasmic loop II of Na\textsubscript{1.5} to facilitate its cell surface expression (9). To determine whether the MOG1 (146–174) region is involved in the interaction with Na\textsubscript{1.5}, GST pulldown assays were performed. GST–Na\textsubscript{1.5}–LII successfully pulled down the full-length MOG1 and the first C-terminal deletion mutant MOG1 (1–174) (Fig. 2, A and B). However, GST–Na\textsubscript{1.5}–LII failed to pull down MOG1 deletion mutants MOG1 (1–146 aa) and MOG1 (1–101 aa) (Fig. 2, A and B). These data suggest that the amino acid residues between 146 and 174 aa are required for MOG1 to increase sodium current density.

**Microdeletion analysis defines the MOG1 functional domain for interaction with Na\textsubscript{1.5} between amino acid residues 146 and 155**

To narrow down the MOG1 functional domain for interaction with Na\textsubscript{1.5}, we created consecutive deletions of 10 amino acid residues for the region between amino acid residues 126 and 174 of MOG1 (Fig. 3A). The whole-cell patch clamp recordings showed that three deletion mutants, MOG1 (166–174 aa deletion), MOG1 (156–165 aa deletion), and MOG1 (126–135 aa deletion), had similar activity as WT MOG1.
increasing sodium current density (Fig. 3, A–C). However, two deletion mutants, MOG1 (146–155 aa deletion) and MOG1 (136–145 aa deletion) failed to enhance sodium current density (Fig. 3, A–C). Consistent with the patch clamp data, mutants MOG1 (146–155 aa deletion) and MOG1 (136–145 aa deletion) failed to be pulled down by GST–Na$_v$1.5–LII, whereas the other three mutants, MOG1 (166–174 aa deletion), MOG1 (156–165 aa deletion), and MOG1 (126–135 aa deletion), were successfully pulled down by GST–Na$_v$1.5–LII (Fig. 4, A and B). Together, these data suggest that amino acid residues between 136 and 155 of MOG1 are involved in MOG1 interaction with Na$_v$1.5–LII to increase sodium current density.

Point mutation analysis identifies critical amino acids of MOG1 required for Na$_v$1.5 function and enhancement of sodium current density

To determine the critical amino acid residues between 136 and 155 of MOG1 required for interaction with Na$_v$1.5–LII and for increasing sodium current density, alanine-scanning mutagenesis was performed for individual amino acid residues in the region (Fig. 5A). Whole-cell patch clamp analysis showed that WT MOG1 increased sodium current density, but MOG1 mutants with mutations at L138A, L139A, D148A, R150A, or S151A failed to increase sodium current density (Fig. 5A). These data identified two MOG1 domains which impaired MOG1-mediated increase of cardiac sodium current density, region I at amino acid residues Leu-138 and Leu-139 and region II at amino acids Asp-148 to Ser-151. GST pulldown assays showed that GST–Na$_v$1.5–LII failed to pull down mutants MOG1 (L138A) and MOG1 (L139A) at region I (Fig. 5, B and C).
The interaction between GST–Nav1.5–LII and MOG1 mutants with mutations at D148A, R150A, or S151A at region II was also decreased compared with that with WT MOG1 (Fig. 5, B and C). The overall effects of MOG1 alanine-scanning mutations at region I (L138A, L139A) were more dramatic than that of similar mutations at region II (D148A, R150A, S151A) (Fig. 5, A–C). To identify the underlying cause, we analyzed the affected amino acid residues against the 3D structure of MOG1 as reported by Bao et al. (15). As shown in Fig. 5D, Leu-138 and Leu-139 are located in the inner part of MOG1, consistent with their characteristics as amino acids with nonpolar side chains (Fig. 5D). Because they are buried inside the structure of MOG1, Leu-138 and Leu-139 cannot be the amino acid residues that interact directly with Nav1.5. Thus, the effects of Leu-138A and Leu-139A could be because of structural changes of MOG1. Nevertheless, our data suggest that Leu-138 and Leu-139 may be involved in establishing the integrity of the MOG1 structure as their mutations to alanine may destabilize the overall structure of MOG1 so that the interaction between MOG1 and Na_{1.5} was disrupted.

Residues Asp-148, Arg-150, and Ser-151 of MOG1 are polar and charged amino acids located on the surface of the molecule (Fig. 5D). To further confirm their functional roles in MOG1, we constructed D148Q, R150Q, and S151Q mutations with more dramatic changes. Whole-cell patch clamp assays showed that unlike WT MOG1, all three mutant MOG1 (D148Q, R150Q, S151Q) failed to increase cardiac sodium current density (Fig. 6, A and B). GST pulldown assays showed that these three mutant MOG1 failed to interact with GST–Na_{1.5}–LII (Fig. 6, C and D). These data suggest that amino acid residues Asp-148, Arg-150, and Ser-151 are the critical amino acids required for MOG1–Nav1.5 interaction, which is required for increased $I_{Na}$ density. We refer to the MOG1 region centered at Asp-148 to Ser-151 as the Na_{1.5} interaction domain of MOG1.

MOG1 increases cardiac sodium current density through facilitating Nav1.5 trafficking to the plasma membrane. To determine the effects of the amino acid residues of the Na_{1.5} interaction domain on the plasma membrane expression level of Nav1.5, mutant MOG1 with mutations L138A, L139A, D148Q, R150Q, or S151Q and pcDNA3-SCN5A were co-transfected into tsA201 cells. The biotinylated plasma membrane proteins were isolated and used for Western blot analysis to determine the expression level of Nav1.5 in the plasma membrane. In contrast to WT MOG1, all five MOG1 mutants failed to increase the level of Nav1.5 in the plasma membrane (Fig. 7, A–D). As a control, the Na_{1.5} expression level in total cell lysates was not affected by the five MOG1 mutants (Fig. 7, A–D).

**Effect of Brugada syndrome mutation E83D of MOG1 on MOG1–Nav1.5 interaction and sodium current density**

A mutation in MOG1, E83D, was identified in a patient with BrS and significantly reduced the density of cardiac sodium current (18). However, the underlying molecular mechanism was poorly understood. Interestingly, our computer structural modeling analysis showed that the Glu-83 residue of MOG1 is located in close proximity to the Na_{1.5} interaction domain (Fig. 5D). Thus, we hypothesized that the Glu-83 residue of...
MOG1 is also involved in the interaction between MOG1 and loop II of Nav1.5, and that the E83D mutation of MOG1 disrupts the interaction between MOG1 and Nav1.5, thus preventing MOG1 from facilitating Nav1.5 trafficking to the cell surface. To test this hypothesis, we performed GST pulldown assays with mutant MOG1 with the E83D mutation. As shown in Fig. 8A, GST–Nav1.5–LII failed to pull down mutant MOG1 (E83D), although the positive control, WT MOG1, successfully pulled down GST–Nav1.5–LII. Moreover, we performed Western blot analysis with biotinylated plasma membrane proteins isolated from the cells co-transfected with pcDNA3-SCN5A and a WT FLAG-MOG1 plasmid, a mutant FLAG-E83D plasmid, or an empty vector control. Overexpression of WT MOG1 significantly increased the expression level of Nav1.5 in the plasma membrane as compared with empty vector control, however, mutant MOG1 with the E83D mutation failed to increase Nav1.5 in the plasma membrane as compared with WT MOG1 (Fig. 8, B and C). As a control, the Nav1.5 expression level in total cell lysates was not affected by MOG1 mutation E83D (Fig. 8, B and C). These results suggest that the mechanism by which MOG1 mutation E83D causes BrS is that it disrupts the interaction between MOG1 and Nav1.5, which significantly decreases the level of Nav1.5 in the plasma membrane, resulting in reduced sodium current density.
Similar effects between MOG1 mutations D148Q, R150Q, and S151Q that disrupt binding to Na1.5–LII and E83D

To determine whether MOG1 mutants D148Q, R150Q, S151Q, and E83D act in a dominant negative manner, we recorded sodium currents from the tsA201 cells co-transfected with an SCN5A expression plasmid and an equal amount of WT MOG1 and each mutant MOG1 as compared with WT MOG1 only. As shown in Fig. 9, A and B, the INa densities for overexpression of WT MOG1 (0.5 μg WT + 0.5 μg vector) were significantly decreased by co-expression of each of the four
mutant MOG1 (0.5 µg WT + 0.5 µg mutant MOG1). The data suggest that MOG1 mutants D148Q, R150Q, S151Q, and E83D all act in a dominant negative manner in reduction of $I_{\text{Na}}$ densities. Consistent with the $I_{\text{Na}}$ data, GST pulldown assays showed that the binding levels between WT MOG1 and Na$_{\text{v}}$1.5–LII were significantly decreased by co-expression of each of the four mutant MOG1 (Fig. 9, C and D).

**Discussion**

Mutations in Na$_{\text{v}}$1.5 cause an inherited arrhythmia of BrS (2), cardiac conduction disease (3), sick sinus syndrome (4), atrial fibrillation (5), dilated cardiomyopathy (5), and sudden infant death syndrome (6). The treatment options for BrS, cardiac conduction disease and sick sinus syndrome are highly limited except for implantation of implantable cardiac defibrillators and pacemakers. Approximately 50% of Na$_{\text{v}}$1.5 mutations are associated with significantly reduced cardiac sodium current density by blocking intracellular trafficking of Na$_{\text{v}}$1.5 to cell surface (20). Therefore, interventions that reverse the effects of Na$_{\text{v}}$1.5 on intracellular trafficking of Na$_{\text{v}}$1.5 to cell surface was proposed as potential strategies to treat patients with BrS, cardiac conduction disease, and sick sinus syndrome (17). We previously showed that MOG1 significantly promoted intracellular trafficking of Na$_{\text{v}}$1.5 to cell surface and increased cardiac sodium current density (9). Moreover, MOG1 overexpression reversed the effects of trafficking-defective mutations D1275N and G1743R of Na$_{\text{v}}$1.5 on cell surface expression of Na$_{\text{v}}$1.5 and sodium current density (17). However, the molecular mechanism by which MOG1 promotes intracellular trafficking of Na$_{\text{v}}$1.5 to cell surface is poorly understood. Studies to identify how MOG1 interacts with Na$_{\text{v}}$1.5 are important for understanding the role of MOG1 in intracellular trafficking of Na$_{\text{v}}$1.5. In this study, we defined the functional domain and precise amino acid residues of MOG1 that are required for its interaction with Na$_{\text{v}}$1.5. By analyzing the interaction of the intracellular loop II of Na$_{\text{v}}$1.5 with WT MOG1 and eight serial deletion mutants of MOG1 and characterizing the effects of deletions on cardiac sodium current density, we defined the Na$_{\text{v}}$1.5-interaction domain of MOG1 between amino acid residue Thr-136 and Pro-155 (Figs. 1–3). Similar analysis but with alanine-scanning mutations identified the specific amino acid residues of MOG1 that are essential for MOG1–Na$_{\text{v}}$1.5 interaction, including Asp-148, Arg-150, and Ser-151. These three amino acid residues are located in an extended outside α-helix loop on the surface of the MOG1 structure (Fig. 5D), which is best suited for contacting Na$_{\text{v}}$1.5.

Interestingly, our deletion and alanine-scanning mutation analyses revealed only one half of the Na$_{\text{v}}$1.5 interaction domain of MOG1. The other half was identified in an interesting manner. Our computer modeling analysis revealed that the Glu-83 residue of MOG1 is located in a close proximity in the 3D structure of MOG1 (Fig. 5D). Functional studies showed that mutation E83D, which is associated with Brugada syndrome, disrupted the interaction between MOG1 and Na$_{\text{v}}$1.5 (Fig. 8), suggesting that the Glu-83 residue is also involved in the interaction between MOG1 and Na$_{\text{v}}$1.5. The Glu-83 residue is located in nearby α-helix loop on the surface of the MOG1 structure (Fig. 5D). Overall, the α-helix loop at Asp-148 to Ser-151 and the α-helix loop at Glu-83 form the two separate sides of a clamp, respectively, which may make MOG1 bind to Na$_{\text{v}}$1.5 by clamping down on a specific structure of Na$_{\text{v}}$1.5 (Fig. 5D). Interestingly, Asp-148, Arg-150, and Glu-83 are all charged amino acid residues, which may interact with Na$_{\text{v}}$1.5 amino acid residues with opposite charges.

During our alanine-scanning mutation analysis, we identified two amino acid residues, Leu-138 and Leu-139, that appeared to disrupt the interaction between MOG1 and Na$_{\text{v}}$1.5 and reduce cardiac sodium current density (Fig. 5, A–C). However, Leu-138 and Leu-139 are nonpolar amino acid residues and computer modeling analysis of the MOG1 structure revealed that these two residues are buried inside the MOG1 structure (Fig. 5D). As such, it is impossible for Leu-138 and Leu-139 of MOG1 to make direct contact with Na$_{\text{v}}$1.5. Instead, Leu-138 and Leu-139 may be essential for maintaining the overall structure of MOG1 so that when either one is mutated to alanine, the overall structure of MOG1 is altered, which disrupts the interaction between MOG1 and Na$_{\text{v}}$1.5 and reduces cardiac sodium current density.
We reported previously that SCN5A is the first gene responsible for Brugada syndrome (2). As MOG1 interacts with and regulates the function of the cardiac sodium channel encoded by SCN5A (9), MOG1 became a candidate gene for Brugada syndrome. Kattynnarath et al. (18) later reported a dominant negative mutation in MOG1, E83D, in a patient with Brugada syndrome. Overexpression of mutant E83D or E83D/H11001 in HEK Na1,5 stable cells failed to increase cardiac sodium current density. However, the molecular mechanism was poorly understood. In this study, we show that the E83D mutation of MOG1 disrupts the interaction between MOG1 and Na1,5 and reduces intracellular trafficking of Na1,5 to cell surface (Fig. 8). Our data provide a mechanistic understanding of why MOG1 mutation E83D significantly reduces cardiac sodium current density, and thereby uncovers a novel molecular mechanism for the pathogenesis of Brugada syndrome. It is interesting to note that a mutation from a glutamic acid residue (E) to an aspartic acid (D) is considered to be a minor change because the mutation does not change the overall negative charge, but simply reduces a methylene bridge (–CH2–) on the side chain.

Figure 9. MOG1 mutations D148Q, R150Q, and S151Q that disrupt the interaction between MOG1 and Na1,5 act in a dominant negative manner as Brugada syndrome mutation E83D. A, effects of MOG1 mutations E83D, D148Q, R150Q, and S151Q on sodium current densities in tsA201 cells co-transfected with 0.2 μg of pcDNA3-SCN5A and an equal amount of WT MOG1 (MOG1-WT). The empty vector (1 μg) was used as a negative control, and MOG1-WT (1 μg) and MOG1-WT (0.5 μg) + vector (0.5 μg) were used as positive controls. Error bars indicate S.E. B, the peak sodium current densities (pA/pF) derived from A: -68.93 ± 32.05 for vector pcDNA3.1, -124.14 ± 43.46 for MOG1-WT (1 μg), -98.20 ± 23.43 for MOG1-WT (0.5 μg) + vector (0.5 μg), -73.62 ± 37.79 for MOG1-WT (0.5 μg) + D148Q (0.5 μg), -72.06 ± 40.67 for MOG1-WT (0.5 μg) + R150Q (0.5 μg), -73.59 ± 40.41 for MOG1-WT (0.5 μg) + Ser-151 (0.5 μg), and -74.77 ± 35.14 for MOG1-WT (0.5 μg) + E83D (0.5 μg). Values are mean ± S.D. C, GST pulldown assays for interaction between GST–Na1,5–LII and WT MOG1, and an equal amount of WT MOG1 and each mutant MOG1 (D148Q, R150Q, S151Q, or E83D). Note that more DNA was needed for transfection for GST pulldown assays than for INa recording. D, quantified data from Western blot analysis as in (C) for the relative binding levels between GST–Na1,5–LII and WT MOG1, and WT/mutants. Error bars indicate S.D. (n = 3).
However, our finding that the E83D mutation is located in the MOG1–Na\(_{v}\)1.5 interaction domain explains why such a weak mutation has a dramatic effect to cause a human disease because a deletion of one methylene bridge (–CH\(_2\)–) in the MOG1–Na\(_{v}\)1.5 interaction pocket can make the interaction weakened, thereby disrupting the interaction and causing a dramatic functional effect.

The data from both patch clamp recordings of \(I_{\text{Na}}\) and GST pulldown assays for the interaction between Na\(_{v}\)1.5–II and MOG1 suggest that MOG1 mutations D148Q, R150Q, and R151Q as well as mutation E83D associated with Brugada syndrome act in a dominant negative manner to antagonize the functional effects of WT MOG1 (Fig. 9). 3D structural studies suggest that MOG1 is a monomer (15). How does the mutant MOG1 interfere with the function of WT MOG1 to exert the dominant negative effect? One possibility is that MOG1 may function as a dimer or multimer \textit{in vivo}, and mutant MOG1 binds to WT MOG1 to disrupt its binding to Na\(_{v}\)1.5 and reduce \(I_{\text{Na}}\) densities. An alternative possibility may be related to the finding that MOG1 is an adaptor protein that can interact with other proteins. MOG1 was shown to interact with Ran GTPase (10, 11). We recently found that small GTPases SAR1A and SAR1B interacted with MOG1, and the interaction was involved in MOG1-mediated cell surface expression and function of Na\(_{v}\)1.5 (26). Therefore, mutant MOG1 may interfere with the interaction between WT MOG1 and SAR1 or other proteins that are in the complex with Na\(_{v}\)1.5 and required for trafficking of Na\(_{v}\)1.5, resulting in reduced \(I_{\text{Na}}\) densities. These and other hypotheses can be tested in the future to further define the detailed molecular mechanism(s) underlying the dominant negative effect of MOG1 mutations.

A limitation of this study is that all experiments were performed in HEK293 cells. We have reported that the functional effects of MOG1 on Na\(_{v}\)1.5 and cardiac sodium currents in HEK293 cells are similar to those found in isolated neonatal cardiomyocytes (9, 17). Moreover, Kattynnarath et al. (18) reported that mutation E83D associated with Brugada syndrome showed a similar effect in HEK293 cells and adult rat atrial cardiomyocytes. Therefore, it is possible that the findings on MOG1 and MOG1 mutations in HEK293 cells may be extended to cardiac myocytes. However, future studies using isolated neonatal cardiomyocytes are needed to test this possibility.

In summary, the data in this study identify the Na\(_{v}\)1.5 interaction domain of MOG1, and define the MOG1–Nav1.5 interaction domain as a potential clamp structure with the \(\alpha\)-helix loop at Asp-148 to Ser-151 as one side of the clamp and the \(\alpha\)-helix loop at Glu-83 as the other side of the clamp. Our data also identify important structural elements, including several charged amino acid residues, Asp-148, Arg-150, and Glu-83, which are essential for the interaction between MOG1 and Na\(_{v}\)1.5, and may make direct contact with Na\(_{v}\)1.5 amino acid residues with opposite charges. Moreover, we uncovered a possible molecular mechanism by which MOG1 mutation E83D causes Brugada syndrome. The identification of the MOG1 domain required for full Na\(_{v}\)1.5 function may facilitate rational design of agents to rescue the trafficking defects of Na\(_{v}\)1.5 mutations associated with Brugada syndrome, sick sinus syndrome, atrial fibrillation, dilated cardiomyopathy, common heart failure, and myocardial ischemia.

**Experimental procedures**

**Plasmids**

WT and mutant MOG1 expression plasmids for electrophysiological analysis in mammalian cells were constructed using the pcDNA3.1- (−) vector. The PCR primers used for amplification of MOG1 cDNA include the forward primer with an EcoR I restriction enzyme site, 5’-ATTGGAATTCGCCACCATGGAGGCCACGAGAGACT-3’ and the reverse primer with a BamHI I restriction site, 5’-CGATGGATCCTTATCTGGG GACCAAGATG-3’. MOG1 deletions were made using overlapping PCR analysis. Alanine-scanning mutations were also created using overlapping PCR-based mutagenesis. The WT and mutant MOG1 expression plasmids for GST pulldown assays were constructed using the p3×FLAG CMV10 vector. The PCR primers used for amplification of MOG1 cDNA include the forward primer with a HindIII restriction enzyme site, 5’-GACCAAGCTTATGGAGCCACGAGAGACT-3’ and the reverse primer with an EcoR I restriction site, 5’-TTGCCGAATTCCTTATCTGGG GACCAAGATG-3’. The expression plasmid for GST–Na\(_{v}\)1.5–loop II (amino acids 940–1200) was cloned into pGEX-4T1 as described previously (9). The mammalian expression plasmid for SCN5A/Na\(_{v}\)1.5 is pcDNA3-SCN5A (pHL3) as described previously (2, 21).

**Cell culture and transfection**

The tsA201 cells were cultured and maintained in Dulbecco’s modified Eagle’s medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS). A stable HEK293 cell line with constant expression of Na\(_{v}\)1.5, Na\(_{v}\)1.5/HEK, was cultured in DMEM (Gibco) with 10% (v/v) FBS and 50 μg/μl of G418. For patch clamping recording, Na\(_{v}\)1.5/HEK cells were cultured on coverslips in 24-well plates to 80% confluence, and transiently transfected using GenJet (SignaGen Laboratories). The amount of plasmid DNA for each 24-well plate well was 0.8 μg of WT, mutant MOG1, or empty vector pcDNA3.1- (−) as negative control, and 0.2 μg of pmaxEGFP as a transfection indicator. For GST pulldown assays, tsA201 cells were cultured in 10-cm dishes, and transfected with 10 μg of plasmids with FLAG-tagged WT or mutant MOG1.

**Electrophysiological analysis**

The transfected Na\(_{v}\)1.5/HEK cells with an approximately equal amount of GFP signal were selected for whole cell voltage clamping recording of sodium current. Electrode resistance ranged from 2 to 4 megohms when filled with the pipette solution (20 mM NaCl, 130 mM CsCl, 10 mM HEPES, 10 mM EGTA, pH 7.2, with CsOH). The composition of bath solution was 70 mM NaCl, 80 mM CsCl, 5.4 mM KCl, 2 mM Ca\(_{2+}\), 1 mM Mg\(_{2+}\), 10 mM HEPES, 10 mM glucose, pH 7.3 with CsOH as described previously (9, 22, 23). Command pulses were generated using a MultiClamp 700B Amplifier (Axon Instruments) under the control of pCLAMP 10 software. The signals were digitized with a Digidata 1440A (Molecular Devices). Data were analyzed with Clampfit software.
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Isolation and characterization of plasma membranes proteins

The tsA201 cells were co-transfected with expression plasmids for Na\textsubscript{v}1.5 (pcDNA3-SCN5A) and WT or mutant FLAG-tagged MOG1. Proteins on plasma membranes were biotinylated using the EZ-Link Sulfo-NHS-SS-Biotin kit (Pierce) according to the manufacturer’s instructions. The cells were then lysed in TNEN buffer (50 mM Tris/HCl, pH 7.5, 150 mM NaCl, 2.0 mM EDTA, 1% Nonidet P-40), and biotinylated proteins were captured using Pierce\textsuperscript{TM} UltraLink\textsuperscript{TM} Immobilized NeutrAvidin\textsuperscript{TM} Resin as described previously (9, 17). Western blot analysis was then performed using an anti-Na\textsubscript{v}1.5 antibody or other control antibodies as described previously (9).

GST pulldown assays

The tsA201 cells were transfected with plasmids for FLAG-tagged MOG1 and lysed in TNEN lysis buffer. GST-tagged Na\textsubscript{v}1.5–loop II (GST–Na\textsubscript{v}1.5–LII) was expressed in BL21 cells induced by IPTG (isopropyl \(\beta\)-thiogalactopyranoside) at 26 °C for 8 h. GST-tagged Na\textsubscript{v}1.5 loop II (amino acids 940–1200) was used as bait to capture WT or mutant FLAG-MOG1 as described by us previously (9). GSH Sepharose 4B beads (GE Healthcare) were used to bind GST–Na\textsubscript{v}1.5–LII or GST alone as negative control. The products pulled down by GST were analyzed by Western blot analysis using an anti-FLAG antibody (Sigma).

Western blot analysis

Western blot analysis was performed as described previously (24, 25). For Western blot analysis for Na\textsubscript{v}1.5, we used 8% gels for separating proteins, whereas for MOG1, we used 15% gels. Primary antibodies used for Western blot analysis are those for FLAG (Sigma, F1804, F3165, 1:2000 dilution), Na\textsubscript{v}1.5 (Protein-tech Group, 23016-A-P, 1:1000; Alomone Labs, ASC-005, ASC-013, 1:1000 dilution), Na/K ATPase (Sigma, 06-520, 1:1000 dilution), and N-cadherin (Novus Biologicals, NB200-592, 1:500 dilution). The secondary antibodies are goat anti-mouse HRP-conjugated secondary antibody (Millipore, 20:000), goat anti-rabbit HRP-conjugated secondary antibody (Millipore, 1:20,000), goat anti-mouse IRDye 680LT (LI-COR Biosciences, 926-68020, 1:20,000), or goat anti-rabbit IRDye 800CW (LI-COR, 926-32111, 1:20000). The intensity of each band from Western blot images was quantified and analyzed by Quantity One analysis software 4.6.2 version (Bio-Rad Laboratories) or Image Studio (LI-COR).

Statistical analysis

Quantitative data were presented as mean ± S.E. for sodium channel current-voltage characteristics and mean ± S.D. for others. The difference between two groups was analyzed by a Student’s \(t\) test. A \(p\) value of < 0.05 was considered to be statistically significant.

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References

1. Willich, S. N., Levy, D., Rocco, M. B., Toffer, G. H., Stone, P. H., and Muller, J. E. (1987) Circadian variation in the incidence of sudden cardiac death in the Framingham Heart Study population. Am. J. Cardiol. 60, 801–806 CrossRef Medline
2. Chen, Q., Kirsch, G. E., Zhang, D., Brugada, R., Brugada, J., Brugada, P., Potenza, D., Moya, A., Borggreve, M., Breithardt, G., Ortiz-Lopez, R., Wang, Z., Antzelevitch, C., O’Brien, R. E., Schulze-Bahr, E., Keating, M. T., Towbin, J. A., and Wang, Q. (1998) Genetic basis and molecular mechanism for idiopathic ventricular fibrillation. Nature 392, 293–296 CrossRef Medline
3. Schott, J. J., Alshinawi, C., Kyndt, F., Probst, V., Hoorn, J. T. M., Hulsbeek, M., Wilde, A. A., Escande, D., Mannens, M. M., and Le Marec, H. (1999) Cardiac conduction defects associate with mutations in SCN5A. Nat. Genet. 23, 20–21 CrossRef Medline
4. Benson, D. W., Silberbach, G. M., Kavanagh-McHugh, A., Cottrill, C., Zhang, Y., Riggs, S., Smalls, O., Johnson, M. C., Watson, M. S., Seidman, J. G., Seidman, C. E., Plowden, J., and Kugler, J. D. (1999) Mutations in the cardiac transcription factor NKX2.5 affect diverse cardiac developmental pathways. J. Clin. Invest. 104, 1567–1573 CrossRef Medline
5. Olson, T. M., Michels, V. V., Ballew, J. D., Reyna, S. P., Karst, M. L., Herron, K. J., Horton, S. C., Rodeheffer, R. J., and Anderson, J. L. (2005) Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. JAMA 293, 447–454 CrossRef Medline
6. Otagiri, T., Kijima, K., Osawa, M., Ishii, K., Makita, N., Matoba, R., Umetsu, K., and Hayakasa, K. (2008) Cardiac ion channel gene mutations in sudden infant death syndrome. Pediatr. Res. 64, 482–487 CrossRef Medline
7. Gellens, M. E., George, A. L., Jr., Chen, L. Q., Chahine, M., Horn, R., Barchi, R. L., and Kallen, R. G. (1992) Primary structure and functional expression of the human cardiac tetrodotoxin-insensitive voltage-dependent sodium channel. Proc. Natl. Acad. Sci. U.S.A. 89, 554–558 CrossRef Medline
8. Wang, Q., Li, Z., Shen, J., and Keating, M. T. (1996) Genomic organization of the human SCN5A gene encoding the cardiac sodium channel. Genomics 34, 9–16 CrossRef Medline
9. Wu, L., Yong, S. L., Fan, C., Ni, Y., Yoo, S., Zhang, T., Zhang, X., Obejero-Paz, C. A., Rho, H. J., Ke, T., Szafrański, P., Jones, S. W., Chen, Q., and Wang, Q. K. (2008) Identification of a new co-factor, MOG1, required for the full function of cardiac sodium channel Na\textsubscript{v}1.5. J. Biol. Chem. 283, 6968–6978 CrossRef Medline
10. Oki, M., and Nishimoto, T. (1998) A protein required for nuclear-protein import, Mog1p, directly interacts with GTP-Gsp1p, the Saccharomyces cerevisiae ran homologue. Proc. Natl. Acad. Sci. U.S.A. 95, 15388–15393 CrossRef Medline
11. Steggerda, S. M., and Paschal, B. M. (2000) The mammalian Mog1 protein is a guanine nucleotide release factor for Ran. J. Biol. Chem. 275, 23175–23180 CrossRef Medline
12. Baker, R. P., Harreman, M. T., Eccleston, J. F., Corbett, A. H., and Stewart, M. (2001) Interaction between Ran and Mog1 is required for efficient nuclear protein import. J. Biol. Chem. 276, 41255–41262 CrossRef Medline
13. Stewart, M., and Baker, R. P. (2000) A \(\alpha\) subunit crystallite structure of the Saccharomyces cerevisiae Ran-binding protein Mog1p. J. Mol. Biol. 299, 213–223 CrossRef Medline
14. Baker, R. P., and Stewart, M. (2000) Crystallization and preliminary X-ray diffraction analysis of the Saccharomyces cerevisiae ran-binding protein Mog1p. Acta Crystallogr. D. Biol. Crystallogr. 56, 229–231 CrossRef Medline
15. Bao, X., Liu, H., Liu, X., Ruan, K., Zhang, Y., Zhang, Z., Hu, Q., Liu, Y., Akram, S., Zhang, J., Gong, Q., Wang, W., Yuan, X., Li, J., Zhao, L., Dou, Z., Tian, R., Yao, X., Wu, J., and Shi, Y. (2018) Mitosis-specific acetylation...
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16. Steggerda, S. M., and Paschal, B. M. (2001) Identification of a conserved loop in Mog1 that releases GTP from Ran. *Traffic* 2, 804–811 CrossRef Medline

17. Chakrabarti, S., Wu, X., Yang, Z., Wu, L., Yong, S. L., Zhang, C., Hu, K., Wang, Q. K., and Chen, Q. (2013) MOG1 rescues defective trafficking of Na (v) 1.5 mutations in Brugada syndrome and sick sinus syndrome. *Circ. Arrhythm. Electrophysiol.* 6, 392–401 CrossRef Medline

18. Kattygnarath, D., Maugenre, S., Neyroud, N., Balse, E., Ichai, C., Denjoy, I., Dilanian, G., Martins, R. P., Fressart, V., Berthet, M., Schott, J. J., Leenhardt, A., Probst, V., Le Marec, H., Hainque, B., Coulombe, A., Hatem, S. N., and Guicheney, P. (2011) MOG1: A new susceptibility gene for Brugada syndrome. *Circ. Cardiovasc. Genet.* 4, 261–268 CrossRef Medline

19. Marfatia, K. A., Harreman, M. T., Fanara, P., Vertino, P. M., and Corbett, A. H. (2001) Identification and characterization of the human MOG1 gene. *Gene* 266, 45–56 CrossRef Medline

20. Gui, J., Wang, T., Jones, R. P., Trump, D., Zimmer, T., and Lei, M. (2010) Multiple loss-of-function mechanisms contribute to SCN5A-related familial sick sinus syndrome. *Plos One* 5, e10985 CrossRef Medline

21. Wang, Q., Shen, J., Splawski, I., Atkinson, D., Li, Z., Robinson, J. L., Moss, A. J., Towbin, J. A., and Keating, M. T. (1995) SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome. *Cell* 80, 805–811 CrossRef Medline

22. Tian, X. L., Yong, S. L., Wan, X., Wu, L., Chung, M. K., Tchou, P. J., Rosenbaum, D. S., Van Wagoner, D. R., Kirsch, G. E., and Wang, Q. (2004) Mechanisms by which SCN5A mutation N1325S causes cardiac arrhythmias and sudden death *in vivo*. *Cardiovasc. Res.* 61, 256–267 CrossRef Medline

23. Yong, S. L., Ni, Y., Zhang, T., Tester, D. J., Ackerman, M. J., and Wang, Q. K. (2007) Characterization of the cardiac sodium channel SCN5A mutation, N1325S, in single murine ventricular myocytes. *Biochem. Biophys. Res. Commun.* 352, 378–383 CrossRef Medline

24. Zhang, T., Yong, S. L., Tian, X. L., and Wang, Q. K. (2007) Cardiac-specific overexpression of SCN5A gene leads to shorter P wave duration and PR interval in transgenic mice. *Biochem. Biophys. Res. Commun.* 355, 444–450 CrossRef Medline

25. Tian, X. L., Kadaba, R., You, S. A., Liu, M., Timur, A. A., Yang, L., Chen, Q., Szafranski, P., Rao, S., Wu, L., Housman, D. E., DiCorleto, P. E., Driscoll, D. J., Borrow, J., and Wang, Q. (2004) Identification of an angiogenic factor that when mutated causes susceptibility to Klippel-Trenaunay syndrome. *Nature* 427, 640–645 CrossRef Medline

26. Wang, Z., Yu, G., Liu, Y., Liu, S., Aridor, M., Huang, Y., Hu, Y., Wang, L., Li, S., Xiong, H., Tang, B., Li, X., Cheng, C., Chakrabarti, S., Wang, F., et al. (2018) Small GTPases SAR1A and SAR1B regulate the trafficking of the cardiac sodium channel Nav1.5. *Biochim. Biophys. Acta. Mol. Basis Dis.* 1864, 3672–3684 CrossRef Medline