DOI: 10.1161/CIRCEP.111.969972

co-transfected with pcDNA3.1-hNav1.5 and EGFP-SLMAP1-WT by about 40 % without any significant changes in the activation and inactivation kinetics of I_{Na}, and the time constants for recovery from inactivation (Table 2, Figure S6).

To exclude a possibility that the EGFP fused to SLMAP might affect the function of SLMAP or hNav1.5, we recorded I_{Na} from cells transiently transfected with pcDNA3.1-hNav1.5 and pIRES-CD8-SLMAP3 with or without mutation/variation (Figure S7 and Table S7). It was observed that pIRES-CD8-SLMAP3-V269I and -E710A decreased the peak current densities of I_{Na} to similar extent as EGFP-fused SLMAPs. The effect of SLMPAP mutations appeared to be exerted by a dominant negative mechanism, as observed for the trafficking impairment.

Binding between SLMAP and hNav1.5

Because the *SLMAP* mutations might modulate I_{Na} through a physical interaction with hNav1.5, we investigated whether SLMAP bound hNav1.5. No direct interaction between SLMAP and hNav1.5 was found under the condition where the binding of hNav1.5 and hNavβ1 could be detected (Figure S8).

Discussion

Arrhythmias can be caused by mutations in the genes encoding ion channels producing action potentials.[30] In BrS, sodium current is more frequently affected than the other currents such as calcium and potassium currents.[31] The affected sodium current is caused by mutations in the gene encoding hNav1.5, *SCN5A*, or genes for modifier proteins.[13,16-18] Prevalence of *SCN5A*

DOI: 10.1161/CIRCEP.111.969972

mutations in BrS is approximately 20%, while the prevalence of mutations in the other genes is relatively low.[31-34] In the present study, genetic analysis of *SLMAP* revealed a low prevalence of mutation two in 190 BrS patients. Functional studies of the mutations suggested that SLMAP might be a modifier protein of hNav1.5 function. Because hNav1.5 and modifier proteins compose the sodium channel complex to generate and regulate the sodium current, functional abnormality of any components of the complex might alter the electrophysiological characters of cardiomyocytes.[30]

BrS-associated mutations in genes for the components of sodium channel complex usually result in loss of hNav1.5 function, including the voltage dependent shift in the steady-state inactivation and activation profile, increased onset of inactivation, and/or decreased I_{Na}.[14] It was reported that mutations in the gene for hNavβ1, an auxiliary subunit of the sodium channel, affected the modulation of hNav1.5 channel gating.[17] Here we demonstrate that the *SLMAP* mutations do not affect the voltage dependence in inactivation or activation profiles, suggesting that the mutations do not biophysically alter the hNav1.5 channel gating. However, the *SLMAP* mutations exerted a biogenic effect by reducing the surface expression of hNav1.5, culminating in decreased peak sodium current density and BrS susceptibility.

SLMAP is a member of tail-anchored proteins, which have a single TM domain at the C-terminal end to determine the subcellular localization. Tail-anchored proteins are involved in a variety of important cellular functions such as apoptosis, protein translocation, and membrane

DOI: 10.1161/CIRCEP.111.969972

fusion in the organelles, where the proteins are anchored by the TM domain.[35] In the present study, we used SLMAPs carrying either TM1 or TM2 domain and demonstrated that the mutation-related functional alteration could be observed similarly in any of the SLMAP isoforms with different TM domains, suggesting that the impaired hNav1.5 trafficking was due to the functional alterations of SLMAP in the endoplasmic reticulum, where SLMAP with either TM1 or TM2 domain could be localized.[24] Interestingly, SLMAP regulates the translocation of insulin-regulated glucose transporter GLUT4 from an intracellular compartment to the plasma membrane in adipose tissue, demonstrating the role of SLMAP in the intracellular trafficking.[36]

We showed that the SLMAP mutants impaired the surface expression of hNav1.5. However, no direct binding of hNav1.5 and SLMAP was detected in this study, speculating that SLMAP might indirectly contribute to the action potential in cardiomyocytes by modulating hNav1.5 localization. It has been demonstrated that MOG1 binds hNav1.5 and a MOG1 mutation causes BrS via intracellular trafficking defect.[13] Then, the mechanism of impaired hNav1.5 trafficking caused by the SLMAP mutations was different from that by the MOG1 mutation. Recently, it was reported that a Z-disc protein, ZASP, formed a macromolecular complex with hNav1.5, but there was no direct interaction between ZASP and hNav1.5, and a *ZASP* mutation disturbed the hNav1.5 function without affecting the localization of hNav1.5.[37] The function of hNav1.5 in cardiomyocytes may be regulated by a fine tuning mechanism where many proteins are directly or indirectly involved. Finally, although the loss of hNav1.5

DOI: 10.1161/CIRCEP.111.969972

function is often associated with prolongation of PR and QRS, no conduction delay was observed in ECGs from both patients carrying the SLMAP mutations. This might be due to the difference in severity of functional loss or depending on the nature of affected genes. Further studies will be required to clarify the mechanisms causing the phenotypic difference in functional loss of hNav1.5.

In summary, we identified two *SLMAP* missense mutations associated with BrS, and the functional analyses indicated that mutant SLMAP biogenically impaired hNav1.5 trafficking. Like many of the BrS-associated auxiliary proteins, SLMAP-mediated BrS joins the most common pathogenic mechanism of BrS, sodium current loss-of-function BrS.

Funding Sources: This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan [(22390157, 23132507 and 23659414)(AK) and (22136007)(NM)], Health and Labor Sciences Research Grant for research on measures of intractable diseases from the Ministry of Health, Labour and Welfare, Japan [(2010-119)(AK) and (2010-145)(NM)], grants for Basic Scientific Cooperation Program between Japan and Korea from the Japan Society for the Promotion of Science (AK), National Research Foundation of Korea Grant (NRF-2010-E00024) (JEP), a research grant from the Association Française contre les Myopathie (TA), Joint Usage/Research Program of Medical Research Institute Tokyo Medical and Dental University (AK), and the Windland Smith Rice Comprehensive Sudden Cardiac Death Program at the Mayo Clinic (MJA). This work was also supported by the follow-up grants provided from the Tokyo Medical and Dental University (AK).

Conflict of Interest Disclosures: MJA is a consultant for Transgenomic/FAMILION. Intellectual property derived from MJA's research program resulted in license agreements in 2004 between Mayo Clinic Health Solutions (formerly Mayo Medical Ventures) and PGxHealth (recently acquired by Transgenomic).

DOI: 10.1161/CIRCEP.111.969972

Appendix: [1]Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan, [2]Division of Cardiology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan, [3]Departments of Medicine (Division of Cardiovascular Diseases), Pediatrics (Division of Pediatric Cardiology), and Molecular Pharmacology & Experimental Therapeutics, Windland Smith Rice Sudden Death Genomics Laboratory, Mayo Clinic, Rochester, MN, USA, [4]Institute of Human Genetics, Helmholtz Center Munich, Neuherberg, Germany, [5]Department of Molecular Medicine, Section of Cardiology, University of Pavia, Pavia, Italy, [6]Department of Cardiology, Fondazione IRCCS Policlinico S. Matteo, Pavia, Italy, [7]Cardiovascular Genetics Laboratory, Hatter Institute for Cardiovascular Research, Department of Medicine, University of Cape Town, South Africa, [8]Department of Medicine, University of Stellenbosch, South Africa, [9]Chair of Sudden Death, Department of Family and Community Medicine, College of Medicine, King Saud University, Riyadh, Saudi Arabia, [10]Division of Cardiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea, [11]Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmacuetical Sciences, Okayama, Japan, [12]Department of Cardiovascular Medicine, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan, [13]Division of Internal Medicine, Asao General Hospital, Kawasaki, Japan, [14]Department of Cardiology, Tokyo Metropolitan Hiroo Hospital, Tokyo, Japan, and [15]Department of Molecular Pathophysiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

References:

1. Brugada P, Brugada J. Right bundle branch block, persistent ST segment elevation and sudden cardiac death: a distinct clinical and electrocardiographic syndrome. A multicenter report. *J Am Coll Cardiol.* 1992;20:1391-1396.

2. Chen PS, Priori SG. The Brugada syndrome. *J Am Coll Cardiol.* 2008;51:1176-1180.

3. Hermida JS, Lemoine JL, Aoun FB, Jarry G, Rey JL, Quiret JC. Prevalence of the brugada syndrome in an apparently healthy population. *Am J Cardiol.* 2000;86:91-94.

4. Miyasaka Y, Tsuji H, Yamada K, Tokunaga S, Saito D, Imuro Y, Matsumoto N, Iwasaka T. Prevalence and mortality of the Brugada-type electrocardiogram in one city in Japan. *J Am Coll Cardiol.* 2001;38:771-774.

5. Antzelevitch C, Brugada P, Borggrefe M, Brugada J, Brugada R, Corrado D, Gussak I, LeMarec H, Nademanee K, Perez Riera AR, Shimizu W, Schulze-Bahr E, Tan H, Wilde A.

Brugada syndrome: report of the second consensus conference: endorsed by the Heart Rhythm Society and the European Heart Rhythm Association. *Circulation.* 2005;111:659-670.

6. Schulze-Bahr E, Eckardt L, Breithardt G, Seidl K, Wichter T, Wolpert C, Borggrefe M, Haverkamp W. Sodium channel gene (SCN5A) mutations in 44 index patients with Brugada syndrome: different incidences in familial and sporadic disease. *Hum Mutat.* 2003;21:651-652.

7. Kapplinger JD, Tester DJ, Alders M, Benito B, Berthet M, Brugada J, Brugada P, Fressart V, Guerchicoff A, Harris-Kerr C, Kamakura S, Kyndt F, Koopmann TT, Miyamoto Y, Pfeiffer R, Pollevick GD, Probst V, Zumhagen S, Vatta M, Towbin JA, Shimizu W, Schulze-Bahr E, Antzelevitch C, Salisbury BA, Guicheney P, Wilde AA, Brugada R, Schott JJ, Ackerman MJ. An international compendium of mutations in the SCN5A-encoded cardiac sodium channel in patients referred for Brugada syndrome genetic testing. *Heart Rhythm.* 2010;7:33-46.

8. Ueda K, Hirano Y, Higashiuesato Y, Aizawa Y, Hayashi T, Inagaki N, Tana T, Ohya Y, Takishita S, Muratani H, Hiraoka M, Kimura A. Role of HCN4 channel in preventing ventricular arrhythmia. *J Hum Genet.* 2009;54:115-121.

9. Medeiros-Domingo A, Tan BH, Crotti L, Tester DJ, Eckhardt L, Cuoretti A, Kroboth SL, Song C, Zhou Q, Kopp D, Schwartz PJ, Makielski JC, Ackerman MJ. Gain-of-function mutation S422L in the KCNJ8-encoded cardiac K(ATP) channel Kir6.1 as a pathogenic substrate for J-wave syndromes. *Heart Rhythm.* 2010;7:1466-1471.

10. Giudicessi JR, Ye D, Tester DJ, Crotti L, Mugione A, Nesterenko VV, Albertson RM, Antzelevitch C, Schwartz PJ, Ackerman MJ. Transient outward current (I(to)) gain-of-function mutations in the KCND3-encoded Kv4.3 potassium channel and Brugada syndrome. *Heart Rhythm.* 2011;8:1024-1032.

11. Burashnikov E, Pfeiffer R, Barajas-Martinez H, Delpon E, Hu D, Desai M, Borggrefe M, Haissaguerre M, Kanter R, Pollevick GD, Guerchicoff A, Laino R, Marieb M, Nademanee K, Nam GB, Robles R, Schimpf R, Stapleton DD, Viskin S, Winters S, Wolpert C, Zimmern S, Veltmann C, Antzelevitch C. Mutations in the cardiac L-type calcium channel associated with inherited J-wave syndromes and sudden cardiac death. *Heart Rhythm.* 2010;7:1872-1882.

12. Amin AS, Tan HL, Wilde AA. Cardiac ion channels in health and disease. *Heart Rhythm.* 2010;7:117-126.

13. Kattygnarath D, Maugenre S, Neyroud N, Balse E, Ichai C, Denjoy I, Dilanian G, Martins RP, Fressart V, Berthet M, Schott JJ, Leenhardt A, Probst V, Le Marec H, Hainque B, Coulombe A,

DOI: 10.1161/CIRCEP.111.969972

Hatem SN, Guicheney P. MOG1: a new susceptibility gene for Brugada syndrome. *Circ Cardiovasc Genet.* 2011;4:261-268.

14. Tfelt-Hansen J, Winkel BG, Grunnet M, Jespersen T. Inherited cardiac diseases caused by mutations in the Nav1.5 sodium channel. *J Cardiovasc Electrophysiol.* 2009;21:107-115.

15. Mohler PJ, Rivolta I, Napolitano C, LeMaillet G, Lambert S, Priori SG, Bennett V. Nav1.5 E1053K mutation causing Brugada syndrome blocks binding to ankyrin-G and expression of Nav1.5 on the surface of cardiomyocytes. *Proc Natl Acad Sci U S A.* 2004;101:17533-17538.

16. Hu D, Barajas-Martinez H, Burashnikov E, Springer M, Wu Y, Varro A, Pfeiffer R, Koopmann TT, Cordeiro JM, Guerchicoff A, Pollevick GD, Antzelevitch C. A mutation in the beta 3 subunit of the cardiac sodium channel associated with Brugada ECG phenotype. *Circ Cardiovasc Genet.* 2009;2:270-278.

17. Watanabe H, Koopmann TT, Le Scouarnec S, Yang T, Ingram CR, Schott JJ, Demolombe S, Probst V, Anselme F, Escande D, Wiesfeld AC, Pfeufer A, Kaab S, Wichmann HE, Hasdemir C, Aizawa Y, Wilde AA, Roden DM, Bezzina CR. Sodium channel beta1 subunit mutations associated with Brugada syndrome and cardiac conduction disease in humans. *J Clin Invest.* 2008;118:2260-2268.

18. London B, Michalec M, Mehdi H, Zhu X, Kerchner L, Sanyal S, Viswanathan PC, Pfahnl AE, Shang LL, Madhusudanan M, Baty CJ, Lagana S, Aleong R, Gutmann R, Ackerman MJ, McNamara DM, Weiss R, Dudley SC, Jr. Mutation in glycerol-3-phosphate dehydrogenase 1 like gene (GPD1-L) decreases cardiac Na+ current and causes inherited arrhythmias. *Circulation.* 2007;116:2260-2268.

19. Brette F, Orchard C. T-tubule function in mammalian cardiac myocytes. *Circ Res.* 2003;92:1182-1192.

20. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danieli GA. Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation.* 2001;103:196-200.

21. Tiso N, Stephan DA, Nava A, Bagattin A, Devaney JM, Stanchi F, Larderet G, Brahmbhatt B, Brown K, Bauce B, Muriago M, Basso C, Thiene G, Danieli GA, Rampazzo A. Identification of mutations in the cardiac ryanodine receptor gene in families affected with arrhythmogenic right ventricular cardiomyopathy type 2 (ARVD2). *Hum Mol Genet.* 2001;10:189-194.

22. Lahat H, Pras E, Olender T, Avidan N, Ben-Asher E, Man O, Levy-Nissenbaum E, Khoury A, Lorber A, Goldman B, Lancet D, Eldar M. A missense mutation in a highly conserved region of CASQ2 is associated with autosomal recessive catecholamine-induced polymorphic ventricular tachycardia in Bedouin families from Israel. *Am J Hum Genet.* 2001;69:1378-1384.

23. Guzzo RM, Sevinc S, Salih M, Tuana BS. A novel isoform of sarcolemmal membrane-associated protein (SLMAP) is a component of the microtubule organizing centre. *J Cell Sci.* 2004;117:2271-2281.

24. Byers JT, Guzzo RM, Salih M, Tuana BS. Hydrophobic profiles of the tail anchors in SLMAP dictate subcellular targeting. *BMC Cell Biol.* 2009;10:48.

25. Wigle JT, Demchyshyn L, Pratt MA, Staines WA, Salih M, Tuana BS. Molecular cloning, expression, and chromosomal assignment of sarcolemmal-associated proteins. A family of acidic amphipathic alpha-helical proteins associated with the membrane. *J Biol Chem.* 1997;272:32384-32394.

26. Benito B, Brugada R, Brugada J, Brugada P. Brugada syndrome. *Prog Cardiovasc Dis.* 2008;51:1-22.

27. Ackerman MJ, Tester DJ, Jones GS, Will ML, Burrow CR, Curran ME. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. *Mayo Clin Proc.* 2003;78:1479-1487.

28. Makita N, Behr E, Shimizu W, Horie M, Sunami A, Crotti L, Schulze-Bahr E, Fukuhara S, Mochizuki N, Makiyama T, Itoh H, Christiansen M, McKeown P, Miyamoto K, Kamakura S, Tsutsui H, Schwartz PJ, George AL, Jr., Roden DM. The E1784K mutation in SCN5A is associated with mixed clinical phenotype of type 3 long QT syndrome. *J Clin Invest.* 2008;118:2219-2229.

29. Abramoff MD, Magelhaes PJ, Ram SJ. Processing with ImageJ. *Biophoton Int.* 2004;11:36-42.

30. Abriel H. Cardiac sodium channel Na(v)1.5 and interacting proteins: Physiology and pathophysiology. *J Mol Cell Cardiol.* 2010;48:2-11.

31. Hedley PL, Jorgensen P, Schlamowitz S, Moolman-Smook J, Kanters JK, Corfield VA, Christiansen M. The genetic basis of Brugada syndrome: a mutation update. *Hum Mutat.*

2009;30:1256-1266.

32. Eckardt L, Probst V, Smits JP, Bahr ES, Wolpert C, Schimpf R, Wichter T, Boisseau P, Heinecke A, Breithardt G, Borggrefe M, LeMarec H, Bocker D, Wilde AA. Long-term prognosis of individuals with right precordial ST-segment-elevation Brugada syndrome. *Circulation.* 2005;111:257-263.

33. Priori SG, Napolitano C, Gasparini M, Pappone C, Della Bella P, Giordano U, Bloise R, Giustetto C, De Nardis R, Grillo M, Ronchetti E, Faggiano G, Nastoli J. Natural history of Brugada syndrome: insights for risk stratification and management. *Circulation.* 2002;105:1342-1347.

34. Crotti L, Kellen CH, Tester D, Castelletti S, Giudessi JR, Torchio M, Medeiros-Domingo A, Savastano S, Will ML, Dagradi F, Schwartz PJ, Ackerman MJ. Spectrum and prevalence of Mutations Involving BrS1-12-Susceptibility Genes in a Cohort of Unrelated Patients Referred for Brugada Syndrome Genetic Testing: Implications for Genetic Testing, *J Am Col Cardiol.* 2012; in press (doi.org/10.1016/j.jacc.2012.04.037)

35. Borgese N, Fasana E. Targeting pathways of C-tail-anchored proteins. *Biochim Biophys Acta.* 2011;1808:937-946.

36. Chen X, Ding H. Increased Expression of the Tail-Anchored Membrane Protein SLMAP in Adipose Tissue from Type 2 Tally Ho Diabetic Mice. *Exp Diabetes Res.* 2011;2011:421982.

37. Li Z, Ai T, Samani K, Xi Y, Tzeng HP, Xie M, Wu S, Ge S, Taylor MD, Dong JW, Cheng J, Ackerman MJ, Kimura A, Sinagra G, Brunelli L, Faulkner G, Vatta M. A ZASP missense mutation, S196L, leads to cytoskeletal and electrical abnormalities in a mouse model of cardiomyopathy. *Circ Arrhythm Electrophysiol.* 2010;3:646-656.

DOI: 10.1161/CIRCEP.111.969972

Table 1. Sequence variations in exons of SLMAP found in BrS patients and controls

	Location in exon	Position at Codon*	Nucleotide change and corresponding amino acid in parenthesis	Asian BrS patients† (n=88)	Japanese Controls (n=94-380)	Caucasian BrS patients (n=102)	dbSNP
1	Exon 1	24	CTG (Leu) to CTA (Leu)	0	0 in 187	1	
2	Exon 1	31	GGC (Gly) to GGT (Gly)	1	3 in 187	0	
3	Exon 2	68	TAT (Tyr) to TTT (Phe)	6	3 in 174	0	
4	Exon 6	193	CTA (Leu) to CTG (Leu)	1	8 in 362	0	
5	Exon 7	217	TTA (Leu) to TTG (Leu)	3	4 in 269	0	rs74857771
6	Exon 8	269	GTT (Val) to ATT (Ile)	1	0 in 380	0	
7	Exon 9	288	CAT (His) to TAT (Tyr)	1	1 in 380	0	
8	Exon 14	408	GGG (Gly) to GGT (Gly)	1	3 in 180	0	
9	Exon 16	447	GAC (Asp) to GAT (Asp)	52	55 in 94	0	rs17058639
10	Exon 19	622	CTT (Leu) to CTA (Leu)	0	0 in 192	4	rs35219531
11	Exon 19	630	CAG (Gln) to CGG (Arg)	0	0 in 192	1	rs35029175
12	Exon 21	681	CAG (Gln) to CAA (Gln)	0	1 in 380	11	rs17745469
13	Exon 21	710	GAA (Glu) to GCA (Ala)	1	0 in 380	0	

*; codon number is that for SLMAP3
†; Japanese patients (n=85) and Korean patients (n=3)

DOI: 10.1161/CIRCEP.111.969972

Table 2. Electrophysiological properties of transfected tsA-201 cells of pcDNA3.1-hNav1.5 and SLMAP constructs

A) with SLMAP3 constructs

	WT-TM1	n	V269I-TM1	n	H288Y-TM1	n	E710A-TM1	n	WT-TM2	n	V269I-TM2	n	H288Y-TM2	n	E710A-TM2	n
Current density at -30mV (pA/pF)	-336.2±63.6	11	-146.3±10.7†	9	-310.3±63.7	7	-169.2±15.7†	16	-373.2±45.9	13	-179.5±26.1†	11	-283.2±55.7	12	-221.4±40.6†	13
Voltage dependence of inactivation ($V_{1/2}$, mV)	-84.72±1.26	12	-86.13±0.98	9	-84.28±1.54	7	-84.47±1.15	15	-86.31±0.94	15	-86.32±0.79	13	-83.4±1.15	15	-85.36±1.37	15
Voltage dependence of activation ($V_{1/2}$, mV)	-46.40±1.85	8	-44.62±1.00	9	-47.46±1.72	7	-43.68±1.09	20	-47.07±1.25	13	-47.23±1.17	9	-45.51±1.59	12	-44.11±1.34	12
Time required for e^{-1} fraction recovery (msec)	8.39±1.16	9	9.34±1.28	9	9.88±1.21	7	9.40±1.29	17	9.31±1.11	11	9.11±1.00	9	9.42±2.11	12	9.40±1.29	14

B) with SLMAP1 constructs and no SLMAP control

	WT-TM1	n	E261A-TM1	n	WT-TM2	n	E261A-TM2	n	EGFP-C1	n
Current density at -30mV (pA/pF)	-413.6±52.6	8	-253.4±44.5†	14	-410.2±39.7	11	-246.6±26.7*	13	-394.0±65.8	15
Voltage dependence of inactivation ($V_{1/2}$, mV)	-86.61±1.29	9	-87.46±1.25	15	-80.46±1.19	12	-81.69±0.98	13	-83.25±1.41	15
Voltage dependence of activation ($V_{1/2}$, mV)	-49.29±2.14	8	-43.19±1.60	14	-45.99±1.28	11	-41.39±1.20	13	-49.83±1.99	15
Time required for e^{-1} fraction recovery (msec)	6.75±0.61	15	7.35±0.55	14	8.67±0.67	10	7.93±1.16	12	7.85±0.89	15

*: $p<0.001$
†: $p<0.05$ *versus* WT

DOI: 10.1161/CIRCEP.111.969972

Figure Legends:

Figure 1. Mutational analysis of *SLMAP* gene in BrS. **A**, Structure of *SLMAP* and sequence variations found in this study. **B** and **C**, Representative ECG records of the patients carrying V269I (B) or E710A (C). Both of them showed no apparent cardiac conduction delay.

Figure 2. Fluorescence images of transiently expressed EGFP-SLMAP in HEK293 cells. **A**, Representative images of HEK293 cells co-transfected with L1-Flag-hNav1.5 and EGFP-SLMAP3-TM1-WT (*a* and *b*), -SLMAP3-TM1-V269I (*c* and *d*), -SLMAP3-TM1-H288Y (*e* and *f*), -SLMAP3-TM1-E710A (*g* and *h*), -SLMAP3-TM2-WT (*i* and *j*), -SLMAP3-TM2-V269I (*k* and *l*), -SLMAP3-TM2-H288Y (*m* and *n*), -SLMAP3-TM2-E710A (*o* and *p*), EGFP-SLMAP1-TM1-WT (*q* and *r*), -SLMAP1-TM1-E261A (*s* and *t*), -SLMAP1-TM2-WT (*u* and *v*), or -SLMAP1-TM2-E261A (*w* and *x*). The cells were permeabilized and stained with anti-Flag Ab (red; *a*, *c*, *e*, *g*, *i*, *k*, *m*, *o*, *q*, *s*, *u*, and *w*). Expression of EGFP-SLMAP is shown in *b*, *d*, *f*, *h*, *j*, *l*, *n*, *p*, *r*, *t*, *v*, and *x* (green). Scale bar, 10μm. **B**, The PTAFI ratio of expressed L1-Flag-hNav1.5 in the transfected cells. Numbers of the analyzed cells are indicated at the bottom of each bar. *, $p<0.001$; †, $p<0.01$

Figure 3. Fluorescence images of transiently expressed EGFP-SLMAP and pcDNA3.1-SLMAP in HEK293 cells. **A**, Representative images of HEK293 cells co-transfected with L1-Flag-hNav1.5 and EGFP-SLMAP3-TM1-WT (*a* and *b*), -V269I (*c* and *d*), -H288Y (*e* and *f*), -E710A (*g* and *h*), EGFP-WT plus pcDNA3.1-V269I (*i* and *j*), EGFP-V269I plus pcDNA3.1-WT (*k* and *l*), EGFP-WT plus pcDNA3.1-E710A (*m* and *n*), or EGFP-E710A plus pcDNA3.1-WT (*o*

DOI: 10.1161/CIRCEP.111.969972

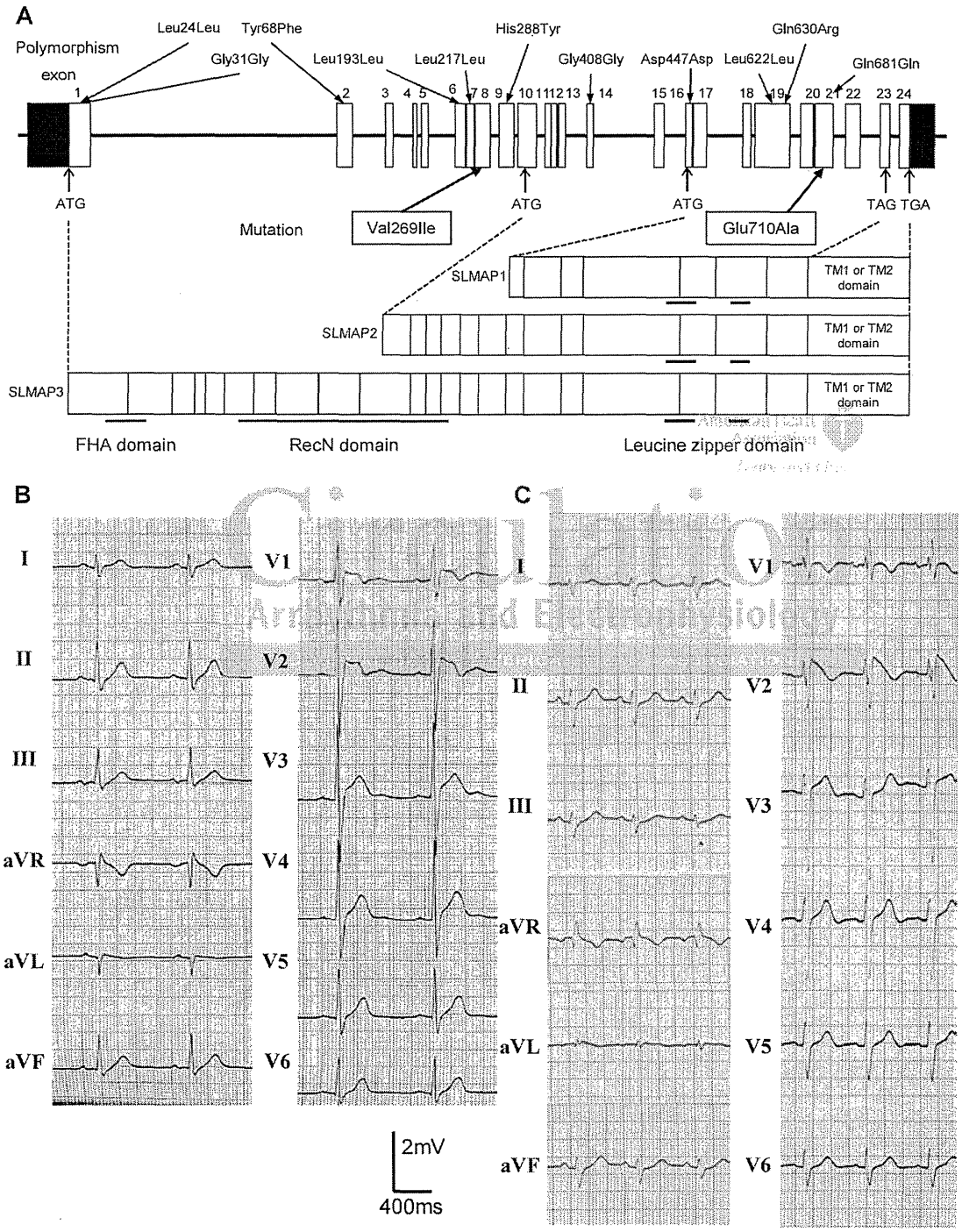
and *p*), The cells were permeabilized and stained with anti-Flag Ab (red; *a*, *c*, *e*, *g*, *i*, *k*, *m*, and *o*). Expression of EGFP-SLMAP is shown in *b*, *d*, *f*, *h*, *j*, *l*, *n*, and *p* (green). Scale bar, 10μm. **B**, The PTAFI ratio of expressed L1-Flag-hNav1.5 in the transfected cells. Numbers of the analyzed cells are indicated at the bottom of each bar. *, p<0.001

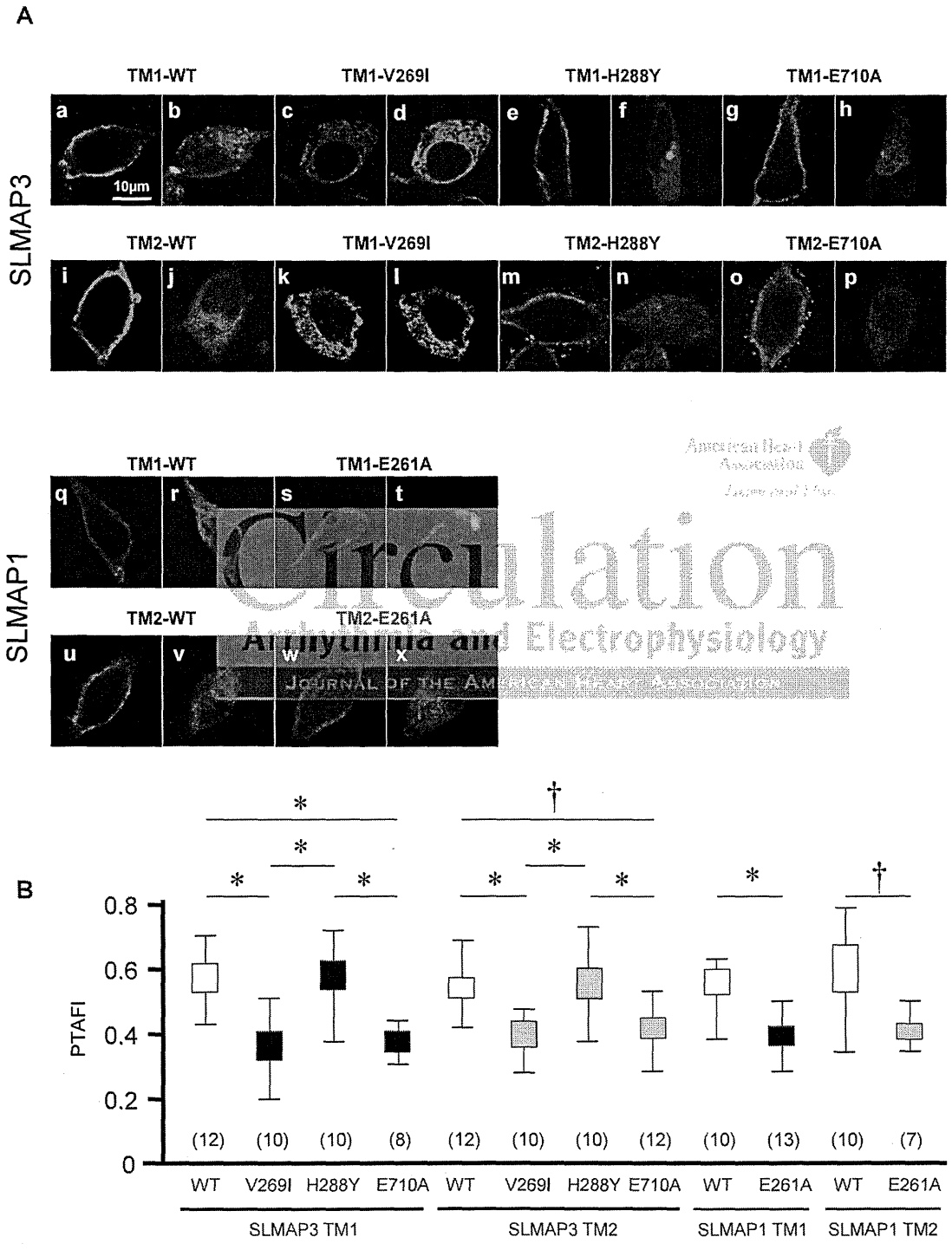
Figure 4. Silencing of transiently expressed *SLMAP3* in HEK293 cells. **A**, Representative images of HEK293 cells co-transfected with L1-Flag-hNav1.5 and EGFP-SLMAP3-TM1-WT (*a*, *b*, *i* and *j*), -SLMAP3-TM1-V269I(*c*, *d*, *k* and *l*), -SLMAP3-TM1-H288Y (*e*, *f*, *m* and *n*), -SLMAP3-TM1-E710A (*g*, *h*, *o* and *p*), -SLMAP3-TM2-WT (*q*, *r*, *y* and *z*), -SLMAP3-TM2-V269I (*s*, *t*, *a'* and *b'*), -SLMAP3-TM2-H288Y (*u*, *v*, *c'* and *d'*), or -SLMAP3-TM2-E710A (*w*, *x*, *e'* and *f'*), in the presence of non-silencing (*a-h* and *q-x*) or pre-designed (*i-p* and *y-f'*) siRNA. The cells were permeabilized and stained with anti-Flag Ab (red, *a*, *c*, *e*, *g*, *i*, *k*, *m*, *o*, *q*, *s*, *u*, *w*, *y*, *a'*, *c'* and *e'*). Expression of EGFP-SLMAP3 was shown in *b*, *d*, *f*, *h*, *j*, *l*, *n*, *p*, *r*, *t*, *v*, *x*, *z*, *b'*, *d'* and *f'* (green). Scale bar, 10μm. **B**, The PTAFI ratio of expressed L1-Flag-hNav1.5 in the transfected cells. Numbers of analyzed cells are indicated at the bottom of each bar. Silencing of EGFP-SLMAP3 by pre-designed siRNA against human SLMAP (s15435) was shown in the lower panel. PTAFIs were compared between the cells transfected with non-silencing siRNA and with SLMAP-siRNA. *, p<0.001; †, p<0.01; ǂ, p<0.05

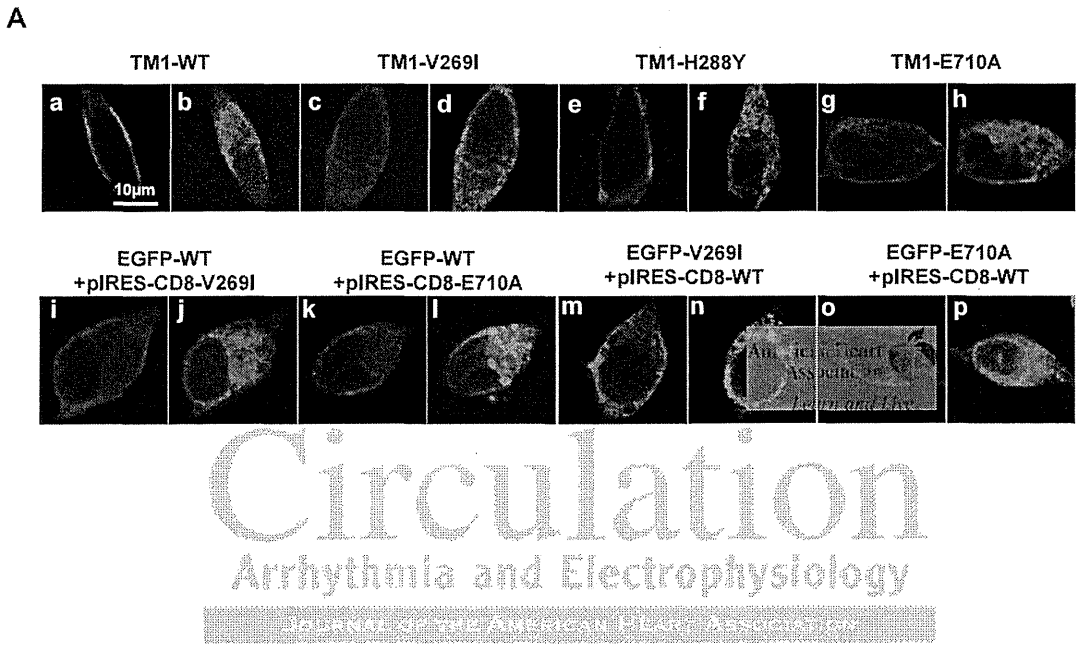
Figure 5. Sodium currents recorded from tsA-201 cells co-transfected with Nav1.5 and SLMAP3-TM1 constructs. **A**, Representative sodium currents were recorded from transfected tsA-201 cells of pcDNA3.1-hNav1.5 with EGFP, EGFP-SLMAP3-WT, -SLMAP3-V269I, -SLMAP3-H288Y, or -SLMAP3-E710A, with TM1 domain. These traces were recorded with

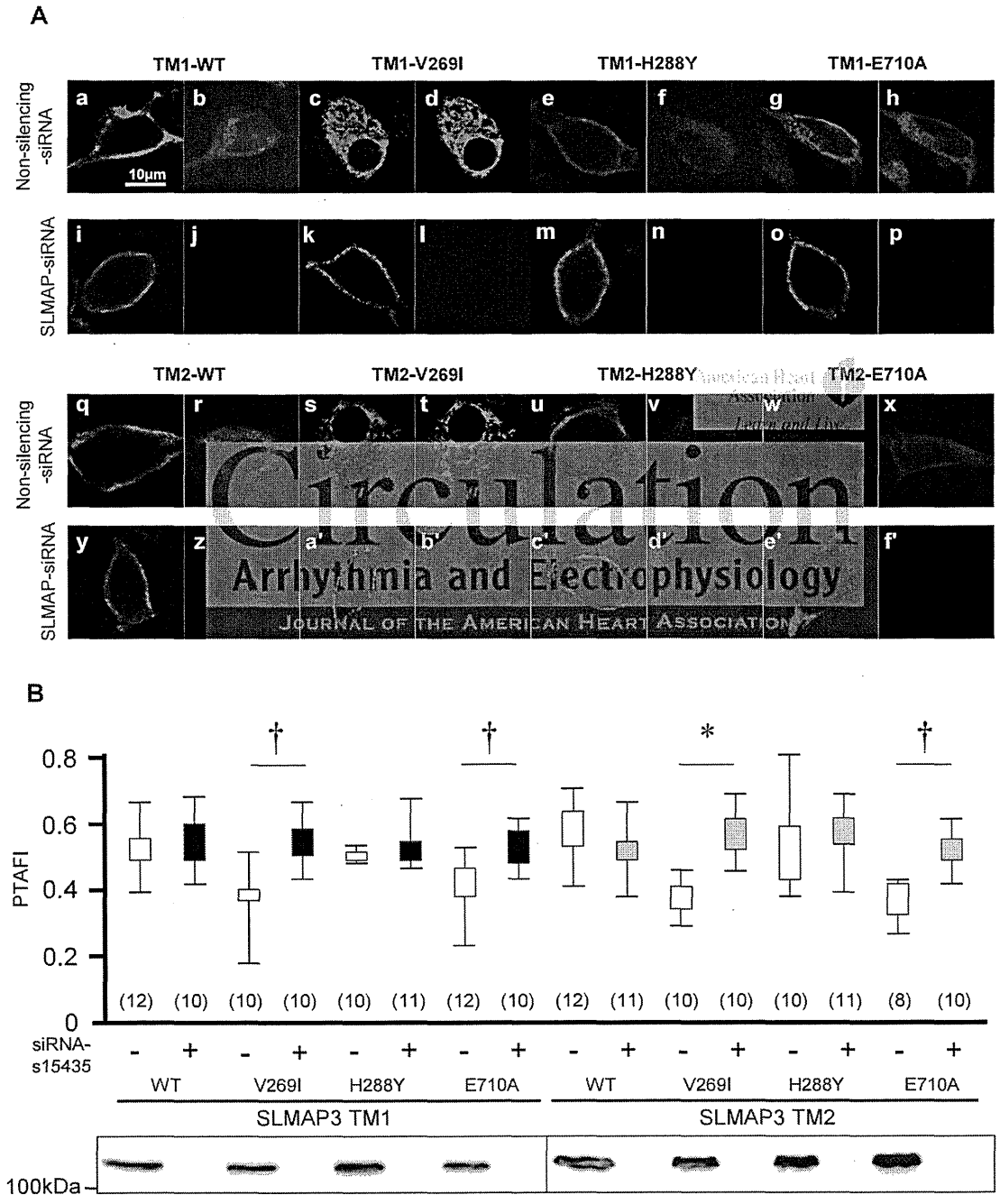
DOI: 10.1161/CIRCEP.111.969972

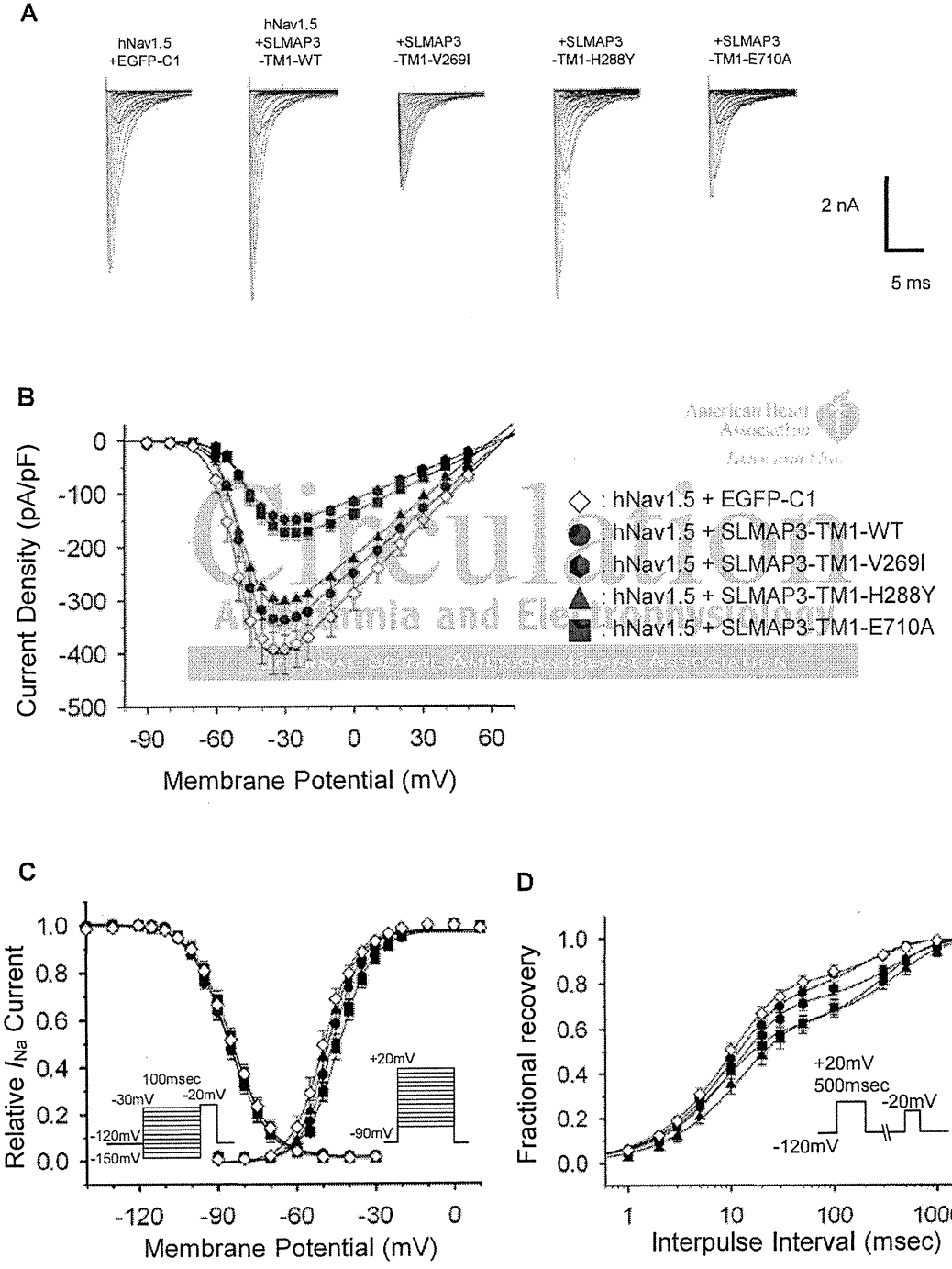
the whole-cell configuration as shown in the inset. **B**, Current-voltage relationship for peak I_{Na}. EGFP-SLMAP3-V269I and -E710A showed a significant decline of peak current densities by 56.5% and 49.7% (n=11 for WT, n=9 for V269I, n=16 for E710A) at -30mV, whereas EGFP-SLMAP3-H288Y (n=7) did not alter the peak current density. **C**, The voltage dependence of steady-state fast inactivation and activation recorded from the transfected cells of pcDNA3.1-hNav1.5 in combination with EGFP, -SLMAP3-V269I, SLMAP3-H288Y or -SLMAP3-E710A with TM1 domain, were similar to that from the cells co-transfected with pcDNA3.1-hNav1.5 and EGFP-SLMAP3-WT, with TM1 domain. **D**, Recovery from inactivation assessed by the double-pulse protocol was nearly identical among WT, V269I, H288Y and E710A in SLMAP3. The 2 pulse protocol is shown in the inset.


A
Polymorphism
exon
Leu24Leu
Tyr68Phe
Gly31Gly
Leu193Leu
Leu217Leu
His288Tyr
Gly408Gly
Asp447Asp
Leu622Leu
Gln630Arg
Gln681Gln
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
ATG
Mutation
Val269Ile
ATG
ATG
Glu710Ala
TAG TGA
SLMAP1
SLMAP2
SLMAP3
TM1 or TM2 domain
FHA domain
RecN domain
Leucine zipper domain
B
I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6
2mV
400ms
C
I
II
III
aVR
aVL
aVF
V1
V2
V3
V4
V5
V6






A
TM1-WT
TM1-V269I
TM1-H288Y
TM1-E710A
Non-silencing -siRNA
SLMAP-siRNA
10μm
TM2-WT
TM2-V269I
TM2-H288Y
TM2-E710A
Non-silencing -siRNA
SLMAP-siRNA
B
PTAFI
0.8
0.6
0.4
0.2
0
(12) (10) (10) (10) (10) (11) (12) (10) (12) (11) (10) (10) (10) (11) (8) (10)
siRNA-s15435
- + - + - + - + - + - + - + - +
WT V269I H288Y E710A WT V269I H288Y E710A
SLMAP3 TM1
SLMAP3 TM2
100kDa


A
hNav1.5
+EGFP-C1
hNav1.5
+SLMAP3
-TM1-WT
+SLMAP3
-TM1-V269I
+SLMAP3
-TM1-H288Y
+SLMAP3
-TM1-E710A
2 nA
5 ms
B
Current Density (pA/pF)
0
-100
-200
-300
-400
-500
-90
-60
-30
0
30
60
Membrane Potential (mV)
◇ : hNav1.5 + EGFP-C1
● : hNav1.5 + SLMAP3-TM1-WT
⬢ : hNav1.5 + SLMAP3-TM1-V269I
▲ : hNav1.5 + SLMAP3-TM1-H288Y
■ : hNav1.5 + SLMAP3-TM1-E710A
C
Relative I_{Na} Current
1.0
0.8
0.6
0.4
0.2
0.0
100msec
-30mV
-20mV
-120mV
-150mV
+20mV
-90mV
-120
-90
-60
-30
0
Membrane Potential (mV)
D
Fractional recovery
1.0
0.8
0.6
0.4
0.2
0.0
+20mV
500msec
-20mV
-120mV
1
10
100
1000
Interpulse Interval (msec)

Advance Publication by-J-STAGE

Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

Novel *SCN3B* Mutation Associated With Brugada Syndrome
Affects Intracellular Trafficking and Function of Nav1.5

Taisuke Ishikawa, BSc; Naohiko Takahashi, MD; Seiko Ohno, MD; Harumizu Sakurada, MD; Kazufumi Nakamura, MD; Young Keun On, MD; Jeong Euy Park, MD; Takeru Makiyama, MD; Minoru Horie, MD; Takuro Arimura, PhD; Naomasa Makita, MD; Akinori Kimura, MD

Background: Brugada syndrome (BrS) is characterized by specific alterations on ECG in the right precordial leads and associated with ventricular arrhythmia that may manifest as syncope or sudden cardiac death. The major causes of BrS are mutations in *SCN5A* for a large subunit of the sodium channel, Nav1.5, but a mutation in *SCN3B* for a small subunit of sodium channel, Navβ3, has been recently reported in an American patient.

Methods and Results: A total of 181 unrelated BrS patients, 178 Japanese and 3 Koreans, who had no mutations in *SCN5A*, were examined for mutations in *SCN3B* by direct sequencing of all exons and adjacent introns. A mutation, Val110Ile, was identified in 3 of 178 (1.7%) Japanese patients, but was not found in 480 Japanese controls. The *SCN3B* mutation impaired the cytoplasmic trafficking of Nav1.5, the cell surface expression of which was decreased in transfected cells. Whole-cell patch clamp recordings of the transfected cells revealed that the sodium currents were significantly reduced by the *SCN3B* mutation.

Conclusions: The Val110Ile mutation of *SCN3B* is a relatively common cause of *SCN5A*-negative BrS in Japan, which has a reduced sodium current because of the loss of cell surface expression of Nav1.5.

Key Words: Brugada syndrome; Electrophysiologic study; Genetics; Ion channels; Sodium

Brugada syndrome (BrS) is a cardiac channelopathy characterized by specific findings, such as accentuated J wave and ST-segment elevation in the right precordial leads on ECG, in the absence of structural heart diseases.[1–3] BrS patients sometimes suffer from syncope, and have a risk of sudden cardiac death caused by rapid polymorphic ventricular tachycardia or ventricular fibrillation.[1–3] Approximately 35% of BrS patients have a family history of the disease, which is consistent with the autosomal dominant inheritance, and mutations in 12 different genes have been reported as associated with BrS, of which the majority are mutations in *SCN5A* encoding a large subunit of the cardiac sodium channel Nav1.5.[3–12] The prevalence of BrS in East Asia including Japan is much higher, reaching 1 in 1,000–2,000, than the worldwide prevalence of approximately 1 in 10,000.[13–15]

Editorial p???

In cardiomyocytes, 5 distinct sodium channel β-subunits, Navβ1, Navβ1b, β2, β3 and β4, are known to be expressed. In particular, Navβ1 and Navβ3, encoded by *SCN1B* and *SCN3B*, are abundantly expressed, and these auxiliary β-subunits and a pore-forming subunit, Nav1.5, comprise the cardiac sodium channel complex.[4,16] Inward sodium current (I_{Na}) generated by the sodium channel complex is crucial for the cardiac action potential,[16,17] and functional alterations of I_{Na} caused by gene mutations have been reported in a wide range of arrhythmias, including long QT syndrome,[18] idiopathic ventricular fibrillation (IVF),[19] sudden infant death syndrome (SIDS),[20] and atrial fibrillation (AF).[21] In BrS patients, disease-causing mutations were found not only in *SCN5A* but also in the genes

Received August 1, 2012; revised manuscript received October 13, 2012; accepted November 16, 2012; released online December 21, 2012 Time for primary review: 35 days

Department of Molecular Pathogenesis, Medical Research Institute, and Division of Genetic Regulation, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo (T.I., T.A., A.K.); Department of Laboratory Examination and Diagnostics, Oita University, Oita (N.T.); Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Otsu (S.O., M.H.); Department of Cardiology, Tokyo Metropolitan Hiroo Hospital, Tokyo (H.S.); Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama (K.N.); Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto (T.M.); Department of Molecular Pathophysiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki (N.M.), Japan; and Division of Cardiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul (Y.K.O., J.E.P.), Korea

Mailing address: Akinori Kimura, MD, PhD, Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo113-8510, Japan. E-mail: akitis@mri.tmd.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-12-0995