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Loss-of-Function of the Voltage-Gated Sodium Channel $Na_V1.5$ (Channelopathies) in Patients With Irritable Bowel Syndrome

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BACKGROUND & AIMS: *SCN5A* encodes the α-subunit of the voltage-gated sodium channel $Na_V1.5$. Many patients with cardiac arrhythmias caused by mutations in *SCN5A* also have symptoms of irritable bowel syndrome (IBS). We investigated whether patients with IBS have *SCN5A* variants that affect the function of $Na_V1.5$. **METHODS:** We performed genotype analysis of *SCN5A* in 584 persons with IBS and 1380 without IBS (controls). Mutant forms of *SCN5A* were expressed in human embryonic kidney-293 cells, and functions were assessed by voltage clamp analysis. A genome-wide association study was analyzed for an association signal for the *SCN5A* gene, and replicated in 1745 patients in 4 independent cohorts of IBS patients and controls. **RESULTS:** Missense mutations were found in *SCN5A* in 13 of 584 patients (2.2%, probands). Diarrhea-predominant IBS was the most prevalent form of IBS in the overall study population (25%). However, a greater percentage of individuals with *SCN5A* mutations had constipation-predominant IBS (31%) than diarrhea-predominant IBS (10%; $P < .05$). Electrophysiologic analysis showed that 10 of 13 detected mutations disrupted $Na_V1.5$ function (9 loss-of-function and 1 gain-of-function function). The p. A997T-$Na_V1.5$ had the greatest effect in reducing $Na_V1.5$ function. Incubation of cells that expressed this variant with mexiletine restored their sodium current and administration of mexiletine to 1 carrier of this mutation (who had constipation-predominant IBS) normalized their bowel habits. In the genome-wide association study and 4 replicated studies, the *SCN5A* locus was strongly associated with IBS. **CONCLUSIONS:** About 2% of patients with IBS carry mutations in *SCN5A*. Most of these are loss-of-function mutations that disrupt $Na_V1.5$ channel function. These findings provide a new pathogenic mechanism for IBS and possible treatment options.

Keywords: Genetics; GI Motility; Voltage-Gated Sodium Channel; Polymorphism.

Irritable bowel syndrome (IBS) is a highly prevalent disorder affecting 15%–20% of the Western world's population.[1] IBS pathophysiology involves abnormalities in gastrointestinal (GI) motility and visceral sensory

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Abbreviations used in this paper: **D, domain; ECG, electrocardiogram; GI, gastrointestinal; GOF, gain of function; GWAS, genomewide association study; HEK, human embryonic kidney; IBS, irritable bowel syndrome; IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; ICC, interstitial cells of Cajal; IDL, interdomain linker; I_{peak}, peak current; LOF, loss of function; Na_V, voltage-gated sodium channel; t_{peak}, time to peak; $V_{1/2a}$, half point of the voltage dependence of activation; $V_{1/2i}$, half point of the voltage dependence of inactivation; δV_a, slope of the voltage dependence of activation; δV_i, slope of the voltage dependence of inactivation.**

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processing.[2] Familial aggregation[3] and twin studies[4] suggest that genetics play a role in IBS. Genotyping supports this notion, but the specific impact of individual genes remains unclear.[5] Ion channels are excellent pathophysiologic and therapeutic targets because they are involved directly in both GI motility[6,7] and visceral pain.[8] Therefore, ion channelopathies may cause IBS in some cases.

Voltage-gated sodium channels (Na_V) are present in the gastrointestinal smooth muscle, including rat fundus,[9] human and canine jejunum,[10,11] and rat and human colon.[12] A particular tetrodotoxin-resistant sodium channel, $Na_V1.5$ (encoded by *SCN5A*), is expressed in human smooth muscle cells and the interstitial cells of Cajal (ICC) of the small intestine and colon.[13,14] Among other functions, ICC are GI pacemakers that generate cyclic depolarizations (slow waves) transmitted to the smooth muscle to provide the electrical stimulus for contraction.[15] In human GI smooth muscle, Na_V channels appear to be excitatory for slow waves[14] and $Na_V1.5$ is functionally relevant in the human GI tract, as pharmacologic block of $Na_V1.5$ is associated with constipation.[16]

SCN5A also is expressed densely in human cardiomyocytes.[17] *SCN5A* rare mutations[18] and common variants[19] are associated with cardiac arrhythmias. Interestingly, patients with arrhythmia-predisposing mutations in *SCN5A* have more gastrointestinal symptoms and an increased prevalence of IBS when compared with patients with other arrhythmia-related ion channelopathies.[20] Conversely, a subset of patients with IBS may have *SCN5A* mutations despite a normal cardiac phenotype. In a pilot study, a rare *SCN5A* missense mutation was found in a patient with IBS and no cardiac conduction abnormalities. This mutation resulted in $Na_V1.5$ channels with decreased peak currents and mechanosensitivity.[21] In the present study, we screened large cohorts of IBS patients to determine the prevalence of *SCN5A* polymorphisms and mutations in IBS, tested whether the identified mutations led to altered $Na_V1.5$ function, and successfully treated an IBS-C patient with the *SCN5A* mutation, which had resulted in the most severe electrophysiology abnormalities. These data may represent a novel pathophysiologic mechanism and provide new therapeutic options for a subset of IBS patients.

Materials and Methods

Subjects

The Mayo Clinic Institutional Review Board approved the study. The mutation discovery cohort included patients (n = 584) aged 18–69 years recruited between February 2004 and July 2005 at the Mayo Clinic Rochester. The cohort used for replication of an independent genome wide association study (GWAS) for *SCN5A* (n = 1745) included additional patients from the United States (Mayo Clinic), and multicenter cohorts from Sweden, Italy, and Greece, as detailed in the Supplementary Materials and Methods section.

Genetic Analysis of SCN5A

Genetic analysis was described previously[22] and the GWAS data and analysis are both detailed in the Supplementary Materials and Methods section.

Expression Vector Construction and Human Embryonic Kidney-293 Cell Transfection

Previously described[21] site-directed mutagenesis (Primers in Supplementary Table 1) and co-transfection (pEGFP-C1 and channel constructs) into human embryonic kidney (HEK)-293 cells are detailed in the Supplementary Materials and Methods section.

Whole-Cell Electrophysiology

Solutions and whole-cell voltage clamp set-up have been described previously[21] and are detailed in the Supplementary Materials and Methods section. Voltage-clamp protocols were designed de novo for this study and are described in detail in the Supplementary Materials and Methods section.

Voltage-Clamp Protocols

Definition of electrophysiologic loss and gain of function. Loss of function (LOF) was defined as a decrease in the overall Na^+ charge flux that would result from a decrease in the peak current (I_{peak}), slowed time to peak (t_{peak}), a depolarization (right-shift) in the half point of the voltage dependence of activation ($V_{1/2a}$), and a decrease in the slope of the voltage dependence of activation (δV_a); or as a hyperpolarization (left-shift) of the half point of the voltage dependence of inactivation ($V_{1/2i}$), a decrease in the slope of the voltage dependence of inactivation (δV_i), and a decrease of the time constants of inactivation (τ_s, τ_f). Gain of function (GOF) was defined as an increase in overall Na^+ charge flux with changes opposite of those described earlier for loss of function.

Clinical case of mexiletine treatment of an A997T-$Na_V1.5$ patient with constipation-predominant IBS. The study team performed a prospective open-label study on the effect of mexiletine on bowel habits and whole-gut transit with 48-hour colon transit measurements completed at baseline and after the 5-day treatment period (Clinicaltrials.gov: NCT01717404). Details are provided in the Supplementary Materials and Methods section.

Results

IBS Patient Cohort

The characteristics of the mutation discovery cohort are shown in Table 1. The median age was 49.5 years; they were predominately Caucasian (94%) women (83%). Diarrhea-predominant IBS (IBS-D, 25%) was more common than constipation-predominant IBS (IBS-C, 10%; Table 1). The remainder of the cohort was mixed-subtype IBS (31%) and those who could not be classified by our questionnaires. IBS patients from the GWAS replication cohorts had similar characteristics (Supplementary Table 2).

IBS Subjects With SCN5A Variations and Confirmation of the Findings in a GWAS

Thirteen of the 584 (2.2%) subjects had unique *SCN5A* amino acid–altering missense mutations (Figure 1). The 13 missense mutations were not observed in 2760 reference alleles. The demographics of the subjects with *SCN5A* mutations (probands) were no different from the cohort

Table 1. Patient Cohort Characteristics

	IBS cases (n = 584)	IBS cases with SCN5A mutation (n = 13)
Median age, *y* (range)	49.5 (18.0–70.0)	49.8 (30.0–62.0)
Female, n (%)	484 (83)	11 (85)
Race		
Caucasian, n (%)	549 (94)	12 (92)
Non-Caucasian, n (%)	35 (6)	
Not stated, n (%)		1 (8)
Region		
Local, n (%)	396 (68)	10 (77)
National, n (%)	187 (32)	3 (23)
Met Rome II criteria, n (%)	328 (59)	
IBS subtype		
IBS-C, n (%)	59 (10)	4 (31)
IBS-D, n (%)	143 (25)	3 (23)
IBS-M, n (%)	181 (31)	3 (23)
Other, n (%)	201 (34)	3 (23)

IBS-M, mixed-subtype IBS.

described earlier (Table 1). However, unlike the IBS patient cohort, the probands were more often IBS-C (31%) than IBS-D (10%; $P < .05$) (Table 1). This subset of IBS subjects with a *SCN5A* mutation had normal QTc (424 ± 22 ms) and PR (164 ± 42 ms) intervals. All electrocardiograms (ECGs) were reviewed and although electrophysiologic abnormalities were discovered, none were formally diagnostic (detailed in the Supplementary Materials and Methods section). There were also 9 distinct polymorphisms detected in 17 (2.9%) subjects. All polymorphisms had been characterized previously and shown to have electrophysiologic abnormalities (Supplementary Table 3).

To independently evaluate the association of *SCN5A* polymorphisms with IBS we inspected data from a Swedish GWAS of IBS, and an association signal of nominal significance was detected for *SCN5A* (Supplementary Figure 1). We followed-up this signal by genotyping 17 *SCN5A* single nucleotide polymorphisms in 1745 additional individuals from 4 independent IBS cohorts from Sweden, Italy, Greece, and the United States. Several gave rise to stronger associations in a meta-analysis of GWAS and replication data (Supplementary Table 4).

Molecular Characteristics of the Identified $Na_V1.5$ Mutations

SCN5A encodes $Na_V1.5$, a 2016–amino acid transmembrane protein with 4 homologous domains (DI-DIV) of 6 transmembrane segments each. One of the 13 identified mutations localized to the N-terminus, 4 to the C-terminus, 6 resided in the interdomain linkers (IDL), and 2 in the transmembrane segments of DI and DIII (Figure 1). Six mutations had been associated previously with cardiac conduction pathologies: 4 were associated with Brugada type 1 (A997T, T220I, G615E, P648L),[23] 2 were associated with long-QT type 3 (G615E,[24,25] T1304M[26]), 1 was associated with sudden infant death syndrome (T1304M[27,28]), 1 was associated with sick sinus syndrome (T220I[29,30]), and 1 was associated with sudden death in women (G615E[31]). The other 7 mutations were novel (I94V, T630M, G1158S, R1512Q, E1780G, A1870D, L1896V, and M1952T). However, even for the previously identified mutations, limited functional data were available for only 3: T220I,[29,30] G615E,[31] and T1304M.[27]

Electrophysiologic Examination of $Na_V1.5$ Mutations

All 13 missense mutations were inserted by site-directed mutagenesis into the most common *SCN5A* background H558/Q1077del (wild type), transfected into HEK-293 cells,

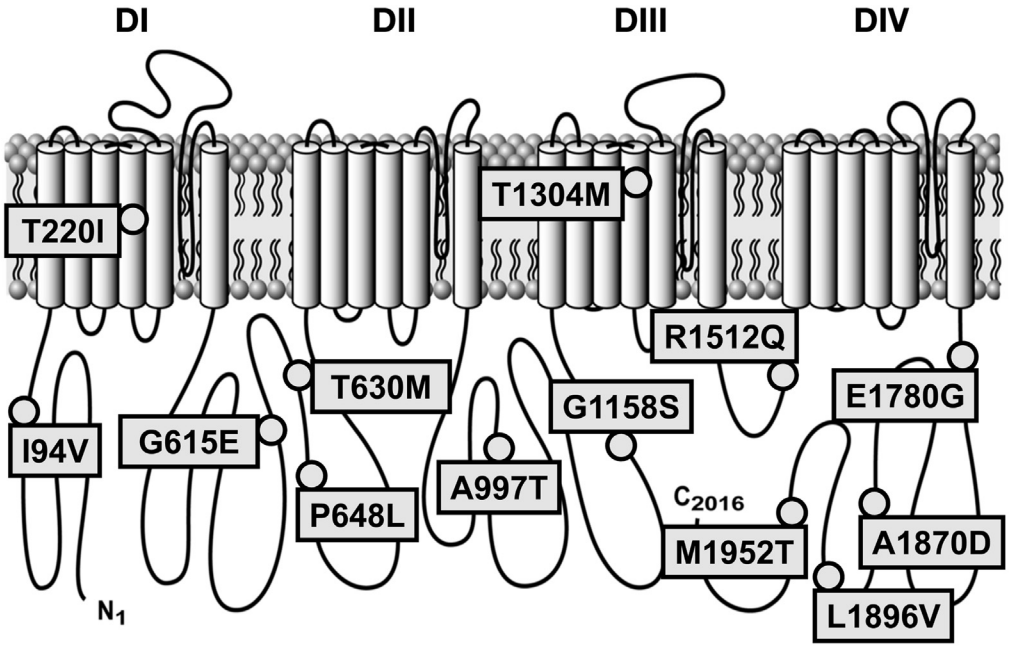


Figure 1. Voltage-gated Na^+ ion channel $Na_V1.5$ topology with missense mutations in *SCN5A* identified in a cohort with IBS. DI–DIV are homologous 6-transmembrane helix domains 1 through 4.

and examined by whole-cell voltage clamp electrophysiology. We explored the functional aspects of $Na_V1.5$ that could contribute to GI electrophysiology.

Ten of the 13 missense mutations (77%) showed functional differences when compared with wild-type controls (Table 2 and Figure 2). Abnormally functioning mutations included 1 in a transmembrane segment (T220I), 5 in IDL1 (G615E, T630M, P648L), 2 in IDL2 (A997T, G1158S), and 2 in the C-terminus (E1780G, L1896V). No statistically significant abnormalities were found in 3 mutations (T1304M, R1512Q, and M1952T).

Functional abnormalities were found in two thirds of all the tested parameters (Table 2). To generalize the pattern of the discovered abnormalities, we separated functional abnormalities into loss of function (Table 2, underlined) or gain of function (Table 2, **bold**). By using this classification, 19 of 21 (90%) abnormal parameters were LOF and 2 of 21 (10%) abnormal parameters were GOF. Overall, 9 of 10 (90%) mutations with electrophysiologic abnormalities were LOF and 1 (10%) was a GOF (Table 2).

Steady-state properties (I_{peak}, $V_{1/2a}$, δV_a, $V_{1/2i}$, δV_i) were affected for 6 mutations. There was a dramatic decrease in peak current (I_{peak}) for A997T (Figure 2*A*). For 3 mutations (T220I, G615E, A997T) there were significant shifts in voltage dependence of activation and/or inactivation, which would predict a decrease in the density and availability of Na^+ current (Figure 2*B*). A decrease in the slope of the voltage dependence of inactivation (δV_i) was the most commonly affected parameter (6 of 13 mutations). The shifts in the half points of voltage dependence ($V_{1/2a}$ and $V_{1/2i}$) and the decrease in the slopes of the voltage dependence of activation and inactivation (δV_a and δV_i) resulted in significant decreases in the window current (Figure 2*C*, area under the curves). The window currents are steady-state currents near resting potential. Therefore, smaller window currents may decrease Na^+ influx and result in hyperpolarization.

Significant changes in kinetic properties were found for 6 mutations, with 3 affecting kinetics of activation (3 of 4 LOF; Figure 2*D*) and 3 affecting kinetics of inactivation (all 3 of 3 LOF; Figure 2*E*). The time to peak (t_{peak}) was significantly slower for 3 mutations and faster for L1896V (Figure 2*A*). Interestingly, given the slower GI kinetics compared with the heart, although the faster of 2 inactivation time constants (τ_{IF}) was not affected for any of the mutations, the slower time constant (τ_s) was significantly slower for 3 mutations (Table 2 and Figure 2*E*).

No abnormalities were found in the fast time constant of inactivation (τ_{IF}), late current (I_{late}), deactivation time constant (τ_D), and rate of recovery from inactivation ($t_{1/2}$).

Correlation of Electrophysiologic Abnormalities With IBS Subtypes

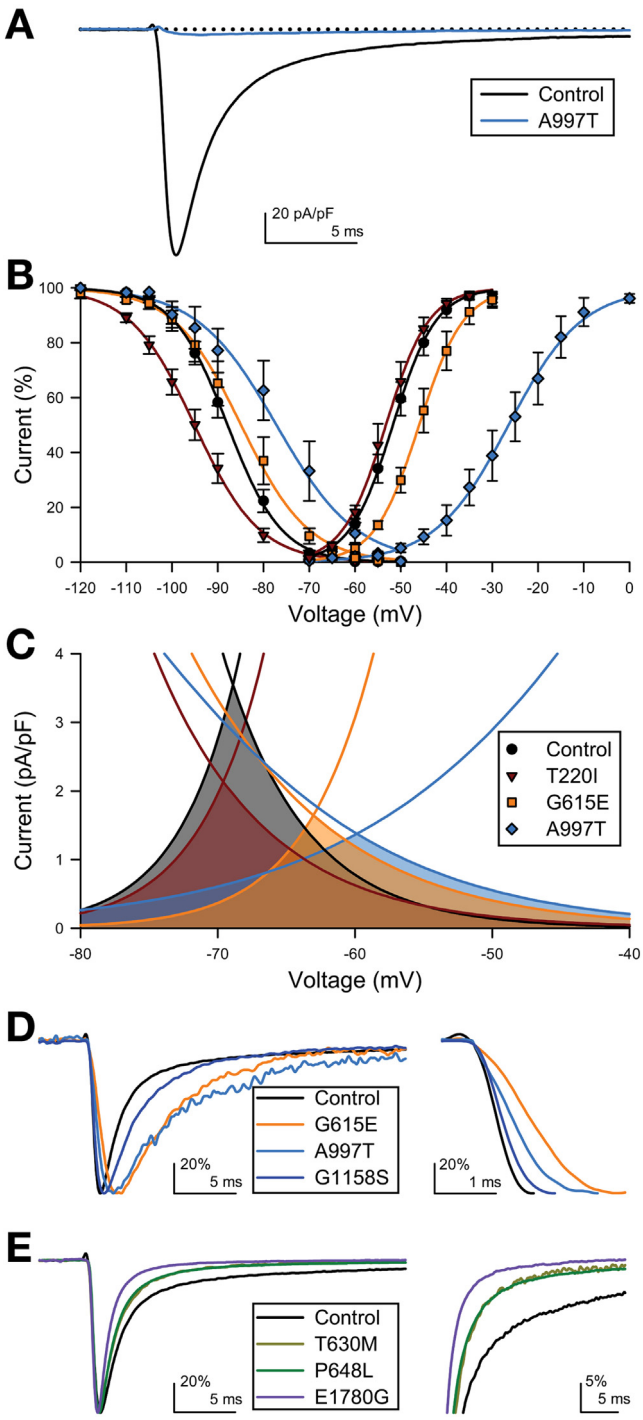
The ion Channel properties that would lead to LOF in both channel activation and inactivation were most common (Table 2 and Figure 3*A*). Most of the LOF abnormalities were found in the 4 IBS-C subjects (9 of 16 [56%]; Figure 3*B*,

Table 2. Functional Parameters of the *SCN5A* Missense Mutations in IBS Patients

Mutation	I_{peak}, pA/pF	t_{peak}, ms	$V_{1/2A}$, mV	δV_A	$V_{1/2i}$, mV	δV_I	τ_{IF}, ms	τ_{IS}, ms	τ_D, ms	I_{LATE}, pA/pF	$t_{1/2REC}$, ms
Wild type	-151 ± 27	1.43 ± 0.04	-58.2 ± 1.0	4.5 ± 0.3	-95.5 ± 1.3	-5.4 ± 0.1	0.84 ± 0.02	6.1 ± 0.3	0.71 ± 0.14	-0.91 ± 0.37	4.2 ± 0.7
I94V	-165 ± 51	1.35 ± 0.03	-58.7 ± 1.8	3.9 ± 0.4	-96.8 ± 1.9	-6.1 ± 0.2[b]	0.79 ± 0.08	5.2 ± 0.3	0.69 ± 0.06	-0.48 ± 0.09	4.0 ± 0.3
T220I	-73 ± 13	1.50 ± 0.16	-60.3 ± 1.2	4.3 ± 0.5	-102.9 ± 1.8[a,b]	-7.0 ± 0.2[a,b]	0.98 ± 0.18	5.8 ± 0.4	0.52 ± 0.08	-0.56 ± 0.19	4.3 ± 0.4
G615E	-55 ± 14	1.96 ± 0.16[a,b]	-52.6 ± 1.5[a,b]	4.8 ± 0.6	-92.4 ± 2.5	-6.4 ± 0.4[a,b]	1.01 ± 0.11	6.8 ± 0.7	0.32 ± 0.06	-0.66 ± 0.27	3.1 ± 0.6
T630M	-104 ± 24	1.37 ± 0.04	-56.7 ± 0.9	5.0 ± 0.2	-94.2 ± 1.4	-5.8 ± 0.2	0.97 ± 0.10	4.6 ± 0.2[b]	0.47 ± 0.06	-0.69 ± 0.13	3.9 ± 0.3
P648L	-149 ± 34	1.28 ± 0.06	-56.2 ± 1.2	4.2 ± 0.3	-96.0 ± 1.9	-5.8 ± 0.2	0.83 ± 0.09	4.3 ± 0.3[b]	0.62 ± 0.11	-0.41 ± 0.08	3.3 ± 0.4
A997T	-16 ± 4[a,b]	**2.52 ± 0.23**[a,b]	-38.6 ± 3.1[a,b]	6.6 ± 0.2[a,b]	**-89.3 ± 1.8**[b]	-6.5 ± 0.3[a,b]	0.96 ± 0.24	5.7 ± 0.6	0.35 ± 0.16	-0.27 ± 0.09	4.8 ± 0.5
G1158S	-54 ± 10	1.77 ± 0.11[b]	-55.0 ± 0.6	4.5 ± 0.3	-98.8 ± 1.5	-6.8 ± 0.1[a,b]	0.90 ± 0.12	5.6 ± 0.3	0.33 ± 0.04	-0.73 ± 0.18	3.1 ± 0.2
T1304M	-102 ± 27	1.76 ± 0.12	-58.5 ± 1.5	4.5 ± 0.6	-94.9 ± 2.5	-5.7 ± 0.3	1.10 ± 0.12	6.4 ± 0.9	0.52 ± 0.05	-0.74 ± 0.20	4.3 ± 0.6
R1512Q	-132 ± 37	1.41 ± 0.07	-55.3 ± 1.3	4.2 ± 0.3	-92.5 ± 1.2	-5.3 ± 0.2	0.90 ± 0.06	6.9 ± 0.4	0.65 ± 0.17	-1.23 ± 0.48	3.2 ± 0.3
E1780G	-94 ± 27	1.24 ± 0.06	-54.6 ± 1.0	4.8 ± 0.2	-93.4 ± 0.7	-5.2 ± 0.2	0.80 ± 0.10	4.8 ± 0.3[b]	0.34 ± 0.03	-3.10 ± 0.97	3.0 ± 0.1
A1870D	-126 ± 45	1.39 ± 0.06	-57.6 ± 1.7	4.5 ± 0.3	-96.6 ± 1.6	-6.0 ± 0.1[b]	1.12 ± 0.12	5.8 ± 0.6	0.88 ± 0.17	-0.49 ± 0.07	5.2 ± 0.4
L1896V	-111 ± 16	**1.26 ± 0.03**[b]	-57.7 ± 0.6	4.7 ± 0.2	-98.1 ± 1.7	-5.3 ± 0.3	0.81 ± 0.07	5.8 ± 0.4	0.57 ± 0.03	-0.44 ± 0.06	4.5 ± 0.4
M1952T	-177 ± 42	1.57 ± 0.07	-58.0 ± 1.1	4.1 ± 0.3	-91.5 ± 1.2	-5.4 ± 0.2	0.90 ± 0.08	6.3 ± 0.5	0.53 ± 0.06	-1.36 ± 0.88	4.9 ± 1.3

NOTE. Mutations are colored for LOF (underlined) and GOF (**bold**) as described in the Materials and Methods section or for no abnormalities (plain text).
I_{peak}, maximum peak current density; t_{peak}, time to peak current at -30 mV; $V_{1/2A}$, voltage dependence of activation; dV_A, slope of steady-state activation;$V_{1/2i}$, voltage dependence of inactivation; dV_I, slope of steady-state inactivation; τ_{IF}, time constant of fast inactivation at -30 mV; τ_{IS}, time constant of slow inactivation at -30 mV; τ_D, time constant of deactivation at -65 mV; I_{LATE}, percentage of peak current remaining after a 200-ms step to -30 mV; $t_{1/2REC}$, half-time to inactivation recovery.
[a]$P < .05$ vs wild type by a 1-way parametric analysis of variance with the Dunnett post-test.
[b]$P < .01$ vs wild type by a 2-tailed unpaired Student *t* test (n=5-12 cells).

red). Interestingly, the majority of LOF in the IBS-C patients were loss in activation (5 of 8), suggesting that lack of $Na_V1.5$ activation may contribute directly to decreased activity in IBS-C. IBS-D subjects had a smaller number of abnormalities (4 of 16 [25%]; Figure 3*B*, blue). Unlike in IBS-C, LOF parameters in IBS-D were mostly in $Na_V1.5$ inactivation (3 of 4). It is presently unclear how loss of $Na_V1.5$ inactivation may affect activity. Finally, only 2 functional abnormalities were discovered in mixed-subtype IBS (2 of 16 [13%]), with 1 being a GOF (t_{peak}) and 1 being a LOF (δV_i) (Figure 3*B*, yellow).



Clinical Case of Patient With A997T $Na_V1.5$

The most striking of our findings was a dramatically smaller, slower, and right-shifted Na^+ current in A997T (Figures 2*A* and 4*A*). This mutation belongs to a 65-year-old Caucasian woman with Rome II IBS-C with lifelong constipation and intermittent abdominal pain and bloating, relieved by defecation. Previous medical history, ECG, laboratory testing, colonoscopy, defecating proctogram, and anorectal manometry were unrevealing. A nuclear medicine, whole-gut transit study found normal gastric emptying and small-bowel transit but a delay in colonic transit at 24 and 48 hours (Supplementary Figure 2).

Electrophysiology analysis showed that p.A997T-$Na_V1.5$ channels are LOF phenotype, with abnormalities in multiple functional parameters (Table 2). Most obvious was a near-complete abolition (98% reduction) of peak current from -151 ± 27 pA/pF for wild-type $Na_V1.5$ to -2.3 ± 0.5 pA/pF for p.A997T-$Na_V1.5$ (n = 9–11; $P < .05$; Figure 4*A* and *B*). Other abnormalities included a depolarized voltage dependence of activation ($V_{1/2a}$) of -49.9 ± 2.9 mV vs -58.2 ± 1.0 mV, and a slower time to peak of 2.52 ± 0.23 ms vs 1.43 ± 0.04 ms (Figure 4*C* and *D*).

Mexiletine is known to rescue expression defects of $Na_V1.5$.[32] We cultured HEK-293 cells that transiently expressed p.A997T-$Na_V1.5$ in the presence of 10 μmol/L mexiletine for 48 hours and measured currents. Mexiletine treatment significantly enhanced p.A997T-$Na_V1.5$ current, increasing peak current 6-fold to -15 ± 4 pA/pF (n = 23–25; $P < .05$ compared with untreated p.A997T-$Na_V1.5$ or treated wild-type; Figure 4*A* and *B*) and restored $V_{1/2a}$ (-52.1 ± 0.8 mV) and time to peak (1.54 ± 0.12 ms; n = 12–17; $P < .05$ vs untreated A997T and $P > .05$ vs wild-type) (Figure 4*C* and *D*).

Figure 2. Electrophysiologic abnormalities in $Na_V1.5$ mutations. (*A*) Representative whole-cell Na^+ current traces (step to -30 mV) from HEK-293 cells transfected with wild-type (control, *black*) or A997T (*blue*). *Dotted line*: 0 pA/pF. (*B*) $Na_V1.5$ current-voltage plot showing shifts in voltage dependence of inactivation for T220I (*maroon*) and A997T (*blue*), and positive shifts in voltage dependence of activation for G615E (orange) and A997T (*blue*). (*C*) Window currents for T220I, G615E, and A997 are the *shaded areas* under the intersecting current-voltage curves. (*D* and *E*) Representative single traces (step to -30 mV, peaks normalized to 100%) of whole-cell Na^+ voltage-dependent current for mutations compared with wild-type (controls) in the (*D*) activation and (*E*) inactivation kinetics. (*D*) Activation kinetics were altered for mutations G615E (orange), A997T (*blue*), and G1158S (violet) compared with wild-type (control, *black*). *Inset*: G615E, A997T, and G1158S activate slower than wild-type. (*E*) Inactivation kinetics were altered for mutations T630M (*maize*), P648L (*green*), or E1780G (*purple*) compared with wild-type (control, *black*). *Inset*: Mutations T630M, P648L, or E1780G inactivate faster than wild-type.

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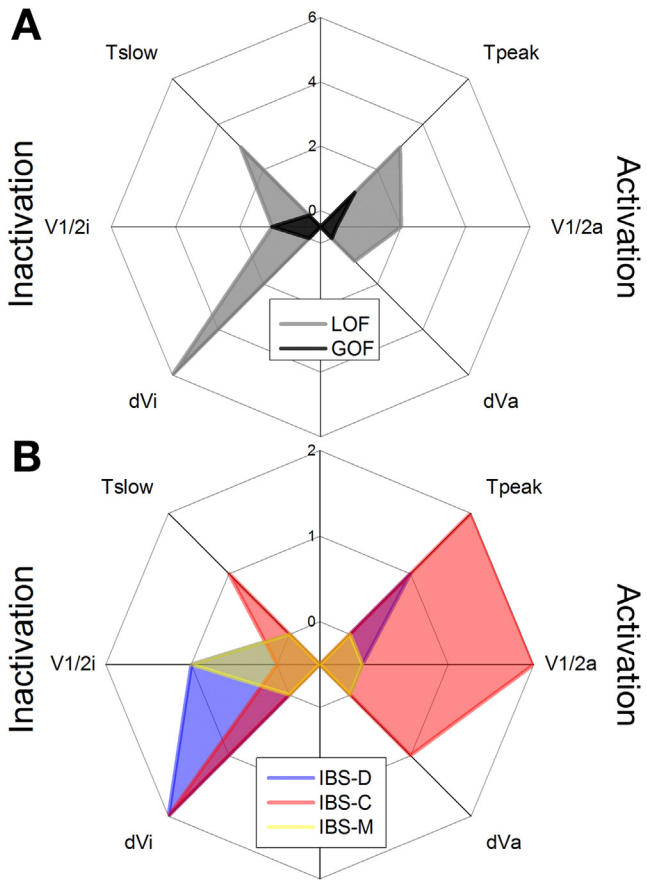

Figure 3. Distribution of the electrophysiologic abnormalities across all mutations. (*A*) Shown in *black* are GOF (2 of 18) and in *grey* are LOF (16 of 18) parameters for all IBS cases (IBS-D, IBS-C, and mixed-subtype IBS [IBS-M]). (*B*) Activation and inactivation LOF abnormalities are shown in IBS-D (4 of 18, *blue*), IBS-C (8 of 16, *red*), and IBS-M (1 of 16, *yellow*).

Mexiletine rescue of p.A997T-$Na_V1.5$ function in vitro led us to hypothesize that this drug also would restore in vivo colonic function. Before drug administration, the patient documented 5 complete spontaneous bowel movements over 3.5 weeks (1.4 ± 0.5/wk) and 5 small hard bowel movements (1.4 ± 1.0/wk) (Figure 5). Mexiletine then was administered orally in increasing doses from 200 to 400 mg every 8 hours over 5 days while the patient was on continuous telemetry. An increase in the QTc interval from study beginning to end (from 441 to 471 ms) was not clinically significant. At the higher doses of mexiletine (300–400 mg), the patient experienced known central side effects including nausea, lightheadedness, and mild ataxia. However, except for missing 1 dose she was able to complete the study. Whole-gut transit after day 5 showed an increase in the rate of gastric emptying (t_{50}, from 132 to 91 min). The patient had 2 spontaneous bowel movements during the transit test but no change in colonic transit was found, likely reflecting passage of unlabeled stool from the left colon. Over a 5-week follow-up period after mexiletine, the patient reported 25 complete spontaneous bowel movements (5 ± 2.0/wk; $P < .05$ compared with pre-mexiletine) and 2 hard small bowel movements (0.4 ± 0.5/wk; $P > .05$ compared with pre-mexiletine). This effect tapered over the 5 weeks off mexiletine (Figure 5).

Discussion

Distinctive SCN5A *Variations in a Subset of IBS Patients*

A number of IBS-related putative genes have been identified, but almost all remain to be validated and each gene contributes to the pathophysiology in 1%–5% of patients.[33] Patients with *SCN5A* mutations predisposing to cardiac conduction disorders also have a higher prevalence of IBS.[20] A pilot study of 49 patients suggested a 2.0% prevalence of *SCN5A* mutations in IBS.[21] In this 584-patient cohort (Table 1) we confirmed a 2.2% prevalence of *SCN5A* mutations in IBS subjects (Figure 1). In particular, the newly identified mutations were absent from 2760 control alleles from Mayo blood donors, as well as from several large exome databases (an additional 7595 samples) that have become publicly available after the initiation of this study (NHLBI[34] and 1000 Genomes[35]), with the exceptions of T220I, G615E, and T1304M, which remain extremely rare among Caucasians (Supplementary Table 5).

We also discovered that an additional 2.9% have previously known, functionally relevant *SCN5A* coding variants (Supplementary Table 3). These results suggest that *SCN5A* status may play a role in 1.7% (electrophysiologically abnormal mutations) to 5.1% (mutations plus other coding polymorphisms) of IBS patients. Therefore, if 15% IBS prevalence is assumed,[1] *SCN5A* coding abnormalities may contribute to IBS pathophysiology in up to 2.3 million IBS patients in the United States. In addition to these, our GWAS-replication data suggest that common, less-damaging *SCN5A* variants and *SCN5A* transcriptional control may be relevant for IBS patients (Supplementary Table 4 and Supplementary Figures 1 and 3).

IBS-Related SCN5A *Mutations Result in Functionally Abnormal* $Na_V1.5$ *Channels*

Cardiology literature analyzing *SCN5A* variants shows that not all mutations are functionally relevant for the heart.[36,37] Thus, functional analysis was required. Three mutations were identified and functionally characterized elsewhere, and our findings agreed with the previous report for T220I,[29] but in contrast to previous reports there was a lack of abnormal findings for T1304M,[27] and a different set of functional abnormalities for G615E.[31] These differences likely were secondary to the stringent statistical cut-off point ($P < .01$) for functional abnormalities used in this study and the GI-focused protocols used vs the cardiac protocols used in previous studies.

We subdivided functional changes into 2 broad categories based on an expected increase (GOF) or decrease (LOF) of Na^+ flux and showed that 90% of the abnormal parameters could be classified as LOF. For the novel mutations, the majority of LOF abnormalities showed a decrease in the slope of steady-state voltage dependence of inactivation (46%; Figure 2*B* and *C*) and of the kinetics of

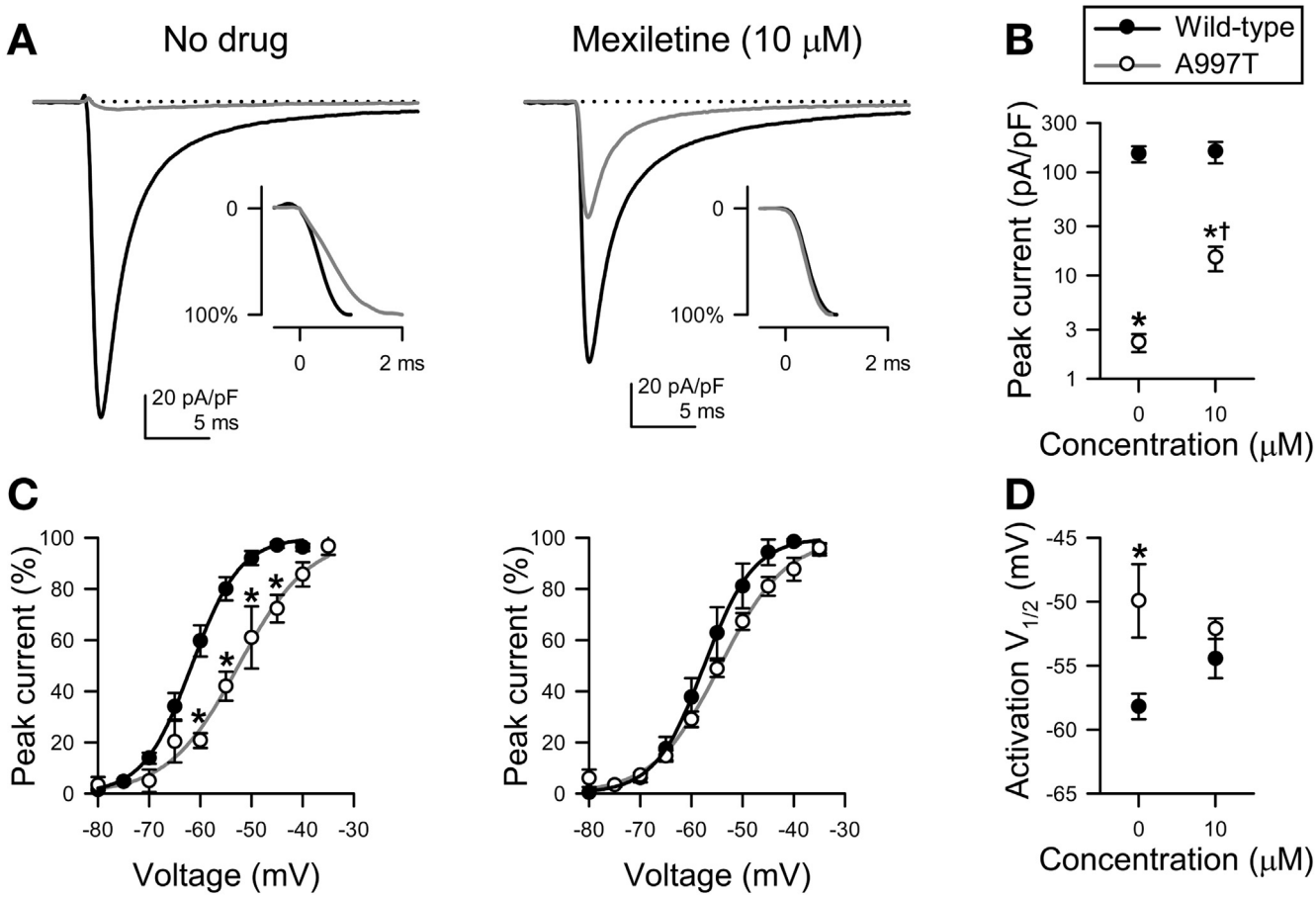

Figure 4. Loss-of-function phenotype in the mutant sodium channel p.A997T-$Na_V1.5$ with partial rescue by mexiletine. (*A*) Representative Na^+ current traces (step to -30 mV) from wild-type (black trace) or p.A997T-$Na_V1.5$ (grey trace) without (*left*) or with (*right*) mexiletine (10 μmol/L). *Inset*: Same traces scaled to peak. (*B*) Averages of peak current densities. *$P < .05$ to wild-type and $^{\dagger}P < .05$ to no drug by a 1-way parametric analysis of variance with the Bonferroni post-test (n = 9–25). (*C*) Voltage dependence of steady-state activation and inactivation of wild-type (*black circles*) or A997T-$Na_V1.5$ (*white circles*) previously exposed to no drug (*left*) or mexiletine (*right*). Boltzmann function fits for wild-type $Na_V1.5$ (*black lines*) or A997T-$Na_V1.5$ (*grey lines*). (*D*) Averages of $V_{1/2}$ of activation. *$P < .05$ compared with wild-type by a 1-way parametric analysis of variance with the Bonferroni post-test (n = 9–25).

activation (30%) and inactivation (23%) (Figure 2*D* and *E*). The decrease in the slope of the voltage dependence of inactivation (δV_i) would predict a smaller window current (Figure 2*C*) and diminish the availability of Na^+ current. Furthermore, the decreases in both δV_i and time to peak (t_{peak}) would contribute to the decrease in the upstroke velocity of the slow waves.

Consistent with the LOF phenotype in vitro, it appears that *SCN5A* mutations may result predominantly in IBS-C. Most of the LOF abnormalities were found in mutation-positive subjects with IBS-C (Figure 3), which were enriched (31%) compared with the overall cohort (10%) (Table 1). It is currently unclear how the LOF abnormalities could yield a predominantly diarrhea phenotype in the mutation-positive IBS-D subjects, and this will require further study.

These data also are consistent with prior reports. $Na_V1.5$ channels are found in human ICC[14] and smooth muscle cells,[10,13] and blockade of the Na_V channels decreases excitability: it hyperpolarizes the resting potential,[14] slows upstroke and prolongs slow wave,[38] and decreases frequency.[14] Furthermore, recent clinical studies have shown that a novel $Na_V1.5$ blocker, ranolazine, is associated with significant constipation.[16] Thus, our findings have implications for involvement of $Na_V1.5$ in the pathophysiology of gastrointestinal motility of IBS.

$Na_V1.5$-A997T Abnormalities Are Rescued In Vitro and In Vivo by Mexiletine

As proof of principle, we performed a detailed evaluation of 1 IBS-C patient with a $Na_V1.5$-A997T mutation. Of the mutations studied, A997T showed significant changes in several parameters, with the most noticeable being a 90% loss of peak current when compared with controls. Our in vitro model showed that short-term treatment with mexiletine reversed many of the A997T-$Na_V1.5$ defects (Figure 4), consistent with previous reports of other defective $Na_V1.5$ channels.[32] Prompted by the in vitro response, we proceeded with a clinical trial of

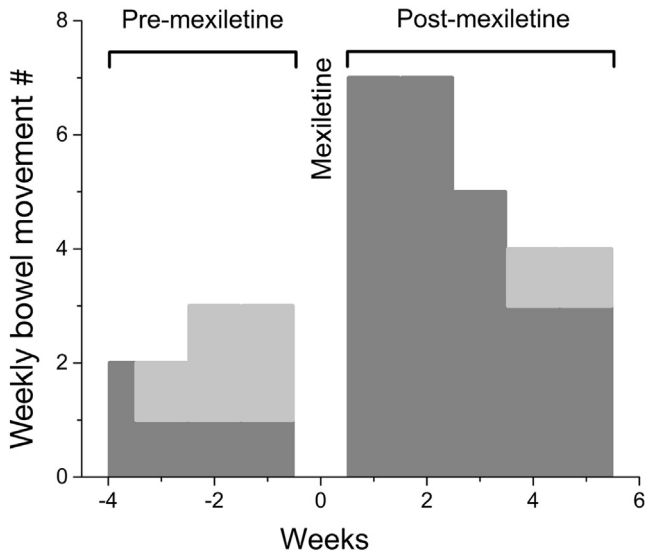

Figure 5. Bowel movement frequency was improved in the A997T-$Na_V1.5$ patient after mexiletine treatment. Complete spontaneous (*dark grey shading*) and small hard (*light grey shading*) bowel movements for the patient for 3.5 weeks preceding and 5 weeks after a 5-day treatment with mexiletine, showing normalization of bowel habits with mexiletine.

mexiletine in this patient with IBS-C. After a 5-day treatment with mexiletine, the patient's bowel movement frequency increased to normal range. Consistent with a likely effect on regulation of expression, the resulting normalization of this patient's GI motility persisted for 5 weeks (Figure 5). Mexiletine carries a risk of cardiac arrhythmia,[39] making it unlikely to be approved by the Food and Drug Administration as an IBS therapy. However, these data do suggest that specific $Na_V1.5$ and other ion channelopathies may benefit from targeted and individualized therapies.

SCN5A *Genotype-Phenotype Relationship May be Organ-or Sex-Specific*

The A997T mutation identified in our patient would result in an almost complete abolition of channel function and a predicted loss of approximately 50% of the $Na_V1.5$ current not only in the GI tract but also in the cardiac myocytes. The loss of 1 *SCN5A* allele can manifest as significant cardiac electrophysiologic abnormalities, particularly in men.[40] Our female patient did not have a personal or family history of cardiac rhythm disturbances or ECG abnormalities before or during this study. It is interesting that despite equal heritability between the sexes, there is a striking 9:1 male:female predominance of cardiac expressivity in subjects with type 1 Brugada syndrome,[41] which is in contrast to the 1:2 male:female IBS prevalence.[42] Indeed, this IBS cohort was predominately female (85%), consistent with known IBS epidemiology.[42] However, in the 2 mutation-positive male subjects, there were more ECG findings than in the rest of the cohort, and both of these *SCN5A* mutations (I94V, P648L) showed LOF changes. Thus, a hypothesis that requires further scrutiny is that loss of $Na_V1.5$ function principally manifests with a cardiac phenotype in males but a GI phenotype in females.

Strengths and Limitations

The strengths of this study were as follows: (1) a large mutation discovery sample size of 584 patients with IBS, (2) independent evidence of *SCN5A* association with IBS in a GWAS and replication study, (3) functional assessment of the discovered mutations, and (4) proof-of-principle study in a patient with a LOF mutation, with normalization of her bowel habits using a drug we showed in vitro to normalize the Na_V channel defect. As any study, there were limitations, including the open-labeled nature of the proof-of-concept study. This study will need to be repeated in another cohort, and there remains an outstanding need to examine in more detail the phenotypes with respect to organ and patient symptoms.

Clinical Implications

In the dawn of the personalized medicine era we are discovering that for genetically complex diseases such as IBS there may be cohorts of patients with well-defined genetic abnormalities. Ion channels are involved directly in the mechanisms of visceral pain and GI motility, therefore, ion channelopathies may be involved in the pathogenesis of IBS. We provide direct molecular and functional evidence for functionally significant *SCN5A* mutations in a subset of IBS patients. As we show in a proof-of-concept study for 1 of these patients, $Na_V1.5$ dysfunction may underlie the IBS pathogenesis and provide personalized treatment options for this subset of patients. In conclusion, the data suggest that a subset of patients with IBS may have an *SCN5A*-encoded $Na_V1.5$ ion channelopathy, which may represent a novel pathophysiologic mechanism and provide novel therapeutic options.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2014.02.054.

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