Loss-of-Function of the Voltage-Gated Sodium Channel NaV1.5 (Channelopathies) in Patients With Irritable Bowel Syndrome

Arthur Beyder,1,* Amelia Mazzone,1,* Peter R. Strage,1,* David J. Tester,2,* Yuri A. Saito,1 Cheryl E. Bernard,1 Felicity T. Enders,3 Veronica E. Ek,4 Peter T. Schmidt,5 Aldona Dlugosz,5 Greger Lindberg,5 Pontus Karling,6 Bodil Ohlsson,7 Maria Gazouli,8 Gerardo Nardone,9 Rosario Cuomo,10 Paolo Usai–Satta,11 Francesca Galeazzi,12 Matteo Neri,13 Piero Portincasa,14 Massimo Bellini,15 Giovanni Barbara,16 Michael Camilleri,1 G. Richard Locke III,1 Nicholas J. Talley,1 Mauro D’Amato,4 Michael J. Ackerman,2,§ and Gianrico Farrugia1,§

1Enteric Neuroscience Program, Division of Gastroenterology and Hepatology, Department of Physiology and Biomedical Engineering, 2Department of Medicine (Cardiovascular Diseases), Department of Pediatrics (Pediatric Cardiology), and Department of Molecular Pharmacology and Experimental Therapeutics and the Windland Smith Rice Sudden Death Genomics Laboratory, 3Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota; 4Department of Biosciences and Nutrition, 5Department of Gastroenterology and Hepatology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; 6Department of Medicine, Umeå University, Umeå, Sweden; 7Department of Clinical Neurophysiology, Skåne University Hospital, Malmö, Sweden; 8Laboratory of Biology, School of Medicine, University of Athens, Athens, Greece; 9Gastroenterology Unit, Department of Clinical Medicine and Surgery, University Federico II, Naples, Italy; 10Digestive Motility Diseases, Department of Clinical Medicine and Surgery, Federico II University Hospital, Naples, Italy; 11S.C. Gastroenterologia, Azienda Ospedaliera G. Brotzu, Cagliari, Italy; 12Department of Medicine, University of Pisa, Pisa, Italy; 13Department of Biomedical Sciences and Human Oncology, Clinica Medica A. Muri, University of Bari Medical School, Bari, Italy; 14Department of Biomedical Sciences and Human Oncology, Clinica Medica A. Muri, University of Bari Medical School, Bari, Italy; 15Gastroenterology Unit, Department of Gastroenterology, University of Pisa, Pisa, Italy; 16Department of Medical and Surgical Sciences, University of Bologna, St. Orsola-Malpighi Hospital, Bologna, Italy

See Covering the Cover synopsis on page 1583.

BACKGROUND & AIMS: SCN5A encodes the α-subunit of the voltage-gated sodium channel NaV1.5. 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 NaV1.5. 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 NaV1.5 function (9 loss-of-function and 1 gain-of-function function). The p. A997T-NaV1.5 had the greatest effect in reducing NaV1.5 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 NaV1.5 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

*Authors share co-first authorship; §Authors share co-senior authorship.

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; Ipeak, peak current; LOF, loss of function; NaN+, voltage-gated sodium channel; tpeak, time to peak; V1/2a, half point of the voltage dependence of activation; V1/2d, half point of the voltage dependence of inactivation; ΔV50, slope of the voltage dependence of activation; ΔV50, slope of the voltage dependence of inactivation.

© 2014 by the AGA Institute
0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2014.02.054
processing. Familial aggregation and twin studies suggest that genetics play a role in IBS. Genotyping supports this notion, but the specific impact of individual genes remains unclear. Ion channels are excellent pathophysiologic and therapeutic targets because they are involved directly in both GI motility and visceral pain. Therefore, ion channelopathies may cause IBS in some cases.

Voltage-gated sodium channels (NaV) are present in the gastrointestinal smooth muscle, including rat fundus, human and canine jejunum, and rat and human colon. A particular tetrodotoxin-resistant sodium channel, NaV1.5 (encoded by SCN5A), is expressed in human smooth muscle cells and the interstitial cells of Cajal (ICC) of the small intestine and colon. 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. In human GI smooth muscle, NaV channels appear to be excitatory for slow waves and NaV1.5 is functionally relevant in the human GI tract, as pharmacologic block of NaV1.5 is associated with constipation.

SCN5A also is expressed densely in human cardiomyocytes. SCN5A rare mutations and common variants 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. 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 NaV1.5 channels with decreased peak currents and mechanosensitivity. 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 NaV1.5 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 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 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 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 (Ipeak), slowed time to peak (t peak), a depolarization (right-shift) in the half point of the voltage dependence of activation (V1/2a), and a decrease in the slope of the voltage dependence of activation (dV/dV1/2a) or as a hyperpolarization (left-shift) of the half point of the voltage dependence of inactivation (V1/2i), a decrease in the slope of the voltage dependence of inactivation (dV/dV1/2i), and a decrease of the time constants of inactivation (τi, τf). 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-NaV1.5 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
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\(_V\)1.5 Mutations**

SCN5A encodes Na\(_V\)1.5, 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\(_V\)1.5 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,
and examined by whole-cell voltage clamp electrophysiology. We explored the functional aspects of Na\textsubscript{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 ($I_{\text{peak}}, V_{1/2a}, \Delta V_a, V_{1/2i}, \Delta V_i$) were affected for 6 mutations. There was a dramatic decrease in peak current ($I_{\text{peak}}$) for A997T (Figure 2A). 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\textsuperscript{+} current (Figure 2B). A decrease in the slope of the voltage dependence of inactivation ($\Delta V_i$) 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 ($\Delta V_a$ and $\Delta V_i$) resulted in significant decreases in the window current (Figure 2C, area under the curves). The window currents are steady-state currents near resting potential. Therefore, smaller window currents may decrease Na\textsuperscript{+} influx and result in hyperpolarization.

Significant changes in kinetic properties were found for 6 mutations, with 3 affecting kinetics of activation (Table 2(245,586),(273,678) and Figure 2D) and 3 affecting kinetics of inactivation (all 3 of 3 LOF; Figure 2E). The time to peak ($t_{\text{peak}}$) was significantly slower for 3 mutations and faster for L1896V (Figure 2A). Interestingly, given the slower GI kinetics compared with the heart, although the faster of 2 inactivation time constants ($\tau_2$) was not affected for any of the mutations, the slower time constant ($\tau_1$) was significantly slower for 3 mutations (Table 2 and Figure 2E).

No abnormalities were found in the fast time constant of inactivation ($\tau_{1p}$), late current ($I_{\text{late}}$), deactivation time constant ($\tau_{d}$), 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 3A). Most of the LOF abnormalities were found in the 4 IBS-C subjects (9 of 16 [56%]; Figure 3B,

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

<table>
<thead>
<tr>
<th>Mutation</th>
<th>$I_{\text{peak}}, \mu A/PF$</th>
<th>$V_{1/2a}, \text{mV}$</th>
<th>$V_{1/2i}, \text{mV}$</th>
<th>$\Delta V_a$</th>
<th>$\Delta V_i$</th>
<th>$t_{\text{peak}}, \mu s$</th>
<th>$t_{1/2}, \text{ms}$</th>
<th>$t_{\text{rec}}, \text{ms}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>-151 ± 27</td>
<td>1.43 ± 0.04</td>
<td>-52 ± 1.8</td>
<td>-95 ± 1.3</td>
<td>-5.4 ± 0.1</td>
<td>0.94 ± 0.02</td>
<td>6.3 ± 0.4</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>T220I</td>
<td>-165 ± 51</td>
<td>1.35 ± 0.03</td>
<td>-56 ± 1.9</td>
<td>-96 ± 1.9</td>
<td>6.7 ± 0.2</td>
<td>0.97 ± 0.02</td>
<td>7.0 ± 0.4</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td>G615E</td>
<td>-104 ± 24</td>
<td>1.30 ± 0.02</td>
<td>-58 ± 1.5</td>
<td>-98 ± 1.9</td>
<td>8.2 ± 0.4</td>
<td>0.98 ± 0.03</td>
<td>8.0 ± 0.5</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>A997T</td>
<td>-16 ± 32</td>
<td>1.77 ± 0.01</td>
<td>-52 ± 1.2</td>
<td>-99 ± 1.8</td>
<td>-5.2 ± 0.2</td>
<td>0.83 ± 0.01</td>
<td>1.6 ± 0.2</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>G1158S</td>
<td>-102 ± 24</td>
<td>1.76 ± 0.02</td>
<td>-56 ± 1.1</td>
<td>-100 ± 1.8</td>
<td>8.4 ± 0.3</td>
<td>0.98 ± 0.01</td>
<td>9.0 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>T1304M</td>
<td>-98 ± 24</td>
<td>1.75 ± 0.02</td>
<td>-56 ± 1.0</td>
<td>-100 ± 1.8</td>
<td>8.5 ± 0.2</td>
<td>0.98 ± 0.01</td>
<td>9.0 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>P648L</td>
<td>-104 ± 24</td>
<td>1.30 ± 0.02</td>
<td>-58 ± 1.5</td>
<td>-98 ± 1.9</td>
<td>8.2 ± 0.4</td>
<td>0.98 ± 0.03</td>
<td>8.0 ± 0.5</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>A997T</td>
<td>-16 ± 32</td>
<td>1.77 ± 0.01</td>
<td>-52 ± 1.2</td>
<td>-99 ± 1.8</td>
<td>-5.2 ± 0.2</td>
<td>0.83 ± 0.01</td>
<td>1.6 ± 0.2</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>G1158S</td>
<td>-102 ± 24</td>
<td>1.76 ± 0.02</td>
<td>-56 ± 1.1</td>
<td>-100 ± 1.8</td>
<td>8.4 ± 0.3</td>
<td>0.98 ± 0.01</td>
<td>9.0 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>T1304M</td>
<td>-98 ± 24</td>
<td>1.75 ± 0.02</td>
<td>-56 ± 1.0</td>
<td>-100 ± 1.8</td>
<td>8.5 ± 0.2</td>
<td>0.98 ± 0.01</td>
<td>9.0 ± 0.4</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>P648L</td>
<td>-104 ± 24</td>
<td>1.30 ± 0.02</td>
<td>-58 ± 1.5</td>
<td>-98 ± 1.9</td>
<td>8.2 ± 0.4</td>
<td>0.98 ± 0.03</td>
<td>8.0 ± 0.5</td>
<td>4.3 ± 0.7</td>
</tr>
</tbody>
</table>

**NOTE.** Mutations are colored for LOF (underlined) and GOF (bold) as described in the Materials and Methods section. Of the 90% abnormalities (gain), there was a dramatic decrease in the density and availability of Na\textsuperscript{+} current (Figure 2B). A decrease in the slope of the voltage dependence of inactivation ($\Delta V_i$) 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 ($\Delta V_a$ and $\Delta V_i$) resulted in significant decreases in the window current (Figure 2C, area under the curves). The window currents are steady-state currents near resting potential. Therefore, smaller window currents may decrease Na\textsuperscript{+} 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 2D) and 3 affecting kinetics of inactivation (all 3 of 3 LOF; Figure 2E). The time to peak ($t_{\text{peak}}$) was significantly slower for 3 mutations and faster for L1896V (Figure 2A). Interestingly, given the slower GI kinetics compared with the heart, although the faster of 2 inactivation time constants ($\tau_2$) was not affected for any of the mutations, the slower time constant ($\tau_1$) was significantly slower for 3 mutations (Table 2 and Figure 2E).

No abnormalities were found in the fast time constant of inactivation ($\tau_{1p}$), late current ($I_{\text{late}}$), deactivation time constant ($\tau_d$), and rate of recovery from inactivation ($t_{1/2}$).
red). Interestingly, the majority of LOF in the IBS-C patients were loss in activation (5 of 8), suggesting that lack of Na\textsubscript{v}1.5 activation may contribute directly to decreased activity in IBS-C. IBS-D subjects had a smaller number of abnormalities (4 of 16 [25%]; Figure 3B, blue). Unlike in IBS-C, LOF parameters in IBS-D were mostly in Na\textsubscript{v}1.5 inactivation (3 of 4). It is presently unclear how loss of Na\textsubscript{v}1.5 inactivation may affect activity. Finally, only 2 functional abnormalities were discovered in mixed-subtype IBS (2 of 16 [13%]), with 1 being a GOF (\(t_{\text{peak}}\)) and 1 being a LOF (\(\Delta V_{i}\)) (Figure 3B, yellow).

**Clinical Case of Patient With A997T Na\textsubscript{v}1.5**

The most striking of our findings was a dramatically smaller, slower, and right-shifted Na\textsuperscript{+} current in A997T (Figures 2A and 4A). 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\textsubscript{v}1.5 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\textsubscript{v}1.5 to -2.3 ± 0.5 pA/pF for p.A997T-Na\textsubscript{v}1.5 (n = 9–11; \(P < .05\); Figure 4A 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 4C and D).

Mexiletine is known to rescue expression defects of Na\textsubscript{v}1.5.\textsuperscript{32} We cultured HEK-293 cells that transiently expressed p.A997T-Na\textsubscript{v}1.5 in the presence of 10 \(\mu\)mol/L mexiletine for 48 hours and measured currents. Mexiletine treatment significantly enhanced p.A997T-Na\textsubscript{v}1.5 current, increasing peak current 6-fold to -15 ± 4 pA/pF (n = 23–25; \(P < .05\) compared with untreated p.A997T and treated wild-type; Figure 4A 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 4C and D).

**Figure 2.** Electrophysiologic abnormalities in Na\textsubscript{v}1.5 mutations. (A) Representative whole-cell Na\textsuperscript{+} 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\textsubscript{v}1.5 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\textsuperscript{+} 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.
Mexiletine rescue of p.A997T-Naᵥ1.5 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 (t₅₀, 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. Patients with SCN5A mutations predisposing to cardiac conduction disorders also have a higher prevalence of IBS. A pilot study of 49 patients suggested a 2.0% prevalence of SCN5A mutations in IBS. 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 (NHBLI44 and 1000 Genomes35), 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, 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 Naᵥ1.5 Channels**

Cardiology literature analyzing SCN5A variants shows that not all mutations are functionally relevant for the heart. Thus, functional analysis was required. Three mutations were identified and functionally characterized elsewhere, and our findings agreed with the previous report for T220I, but in contrast to previous reports there was a lack of abnormal findings for T1304M, and a different set of functional abnormalities for G615E. 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 2B and C) and of the kinetics of

---

**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).**
activation (30%) and inactivation (23%) (Figure 2D and E).
The decrease in the slope of the voltage dependence of inactivation (dVi) would predict a smaller window current (Figure 2C) and diminish the availability of Na⁺ current. Furthermore, the decreases in both dVi and time to peak (tpeak) 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. Naᵥ1.5 channels are found in human ICC⁵ and smooth muscle cells,¹⁰,¹³ and blockade of the Naᵥ 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 Naᵥ1.5 blocker, ranolazine, is associated with significant constipation.¹⁶ Thus, our findings have implications for involvement of Naᵥ1.5 in the pathophysiology of gastrointestinal motility of IBS.

Naᵥ1.5-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 Naᵥ1.5-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-Naᵥ1.5 defects (Figure 4), consistent with previous reports of other defective Naᵥ1.5 channels.³⁵ Prompted by the in vitro response, we proceeded with a clinical trial of
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, making it unlikely to be approved by the Food and Drug Administration as an IBS therapy. However, these data do suggest that specific NaV1.5 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 NaV1.5 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. 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, which is in contrast to the 1:2 male:female IBS prevalence. Indeed, this IBS cohort was predominately female (85%), consistent with known IBS epidemiology. 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 NaV1.5 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 NaV 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, NaV1.5 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 NaV1.5 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.

References


