Sodium channel mutation in irritable bowel syndrome: evidence for an ion channelopathy

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1Enteric Neuroscience Program, 2Departments of Medicine (Cardiovascular Diseases), Pediatrics (Pediatric Cardiology), and Molecular Pharmacology and Experimental Therapeutics and the Windland Smith Rice Sudden Death Genomics Laboratory, and 3Miles and Shirley Fiterman Center for Digestive Diseases, Mayo Clinic, Rochester, Minnesota; and 4Department of Medicine, University of Wisconsin, Madison, Wisconsin

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Saito YA, Streege PR, Tester DJ, Locke GR 3rd, Talley NJ, Bernard CE, Rae JL, Makielski JC, Ackerman MJ, Farrugia G. Sodium channel mutation in irritable bowel syndrome: evidence for an ion channelopathy. Am J Physiol Gastrointest Liver Physiol 296: G211–G218, 2009. First published December 4, 2008; doi:10.1152/ajpgi.90571.2008.—The SCN5A-encoded Na+ channel is expressed in interstitial cells of Cajal and smooth muscle in the circular layer of the human intestine. Patients with mutations in SCN5A are more likely to report gastrointestinal symptoms, especially abdominal pain. Twin and family studies of irritable bowel syndrome (IBS) suggest a genetic basis for IBS, but no genes have been identified to date. Therefore, our aims were to evaluate SCN5A as a candidate gene involved in the pathogenesis of IBS and to determine physiological consequences of identified mutations. Mutational analysis was performed on genomic DNA obtained from 49 subjects diagnosed with IBS who reported at least moderately severe abdominal pain. One patient hosted a loss-of-function missense mutation, G298S, that was not observed in >3,000 reference alleles derived from 1,500 healthy control subjects. Na+ currents were recorded from the four common human SCN5A transcripts in transfected HEK-293 cells. Comparing Na+ with G298S-SCN5A versus wild type in HEK cells, Na+ current density was significantly less by 49–77%, and channel activation time was delayed in backgrounds that also contained the common H558R polymorphism. Single-channel measurements showed no change in Na+ current. Mechanosensitivity was reduced in Na+ current and loss of function of Na+ current, suggesting SCN5A as a candidate gene in the pathophysiology of IBS.

SCN5A; Na+, current density; mechanosensitivity

THE SCN5A-ENCODED Na+ channel is expressed in cardiac myocytes, and loss-of-function or gain-of-function mutations in this gene are known to result in cardiac channelopathies such as Brugada syndrome and type 3 long-QT (LQT) syndrome, respectively (15). Recent data show that Na+ expression is not limited to the heart but that this sodium channel is also expressed in human intestinal interstitial cells of Cajal (25) and smooth muscle (18). Modulation of the mechanosensitive sodium channel results in changes in human smooth muscle membrane potential and slow wave upstroke, suggesting a role of intestinal Na+ in the regulation of gastrointestinal function. In response to a gastrointestinal symptom questionnaire sent to families genotyped for mutations in SCN5A, individuals with SCN5A mutations reported more gastrointestinal symptoms, especially abdominal pain, compared with patients in the same families without mutations, with an odds ratio of 5.7 (95% CI: 1.3–24.4) (11). This finding appeared to be specific to SCN5A, because no association was seen with KCNH2, a potassium channel-encoding gene responsible for type 2 LQT syndrome that is expressed in both heart and gastrointestinal tract.

Characterized by chronic or recurrent abdominal pain or discomfort, irritable bowel syndrome (IBS) is a very common disorder affecting 10–20% of Americans and accompanied closely by diarrhea- or constipation-type symptoms (20). Several studies have demonstrated that IBS aggregates in families (9, 12, 22, 23), lending support for the idea that either shared environmental factor(s) or genetic factor(s) (or both) may subserve the familial clustering. Furthermore, twin studies support both an environmental and a genetic basis for IBS (3, 10, 16). Thus IBS appears to be a complex, heterogeneous multifactorial disorder; both environmental and genetic interactions may result in symptom development, and this perhaps explains the clinical heterogeneity inherent to IBS. However, specific IBS-predisposing genetic defects have yet to be identified.

Given the association between genetic defects in SCN5A and gastrointestinal symptoms (11), the aims of this study were to evaluate SCN5A as a candidate gene involved in the pathogenesis of IBS and to determine the role of any identified mutation on Na+ current density, kinetics, mechanosensitivity, and single-channel conductance.

METHODS

This study was approved by the Mayo Clinic Institutional Review Board.

Subjects

Cases. DNA samples were collected from 49 patients participating in an ongoing genetic IBS study in which gastrointestinal symptom data were collected from cases. Subjects were outpatients with IBS,
ages 18–69 yr, seen in the Division of Gastroenterology of the Mayo Clinic, who were recruited between February 2004 and July 2005. All subjects were then asked to complete a self-reported bowel disease questionnaire (26) and donate 20 ml of blood. For the present study, those IBS cases who met Rome II criteria (27) for IBS and reported abdominal pain at least 2 yr in duration and rated as at least moderately severe (not mild) were selected. Lymphocyte DNA was extracted with a Gentra Autopure automated DNA extractor, and DNA quantification was performed with a spectrophotometer (SPECTRAMax PLUS 384).

Control subjects/reference group. Previously, genomic DNA from 829 apparently healthy anonymized subjects [319 African Americans (blacks), 295 Caucasians of European ancestry (whites), 112 Asians, and 103 Hispanics], obtained either from the Human Genetic Cell Repository sponsored by the National Institute of General Medical Sciences and the Coriell Institute for Medical Research (Camden, NJ) or from volunteer blood donors, underwent comprehensive SCN5A open reading frame/splice site genetic analysis (2). Ongoing SCN5A analysis now exceeds 3,000 reference alleles derived from at least 1,500 healthy volunteers. Volunteer donors were apparently healthy at the time of collection and self-reported their ethnicity. To be included in a specific ethnic group, each subject represented that all four grandparents were from the same group.

Genetic Analysis for SCN5A Mutations

With previously published primers (29), SCN5A mutational analysis was performed with polymerase chain reaction, denaturing high-performance liquid chromatography (dHPLC, WAVE, Transgenic, San Jose, CA), and dye terminator cycle sequencing (ABI Prism 377, PE Biosystems, Foster City, CA) as previously described (1). Only nonsynonymous polymorphisms (amino acid-altering variants) absent in control subjects were considered potentially pathogenic. Synonymous polymorphisms (silent nucleotide substitutions that did not alter the primary amino acid sequence) were excluded from this analysis.

Expression Vector Construction and HEK-293 Transfection

To assess the physiological significance of the observed mutation(s) in the IBS cases, wild-type and mutated SCN5A were transfected with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) in HEK-293 cells (American Type Culture Collection, Manassas, VA) together with green fluorescent protein (GFP) pEGFP-C1 (Clontech, Palo Alto, CA). The expression vectors containing the human jejunum Na+ channel SCN5A-encoded α-subunit were described previously (13, 19). HEK cells expressing SCN5A were cultured for 1 day after transfection. Transfected cells were identified by fluorescence microscopy and patch-clamped to record whole cell currents, and on-cell patches were obtained to study single-channel currents.

To accurately depict the functional consequence of any putative SCN5A mutation, it is important to express putative Na+ channel mutations in their proper context or genetic background. All humans express two abundant SCN5A transcripts (1 lacking the codon for Q1077 and 1 encoding Q1077) resulting from alternative splicing. Thus two different Na+,1.5 channels, designated Q1077del-SCN5A and Q1077-SCN5A, are expressed at all times in all humans and may demonstrate unique phenotypes when coexpressed with additional variants (13). Additionally, a common polymorphism, H558R (histidine 558 arginine), present with a 35% heterozygous frequency among white subjects, has been shown to alter the phenotypic expression of some type 3 LQT (LQT3)/Brugada syndrome SCN5A mutations (13). The patient identified with the G298S-SCN5A mutation (see below) was heterozygous for H558R-SCN5A; therefore, her cardiac and intestinal cells presumably express all four SCN5A transcripts with relative abundances as follows: H558/Q1077del = R558/Q1077del > H558/Q1077 = R558/Q1077. Since tissue was not available to extract mRNA needed to determine whether G298S resided on the H558-encoded allele or the R558-encoded allele, patch-clamp records were obtained from cells with and without the missense mutation G298S in the context of all four Na+ channel genetic backgrounds.

Patch-Clamp Recordings

Whole patch-clamp recordings were obtained at 22°C with standard whole cell and on-cell techniques (6, 7). Microelectrodes were pulled from Kimble KG-12 glass for the whole cell records and Schott 8250 for the single-channel records on a P-97 puller (Sutter Instruments, Novato, CA). Electrodes were coated with R6101 (Dow Corning, Midland, MI) and fire-polished to a final resistance of 3–5 MΩ for the whole cell records and 10–15 MΩ for the single-channel records. Currents were amplified, digitized, and processed with an Axopatch 200B amplifier, Digidata 1322A, and pCLAMP 9 software (Molecular Devices, Union City, CA). Whole cell records were sampled at 10 kHz and filtered at 4 kHz with an eight-pole Bessel filter. Cells were held at a holding potential of −100, −90, −80, −70, or −60 mV and then stepped from −80 to +35 mV in 5-mV intervals for 50 ms each. An 80–95% series resistance compensation with a lag of 10–60 μs was applied during each recording. Resistance after access was established was 5–10 MΩ. Single-channel records were sampled at 10 kHz and filtered at 5.1 kHz. Cells were held at transmembrane voltages ranging from −70 to −120 mV, briefly stepped to −50 mV for 100 μs to open the channel, and then ramped from −60 to +60 mV.

Drugs and Solutions

For whole cell experiments, the intracellular solution contained (in mM) 130 Cs+, 125 methanesulfonate, 20 Cl−, 5 Na+, 5 Mg2+, 5 HEPES, 2 EGTA, 2.5 ATP, and 0.1 GTP. The extracellular solution before, during, and after perfusion was normal Ringer solution (in mM): 149.2 Na+, 159 Cl−, 4.74 K+, 2.54 Ca2+, 5 HEPES, and 5 glucose. Intra- and extracellular solutions were equilibrated to pH 7.0 and pH 7.35 with CsOH or NaOH, respectively. Osmolality of either solution was 300 mosmol/kgH2O. The flow rate of solution during perfusion to test for mechanosensitivity was 10 ml/min, and this flow rate was applied for 60 s. For single-channel experiments, the pipette and bath solutions contained (in mM) 149.2 Na+, 159 Cl−, 4.74 K+, 2.54 Ca2+, and 5 HEPES, equilibrated to 7.35 with NaOH.

Data Analysis

For the genotyping portion of the study, demographic comparisons used t-tests and χ2-tests for continuous and categorical data, respectively. Allele and genotype frequencies (%) were calculated for each polymorphism among the IBS cases and the reference group. Comparison of these frequencies between IBS cases and the reference group was performed with χ2-analysis (or Fisher’s exact tests) and Armitage trend tests with SAS statistical software (SAS Institute, Cary, NC).

For the functional studies of sodium channel properties, data were analyzed with Clampfit (Molecular Devices), Excel (Microsoft, Redmond, WA), GraphPad InStat (GraphPad Software, San Diego, CA), and SigmaPlot (SPSS, Chicago, IL). For current density-voltage graphs, currents (in pA) were expressed as a fraction of each whole cell capacitance (pF). For steady-state kinetics, the peak inward current of a single control trace was normalized to 1 with the equation

\[ I_{\text{NORM}} = 100 \times (I/I_{\text{MAX}}), \]

where \( I_{\text{NORM}} \) is normalized current, \( I \) is measured current at a given voltage, and \( I_{\text{MAX}} \) is maximum peak inward current of the control trace. Thus peaks of all other traces per cell were expressed as a fraction of 100. For perfusion experiments, data are expressed as values normalized to the amount of baseline current measured before perfusion (100%). Statistical comparisons were made with a paired, two-tailed Student’s t-test or one-way ANOVA. Statistical significance was accepted when \( P \) values were <0.05. Data are expressed as means ± SE. Single-channel conduc-
RESULTS

Characteristics of Subjects

The median age of the 49 patients with IBS was 33 (range 18–49) yr. Of this sample, 43 (88%) were female and 6 (12%) were male. The racial distribution by self-report was as follows: 47 (96%) reported only Caucasian backgrounds; 1 (2%) reported Caucasian, black or African American, and Hispanic or Latino backgrounds; and 1 (2%) reported Caucasian and Hispanic or Latino backgrounds. The usual bowel habit in the last year was reported to be diarrhea by 25 (51%), constipation by 15 (31%), mixed by 8 (16%), and normal by 1 (2%).

SCN5A Genetic Polymorphisms

H558R polymorphism. The genotype and allele frequencies for the known H558R polymorphism are shown in Table 1. No difference in genotype frequency was observed between IBS cases and the reference group. Although the frequency of the R allele was numerically greater than in the reference group (28% vs. 22%), this was not statistically significant (P = 0.18).

G298S missense mutation. One of the 49 patients (2%) hosted a G298S missense mutation. She was also heterozygous for the H558R common polymorphism. For the known H558R polymorphism are shown in Table 1. No difference in genotype frequency was observed between IBS cases and the reference group. Although the frequency of the R allele was numerically greater than in the reference group (28% vs. 22%), this was not statistically significant (P = 0.18).

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Table 1. Genotype and allele frequencies of H558R common polymorphism in irritable bowel syndrome cases and control reference group

<table>
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<tr>
<th>Genotypes</th>
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<tr>
<td>HH</td>
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<tr>
<td>HR</td>
<td>72%</td>
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<td>RR</td>
<td>72%</td>
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<td>Control</td>
<td>72%</td>
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Genotype frequency values are numbers of subjects (%).
sulted in reduced mechanosensitivity in the setting of a com-
mon SCN5A transcript, H558/Q1077del. Furthermore, we
found this marked loss-of-function sodium channel mutation in
a patient with IBS, raising the possibility that ion channel
mutations may underlie a subset of patients with this hetero-
geneous and poorly understood disorder. To date, there are
over a hundred rare mutations in SCN5A that have been
associated with either LQT3 syndrome, type 1 Brugada syn-

**Fig. 1.** G298S mutation reduces Na⁺ current density from SCN5A transfected in HEK cells. Current-voltage relationships of Na⁺ current expressed in HEK cells from SCN5A transcript backgrounds H558/Q1077del (A), H558R/Q1077del (B), H558/Q1077 (C), or H558R/Q1077 (D) with the G298 wild-type allele or G298S missense mutation are shown. Insets: representative Na⁺ currents recorded from channels with G298 or G298S. *P < 0.05; n values are indicated in parentheses.

**Fig. 2.** G298S mutation reduces Na⁺ current density at multiple holding voltages. Peak Na⁺ current densities sorted by holding voltage and recorded from HEK cells expressing SCN5A transcript backgrounds H558/Q1077del (A, n = 14–30), H558R/Q1077del (B, n = 9–17), H558/Q1077 (C, n = 5–10), or H558R/Q1077 (D, n = 5–10) with the G298 wild-type allele or the G298S missense mutation are shown. *P < 0.05.
drome, inherited cardiac conduction defects, sudden unexpected nocturnal death syndrome (SUNDS), or sudden infant death syndrome (SIDS) (15). None has been reported in gastrointestinal diseases. Several studies suggest that there may be an underlying genetic basis to IBS. While several candidate gene association studies have been performed evaluating neurotransmitter processing proteins and inflammatory mediators (21), no genetic abnormalities in genes encoding intestinal channel proteins have been evaluated thus far. In light of previous data from our group suggesting that congenital LQT syndrome and

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**Fig. 3.** G298S mutation slows activation kinetics. Time to peak of Na⁺ current expressed in HEK cells from transcript backgrounds H558/Q1077del (A), H558R/Q1077del (B), H558/Q1077 (C), and H558R/Q1077 (D) with the G298 wild-type allele or the G298S mutation is shown. *P < 0.05 from 0 to 35 mV; n values are indicated in parentheses.

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**Fig. 4.** G298S mutation does not alter inactivation kinetics. Fast (τ₂) and slow (τ₁) time constants of inactivation of Na⁺ current from SCN5A with G298 wild-type allele or G298S mutation are shown. No change in inactivation kinetics was noted at any voltage for transcript backgrounds H558/Q1077del (A, n = 16–28), H558R/Q1077del (B, n = 9–12), H558/Q1077 (C, n = 8 or 9), or H558R/Q1077 (D, n = 6–9).
Brugada syndrome patients with rare mutations in the SCN5A gene have more gastrointestinal symptoms (11), this study aimed to determine whether genetic variants in SCN5A might be associated with IBS. Thus sequence analysis of this gene in 49 patients with IBS and moderate to severe pain was performed. In 1 of the 49 patients studied, all of whom fulfilled the Rome II criteria for IBS, we found a mutation in SCN5A (G298S). This G to A substitution at nucleotide 892 on exon 7 results in a glycine (G) to serine (S) missense mutation involving residue 298 (G298S) that localizes to the domain I/S5-S6 loop (pore) region of the sodium channel. Notably, this specific loss-of-function mutation was reported previously in two siblings with an atrioventricular conduction defect (28). Given that this report predated our association of gastrointestinal symptoms in cardiac patients with SCN5A mutations, it is not surprising that there was no mention of gastrointestinal symptomatology. G298S was not observed in our reference group, nor has it been reported in over 3,000 reference alleles worldwide. Interestingly, the patient in the present IBS study had no evidence of a cardiac conduction defect and a completely normal ECG despite the marked changes in Na\(^+/\)H\(^{-}\) current density. The patient has declined a provocation study with procainamide to see whether a Brugada ECG pattern is inducible.

Nav1.5 is present in the population as several distinct backgrounds. The most common are H558/Q1077del, H558R/Q1077del, H558/Q1077, and H558R/Q1077, which are estimated to be present in the population with frequencies of 45%, 20%, 25%, and 10%, respectively (13). These genetic backgrounds are functionally distinct, with the H558R/Q1077 minor transcript associated with reduced sodium current compared with the three other main transcripts (13). This patient was heterozygous for the common H558R polymorphism and therefore expressed all four transcripts. Since tissue was not available to extract mRNA needed to determine whether the G298S missense mutation resided on the H558-encoded allele or the R558-encoded allele, all eight possible SCN5A transcripts were engineered (i.e., with and without G298S).

We found that the mutation was functionally significant and that the functional effects of the mutation were in fact dependent on the channel background. The effect of the G298S mutation on whole cell Na\(^+/\)H\(^{-}\) channel current density was characterized previously by Wang et al. (28) in the context of the hH1 Na\(^+/\)H\(^{-}\) channel clone (H558/Q1077). While we saw a 65% decrease in current in H558/Q1077, this did not reach statistical significance in our study, likely because of the high variability in the control subjects. In this study we also extend the finding to H558/R1077del (49% decrease), H558R/Q1077del (55% decrease), and H558R/Q1077 (77% decrease). These data fit with the observed changes in activation kinetics. Time to peak was increased by the G298S missense mutation in both H558R-containing backgrounds, which also showed a decrease in peak current. The combination of slower activation and unchanged inactivation kinetics would result in a decrease in peak Na\(^+/\) current density. However, the decrease in peak current density in G298S/H558/Q1077del-SCN5A suggests...
that the delay in activation kinetics is not the sole cause of the smaller current. A smaller current can be due to a change in channel kinetics, a change in open probability, or a change in the number of channels available. We did not see a change in single-channel conductance, suggesting that the number of available channels was decreased. Whether this is due to defective trafficking or a change in the number of channels available to open was not determined in this study.

There is increasing evidence that Na$_1$5.1 is a mechanosensitive channel. This was first shown in whole cell current experiments (24, 25). Mechanosensitivity is dependent on an intact cytoskeleton and is abolished when the link is severed between the PDZ domain of syntrophin $\gamma$ and the COOH terminus of the SCN5A-encoded $\alpha$-subunit (19). Recently, mechanosensitivity has been shown at the level of single sodium channels (17). Mechanosensitivity was reduced when the G298S mutation was expressed in the most abundant sodium channel transcript, H558/Q1077del. Reduced mechanosensitivity in the gut, together with the observed changes in peak current density, may also contribute to the development of an observable phenotype. Of note, the degree of mechanosensitivity in all four G298S-containing transcripts was approximately the same; the relative difference observed in the context of the H558/Q1077del transcript was predominantly due to the greater mechanosensitivity in the common wild-type sodium channel, G298/H558/Q1077del. We therefore tripled the $n$ value of our observations to determine whether this was a statistical anomaly. Even with this increased number of observations, the same result was obtained, suggesting that G298S in the context of H558/Q1077del-SCN5A does indeed reduce mechanosensitivity.

The mutation in the patient with IBS therefore appears to be physiologically significant. It is at present unclear what contribution this mutation made to the patient’s symptoms. Our present understanding is that Na$_1$5.1 in the human gut is expressed in smooth muscle cells and interstitial cells of Cajal. Expression of Na$_1$5.1 in enteric nerves is not known. Given the role that smooth muscle and interstitial cells of Cajal play in regulation of gastrointestinal motility, attribution of motility-like symptoms such as diarrhea and constipation to changes in ion channels expressed in these cells types is plausible. In this regard a preliminary report (5) from a second institution has also linked mutations in SCN5A (in patients with Brugada syndrome) with gastrointestinal symptoms. The finding of a predominance of abdominal pain in our previous study (11) and again in this patient with abdominal pain, however, suggests that additional mechanisms aside from changes in motility are also operative. Intestinal cells of Cajal are associated closely with vagal afferents and may be involved in transduction ofafferent signals (4, 8). Also, the significant role that central processing plays in the expression of symptoms in functional bowel diseases such as IBS may result in pain as the dominant symptom in some patients while not in others, as a result of differential central processing of peripheral motor signals (14). It is also possible that the expression of Na$_1$5.1 in the gut is more widespread than currently thought.

If our novel discovery of a rare, functionally significant missense mutation in a patient with idiopathic IBS is a real pathogenic association, this represents a major breakthrough in helping to understand possible mechanisms underlying IBS, at least in a subset of patients with this heterogeneous disorder. This patient underwent an extensive work-up ruling out other causes for her IBS symptoms, making a misdiagnosis of IBS unlikely. However, this mutation was the only mutation discovered in SCN5A and was only found in 1 of 49 IBS patients tested. IBS is quite variable in presentation, suggesting that there may be underlying mechanistic heterogeneity (i.e., multiple genes and environmental risk factors) responsible for this common disorder that affects 12–20% of the general population. Our study suggests that there are other disease susceptibility loci for IBS that remain to be discovered.

In summary, these data suggest that a rare missense mutation in SCN5A (G298S) results in loss of whole cell current density, delayed activation kinetics, and, in the setting of the most abundant alternatively spliced transcript, H558/Q1077del-SCN5A, an attenuation in mechanosensitivity. This mutation was found in a patient with IBS and suggests that perturbations in SCN5A may confer susceptibility to the development of IBS. Additional studies are needed to determine the potential role of this mutation specifically and the prevalence of rare SCN5A mutations generally in a larger sample of patients with IBS. The concept of an intestinal channelpathy resulting in IBS or IBS-like symptoms deserves further investigation.

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