

IMMEDIATE COMMUNICATION**Sodium channels *SCN1A*, *SCN2A* and *SCN3A* in familial autism**LA Weiss¹, A Escayg¹, JA Kearney¹, M Trudeau¹, BT MacDonald¹, M Mori², J Reichert³, JD Buxbaum³ and MH Meisler¹¹Department of Human Genetics, University of Michigan, Ann Arbor, MI, USA; ²Department of Physiological Sciences, Graduate University for Advanced Studies, National Institute for Physiological Sciences, Okazaki, Japan; ³Seaver Autism Research Center, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY, USA

Autism is a psychiatric disorder with estimated heritability of 90%. One-third of autistic individuals experience seizures. A susceptibility locus for autism was mapped near a cluster of voltage-gated sodium channel genes on chromosome 2. Mutations in two of these genes, *SCN1A* and *SCN2A*, result in the seizure disorder GEFS+. To evaluate these sodium channel genes as candidates for the autism susceptibility locus, we screened for variation in coding exons and splice sites in 117 multiplex autism families. A total of 27 kb of coding sequence and 3 kb of intron sequence were screened. Only six families carried variants with potential effects on sodium channel function. Five coding variants and one lariat branchpoint mutation were each observed in a single family, but were not present in controls. The variant R1902C in *SCN2A* is located in the calmodulin binding site and was found to reduce binding affinity for calcium-bound calmodulin. R542Q in *SCN1A* was observed in one autism family and had previously been identified in a patient with juvenile myoclonic epilepsy. The effect of the lariat branchpoint mutation was tested in cultured lymphoblasts. Additional population studies and functional tests will be required to evaluate pathogenicity of the coding and lariat site variants. SNP density was 1/kb in the genomic sequence screened. We report 38 sodium channel SNPs that will be useful in future association and linkage studies.

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Introduction

Autism [MIM 209850] is a complex psychiatric disorder characterized by impaired communication

and social skills, and restricted and repetitive behaviors.¹ Half of the children with autism are nonverbal and between one half and three-quarters are mentally retarded. Epilepsy is a complication in one-third of autistic children, with the most severe seizures occurring in adolescence.² In one study, 82% of children with regressive autism exhibited epileptiform activity.³ The population incidence of autism is approximately 0.1%.⁴

Autism appears to have a strong genetic component, with an estimated heritability above 90%.⁵ The disease risk of siblings is 20–40 times higher than the general population.⁶ Genetic modelling suggests that epistatic interaction between 3 and 15 genes may be responsible for the disorder.^{6,7} The broad phenotypic variation in autism spectrum disorders is consistent with the evidence of genetic heterogeneity. Specific rearrangements of chromosomes 7, 15 and X have been observed in autistic individuals, and evidence for linkage to markers on chromosome 7q was obtained in large-scale genome scans.⁶ Mutations in the X-linked Rett syndrome gene *MECP2* have been identified in some patients with infantile autism,⁸ but were not observed in another survey of 59 patients.⁹ One chromosome region that generated positive LOD scores in early genome scans of autistic families is on Chr 2q.^{10,11} In recent studies, LOD scores

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approaching 4 were obtained with markers located between 176 and 180 cM on chromosome 2.^{12–14} Restriction to cases with delayed onset of phrase speech (PSD) increased the evidence for linkage on chromosome 2.¹²

The sodium channel alpha subunit genes *SCN1A*, *SCN2A* and *SCN3A* are located at approximately 175 cM on chromosome 2, band 2q24.¹⁵ These voltage-gated sodium channels are expressed in neurons and glia throughout the central and peripheral nervous system^{16,17} and have been highly conserved during invertebrate and vertebrate evolution.¹⁸ Missense mutations in *SCN1A* and *SCN2A* have been identified in the inherited seizure disorder Generalized Epilepsy with Febrile Seizures Plus (GEFS+2, MIM 604236, 604233).¹⁹ *De novo* null mutations in *SCN1A* result in severe myoclonic epilepsy of infancy, a syndrome that includes mental retardation.²⁰ Mice expressing a mutated *SCN2A* channel with persistent current exhibit seizures and repetitive behaviors.²¹ The association of seizures with mutations in the sodium channel genes, and their location near an autism susceptibility locus, suggested that they might play a role in susceptibility to autism.

The Autism Genetic Research Exchange (AGRE) was developed to facilitate collaborative genetic research into the etiology of autism, by making biomaterials from well-characterized families widely available to the scientific community.^{4,22} Each AGRE family includes two parents and 2–6 sibs, at least 2 of whom are affected. Genomic DNA and immortalized cell lines are available for several hundred families. We have studied these multiplex families in order to identify novel mutations in *SCN1A*, *SCN2A* and *SCN3A* and evaluate their cosegregation with the disorder.

Material and methods

Subjects

DNA (10 μ g) from one affected individual in each of 229 multiplex families was purchased from the AGRE. Diagnosis was based on the autism diagnostic interview-revised protocol (ADI-R)¹ and reflects the accepted criteria of the DSM-IV/ICD-10.²² A representative subset of AGRE families was analyzed. Linkage data for these families were not available at the outset of the study. In all, 11 additional samples were obtained from patients with sib-pair analysis consistent with linkage to chromosome 2.¹² polyA + RNA was extracted from immortalized lymphoblast cell lines obtained from AGRE and cultured under standard conditions.

Mutation detection

A total of 26 coding exons of *SCN1A* were amplified in 28 PCR reactions and analyzed by conformation sensitive gel electrophoresis (CSGE) as previously described.^{15,23} PCR products containing variants were gel purified using Qiaex II or QIAquick (Qiagen) and manually sequenced with ThermoSequenase (USB).

PCR primers flanking the exons of *SCN2A* and *SCN3A* were designed from intronic sequences in the public databases. The polymorphism C(6015 + 856)T in the 1.7 kb 3'UTR of *SCN2A* was detected in the 499 bp fragment obtained by amplification of genomic DNA with the primers F2 (AGG TTG TTT ACT ATT ATA TGT GAC) and R2 (TTT TTA TTT TAT GCC AAA GGACAC). *SCN1A* and *SCN2A* were screened in 117 AGRE families and *SCN3A* was screened in a subset of 32 families consistent with Chr 2 linkage.

RT-PCR of lymphoblast RNA and primer extension reaction

To evaluate the effect of the lariat mutation in intron 6, total RNA was isolated from lymphoblast cell lines. Reverse transcription of 5 μ g of total RNA from lymphoblast cell lines was performed with poly(dT) and random hexamers as previously described.²⁴ First-strand cDNA (1 μ l) was amplified with the forward primer 6F in exon 6 (TCC TTT GAA ATA AAT ATC ACT TCC) and the reverse primer 10R in exon 10 (TAG ATG CTA CTG AAG AAC TCT CTG). For the primer extension reaction, RT-PCR was carried out with primers F2 and R2 (above). PCR products were gel purified using the Qiaquick gel extraction kit (Qiagen). The reverse primer R5 (GAC TGT GAA TGA CAA TGT AAT GGT A) was gel purified and used for the primer extension reaction with 1 μ l of PCR product as template, using the ThermoSequenase kit (USB) in the presence of 0.1 μ Ci [α -³³P] ddATP and unlabelled dCTP, dTTP and dGTP. Reaction products were resolved on a 12% denaturing polyacrylamide gel and visualized by autoradiography.

Calmodulin binding

The interaction between calmodulin and the synthetic peptide NaIQ was measured by fluorescence spectroscopy using dansylated calmodulin as previously described.²⁵ Fluorescence spectra from the wild-type peptide were compared with a peptide containing the *SCN2A* variant R1902C. Peptide titration data were analyzed using the MicroCal Origin software and fitted with a two-site binding model to obtain values for the dissociation constants, using the formula $y = B_{\max 1}^* x / (k_1 + x) + B_{\max 2}^* x / (k_2 + x)$.

Results

Five rare coding variants in autism families

The 26 coding exons of *SCN1A* and *SCN2A* were screened in an affected individual from 117 AGRE families. The four exons containing variants were then screened in an additional 112 families. Five missense variants were identified, each present in 1/229 families: R542Q, I1034T, F1038L, I1955T and R1902C (Table 1). The positions of the amino acid substitutions in the transmembrane channel protein are shown in Figure 1. The mutation R542Q in *SCN1A* was inherited by 2 affected sibs (Figure 2), and was previously observed in a patient with

Table 1 Coding variants in sodium channel genes identified in patients with autism

Gene	Amino acid substitution	Type	Frequency in AGRE families	Frequency in controls	Concordance	
					Affected	Unaffected
SCN1A	R542Q	R	1/229 (AU0114)	0/96	2/2	0/0
	I1034T	R	1/229 (AU0427)	0/96	1/2	0/1
	F1038L	R	1/229 (AU0044)	0/96	2/2	0/0
	T1067A	P	40/117	32/96 ^a	Polymorphic	
	I1955T	R	1/229 (AU0203)	0/48	Not available	
SCN2A	R19K	P	6/117	3/48	Polymorphic	
	R1902C	R	1/229 (AU0247)	0/296	1/2	0/0
SCN3A	ΔN43	P	12/117	5/57	Polymorphic	
	G1813S	P	3/117	2/121	Polymorphic	

^aFrom Escayg *et al* (2001) (Ref. 15).

Positions of these variants in the cytoplasmic domains of the sodium channel protein are shown in Figure 1. All individuals carrying these variants were heterozygotes. Type: P, polymorphic (allele frequency >1%); R, rare (frequency <1%).

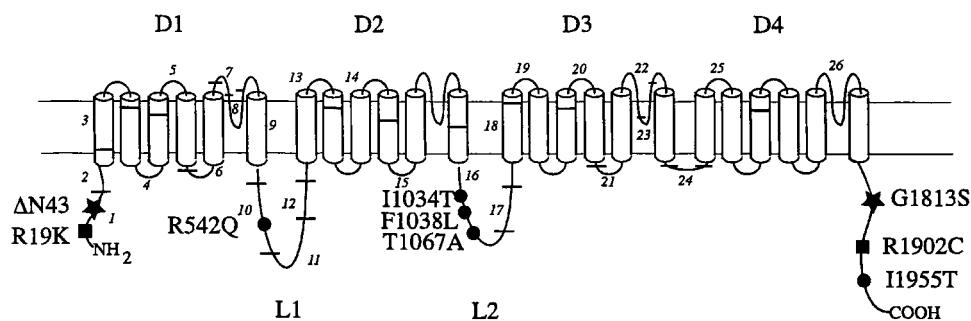


Figure 1 Positions of rare and polymorphic coding substitutions in three sodium channel proteins: L1, cytoplasmic loop 1; L2, cytoplasmic loop 2. SCN1A, circles; SCN2A, squares; SCN3A, stars. Exons are numbered.

juvenile myoclonic epilepsy.¹⁵ This mutation disrupts the predicted tyrosine kinase site RLTYEKRY in cytoplasmic loop 1. The mutation I1034T in SCN1A was present in 1 of 2 affected sibs and not in the unaffected sib (Figures 1 and 2). The mutation F1038L in SCN1A was present in 2 affected sibs (Figure 2). The missense mutation R1902C in SCN2A was detected in 1 affected individual from family AU0247 but not in his affected sib (Figure 2) nor in 296 controls. Residue R1902 is conserved in mammalian and invertebrate sodium channels and is located within a calmodulin binding site (see below). The mutation I1955T was identified in an affected individual from family AU0203; the other members of this family were not available for testing (pedigree not shown).

The 26 coding exons of SCN3A were screened in a subset of 32 AGRE families; in these families, markers for chr 2q24 were concordant with autism. No rare coding variants were observed; two polymorphic coding variants of SCN3A are described in a later section (Table 1).

Functional effect of R1902C on calcium-calmodulin binding

R1902C is located within a 27 amino-acid peptide of SCN2A that contains a functional binding site for calmodulin.²⁵ The mutant peptide was synthesized and the binding affinity for calcium-calmodulin was determined. The K_d was 304 nM for the mutant peptide and 141 nM for the wild-type peptide (Figure 3), demonstrating reduced affinity of the mutant peptide for the low-affinity binding site for calcium-bound calmodulin. The binding constant for calcium-free calmodulin was not affected (data not shown), consistent with evidence that interaction with calcium-free calmodulin occurs at the IQ motif residues that are not affected by this mutation.²⁵

A branchpoint mutation in intron 6 of SCN2A

The nucleotide substitution A(970–32)G was identified in an affected individual from family AU0207. This variant alters the essential adenosine residue of a lariat consensus element (YNYRAY) that is located 32 bp upstream of exon 7 in SCN2A (Figure 4).

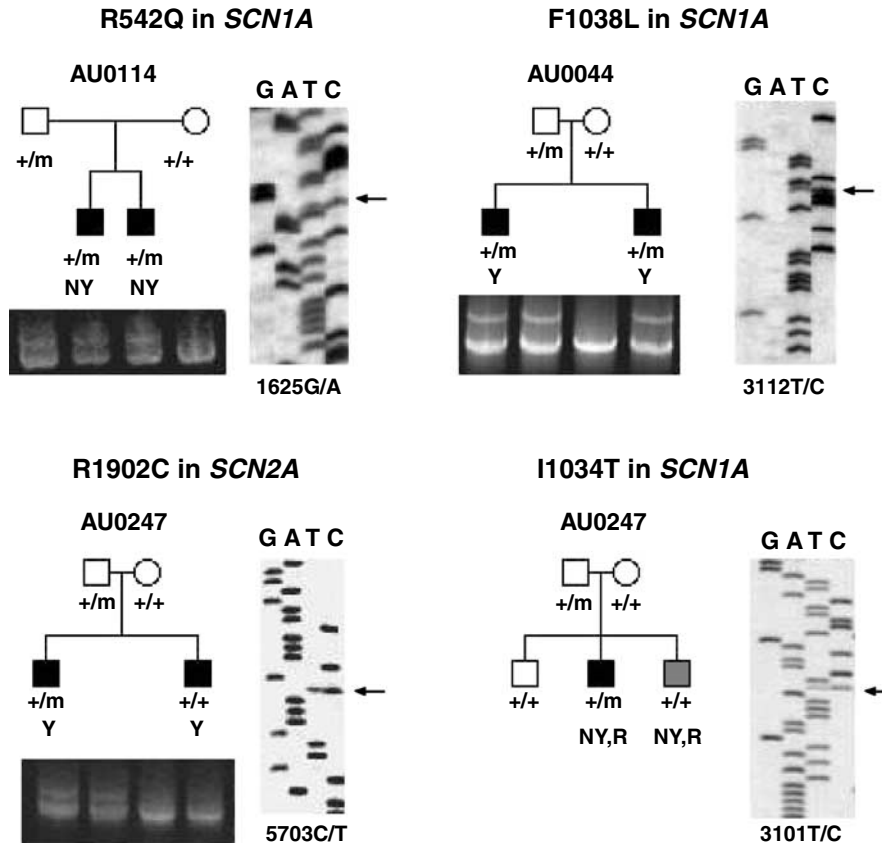


Figure 2 Segregation of rare coding variants in multiplex autism families. Beneath each pedigree, the results of conformation-sensitive electrophoresis are shown. Heterozygosity for sequence variation is presented for one individual from each family. Solid symbols, autism; shaded symbol, pervasive developmental disorder; m, mutant allele; N, nonverbal; V, verbal; R, regressive disorder.

Substitution of G for A at the lariat branchpoint is known to reduce splicing efficiency,^{26,27} suggesting that this intronic variant could have functional significance. We therefore screened an additional 112 patients and identified 2 more families carrying the same mutation (Figure 4). The heterozygote frequency was 3/229 in unrelated autism patients and 3/450 in a group of unrelated controls. In 2 families, the branchpoint variant was inherited by affected sibs only, but in family AU0509, 5 unaffected offspring also carry the mutation (Figure 4). It is interesting that almost all the male heterozygotes in these families are affected (7/8), while none of the female heterozygotes are affected (0/7) ($P < 0.01$).

To investigate the effect of the intron 6 branchpoint mutation on splicing of exon 7, we analyzed lymphoblast RNA from family AU0207. RT-PCR was carried out using a forward primer in exon 6 and a reverse primer in exon 10 (Figure 5a). The product obtained from II-1 was identical in length to the wild-type product from I-1, indicating that splicing is qualitatively normal (Figure 5b).

To evaluate the effect of the branchpoint mutation on splicing efficiency, we identified individuals who

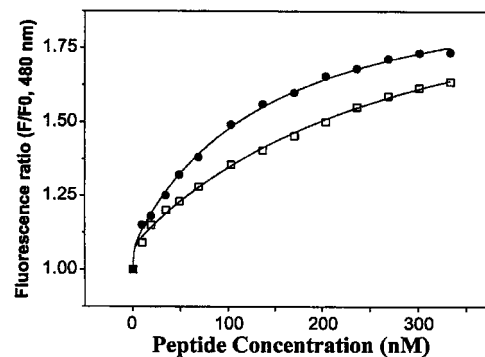


Figure 3 Reduced binding of calcium-calmodulin because of the amino-acid substitution R1902C in *SCN2A*. Dansylated synthetic peptides containing the wild-type or mutant sequence were incubated with calcium-calmodulin as previously described.²⁵ Closed circles, wildtype; open squares, mutant.

were heterozygous for both the branchpoint mutation and an exonic SNP that would enable us to distinguish between the two transcripts. We sequenced a 499 bp fragment from the 3' UTR and determined that

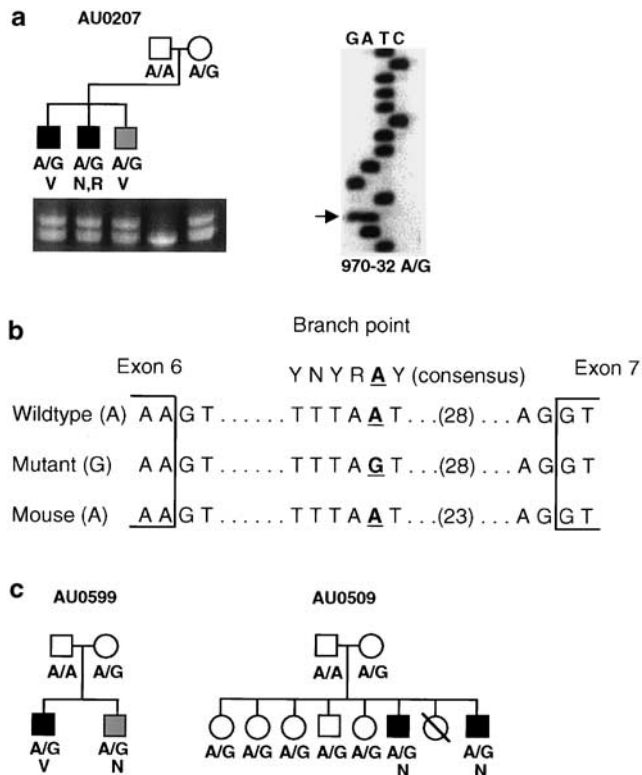


Figure 4 Segregation of the branchpoint mutation A(970–32)G in three autism families. (a) Segregation, conformation-sensitive electrophoresis and heterozygous sequence; symbols as defined in Figure 2. (b) Position of the branch site mutation. (c) Genotypes of two additional families. N, nonverbal; V, verbal; R, regressive disorder.

an affected male and his unaffected father (Figure 4a) are heterozygous for the variant C(6015 + 856)T. The affected male is also heterozygous for the branchpoint mutation while the father is homozygous for the wild-type A at the branchpoint. To compare the abundance of the two *SCN2A* transcripts in these individuals, the 3' UTR was amplified by RT-PCR and the products were analyzed with a primer extension assay using a primer downstream of the C/T variant in the presence of one radiolabelled dideoxy nucleotide triphosphate. The radiolabelled primer extension products were 31 bp for the C allele and 27 bp for the T allele. Analysis of genomic DNA served as a control demonstrating the products obtained from equal abundance of the two alleles (Figure 5c, lane 3). The same ratio was observed with RNA from the branchpoint A/G heterozygote and the A/A homozygote (Figure 5c). This result demonstrates that the branchpoint mutation did not alter splicing efficiency of the *SCN2A* transcript in lymphoblast cells.

Polymorphic coding variants (cSNPs)

Four polymorphic coding variants were observed at equal frequencies in patients and controls: T1067A, R19K, Δ N43 and G1813S (Figure 1 and Table 1). The 2 alleles of T1067A in *SCN1A* exhibit frequencies of 0.67 and 0.33 in the patient population (Table 1), in agreement with our previous analysis of a control population.¹⁵ The variant R19K in *SCN2A* was detected in 6/117 affected individuals and 3/48 controls. The deletion Δ N43 in *SCN3A* was present in 12/117 patients and 5/57 controls. The rat and

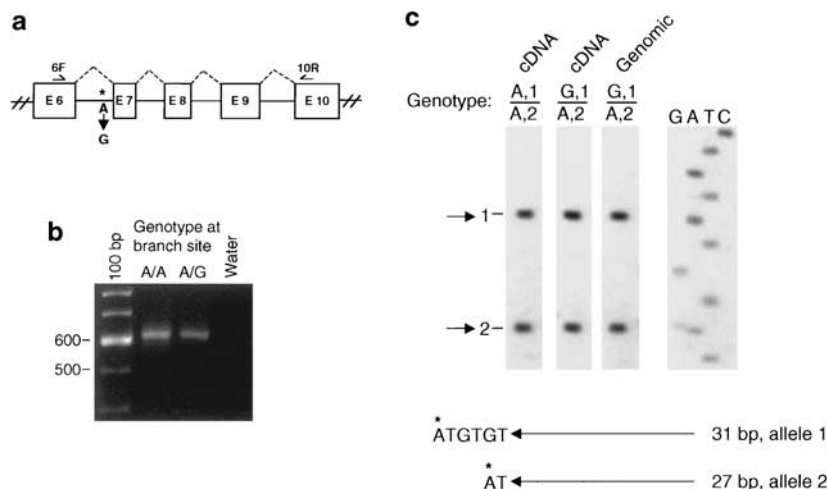


Figure 5 Effect of the branchpoint mutation on splicing of exon 6. Total RNA was isolated from cultured lymphoblasts and analyzed by RT-PCR and primer extension (see text for details). (a) Location of primers in exons 6 and 10. (b) Length of RT-PCR product obtained with these primers is not affected by the branchpoint mutation, indicating that exon 6 is spliced normally. (c) Primer extension analysis of the effect of the branchpoint mutation on transcript abundance. Genotypes are indicated for the branchpoint nucleotide (A or G) and for the SNP in the 3' UTR (allele 1 = C(6015 + 856), allele 2 = T(6015 + 856)). On the reverse strand, allele 1 incorporates a G at the variable position and gives an A-labelled product of 31 bp; allele 2 incorporates an A at the variable position to give a product of 27 bp. Primer extension products were separated on a 12% acrylamide gel.

mouse orthologs of human *SCN3A* contain the N43 residue, indicating the minor human allele is the product of a recent 3 bp deletion that occurred within an imperfect trinucleotide repeat (Figure 6). The residue G1813 in *SCN3A* is located in an evolutionarily conserved region of the C-terminal domain. Either glycine or alanine is present in all of the sequenced vertebrate and invertebrate sodium channels represented in GenBank. The variant G1813S was detected in 3/117 patients and in 2/121 controls. G1813S did not cosegregate with autism in the 3 patient families (not shown).

Noncoding variants in the sodium channel genes

The sodium channel primer pairs amplified a total of 27 kb of coding sequence and 3 kb of adjacent intronic sequence from genomic DNA. We detected 37 noncoding SNPs, 2 single-base insertions, 2 single-base deletions, and 1 dinucleotide deletion (Tables 2 and 3), for a density of 1 variant per 700 bp. Many of the

human	Q	D	N	D	D	E	N
human	C A A	G A T	A A T	G A T	G A T	G A G	A A C
ΔN43	C A A	G A T	- - -	G A T	G A T	G A G	A A C
rat	C A A	G A C	A T T	G A C	G A T	G A G	A A C
mouse	C A A	G A C	A T T	G A C	G A T	G A G	A A C

Figure 6 Simple sequence surrounding the ΔN43 variant of *SCN3A*. The wild-type allele in human, mouse and rat contains an amino acid that is deleted in the variant. The population frequency of the deletion allele is 0.05 (Table 1).

variants were restricted to 1 or 2 individuals. Haplotype frequencies for *SCN1A* in the AGRE families were in agreement with those previously described,¹⁵ except that a novel *SCN1A* haplotype was observed in 2 autistic individuals (Table 4).

Discussion

Potential disease mutations

Six potential disease mutations were observed, each in only 1 AGRE family. The substitution of G for the branchpoint site A in intron 6 of *SCN1A* (TTTTAAT to TTTTAGT) alters the consensus sequence YNY-TRAY and is predicted to reduce splicing efficiency.^{26,27} The association of haploinsufficiency of *SCN1A* with severe seizures²⁰ supports the possibility that a hypomorphic allele of *SCN2A* could contribute to autism. However, this mutation did not reduce the efficiency of removal of intron 6 in lymphoblasts. In view of other examples of neuron-specific splicing defects, for example, in familial dysautonomia,²⁸ we cannot exclude the possibility that this mutation reduces splicing efficiency in neurons. It is interesting that 7/8 of heterozygous males in the AGRE families are affected, while none of the 7 heterozygous females are affected ($P < 0.01$) (Figure 4). If we include the 2 heterozygous female controls and 1 heterozygous male control, the incidence of autism is 7/9 in male heterozygotes and 0/9 in female heterozygotes. This may indicate that males are more sensitive to the branchpoint mutation.

The R1902C substitution is located within the Ca^{2+} -calmodulin binding site of *SCN2A*, which was

Table 2 Polymorphic noncoding SNPs in *SCN1A*, *SCN2A* and *SCN3A*. Allele frequencies did not differ between autism patients and controls. nd, frequencies not determined

Gene	Location in gene	cDNA nucleotide	SNP alleles major/minor	Frequency in AGRE families	Allele frequency in AGRE families	Allele frequency in controls
<i>SCN1A</i>	Intron 6	965–21	C/T	nd	nd	0.33 ^a
	Intron 7	1028 +21	T/A	nd	nd	nd
	Intron 8	1171–9,10	+/ Δ TT	7/117	0.03	0.04 ^a
	Exon 9	1212	A/G	62/117	0.34	0.33 ^a
	Exon 13	2292	T/C	62/117	0.34	0.33 ^a
	Intron 13	2416–72	G/A	40/117	0.17	nd
	Intron 13	2416–37	A/C	63/117	0.34	0.33 ^a
<i>SCN2A</i>	Intron 7	1034–3	T/C	33/117	0.15	0.10 (n=30)
	Exon 10	1388	A/C	nd	nd	0.20 (n=30)
	Intron 10	1671–16	C/T	74/117	0.42	0.48 (n=30)
	intron 21	4254–31	A/G	33/117	0.14	0.12 (n=29)
	Exon 26	4913	T/A	61/117	0.31	0.28 (n=30)
<i>SCN3A</i>	Intron 2	264+81	C/T	5/32	0.08	0.16 (n=29)
	Intron 5	692–46	G/A	5/32	0.08	0.12 (n=30)
	Intron 8	1170+69	G/A	13/32	0.20	0.23 (n=30)
	Exon 10	1438	C/T	nd	nd	nd
	Exon 12	1881	A/T	11/32	0.17	0.23 (n=30)

^aFrom Escayg et al (2001) (Ref. 15).

Table 3 Rare noncoding SNPs in *SCN1A*, *SCN2A* and *SCN3A*

Gene	Location in gene	cDNA nucleotide	SNP alleles major/minor	Frequency in AGRE families	Allele frequency
<i>SCN1A</i>	Exon 2	345	T/C	2/117	0.009
	Exon 2	363	T/C	1/117	0.004
	Intron 2	384–20	G/C	2/117	0.009
	Intron 10	1662+9	C/A	1/117	0.004
	Exon 15	2889	T/C	2/117	0.009
	Intron 23	4476+33	G/A	1/117	0.004
	Exon 26	4974	G/C	1/117	0.004
	Exon 26	5418	G/A	2/117	0.009
<i>SCN2A</i>	Intron 1	266+51	G/A	2/117	0.009
	Intron 2	385+46	C/A	1/114	0.004
	Intron 2	386–10	G/A	1/117	0.004
	Intron 6	970–32	A/G	1/117	0.004
	Exon 11	1784	T/C	3/117	0.013
	Intron 16	3399–74	+/insG	3/98	0.015
	Intron 16	3399–71	C/T	1/117	0.004
	Exon 26	5387	G/A	1/117	0.004
	Exon 26	5930	T/C	1/117	0.004
<i>SCN3A</i>	Intron 1	264+36	+/ Δ A	1/32	0.016
	Intron 2	384–68/69	+/insT	1/32	0.016
	Intron 6	965–20	G/T	1/32	0.016
	Intron 6	965–12	+/ Δ T	2/32	0.030
	Intron 9	1378–34	G/A	nd	nd
	Exon 26	5496	C/T	1/117	0.01

Table 4 Common haplotypes for *SCN1A* and *SCN3A*

<i>SCN1A</i> haplotype	Number of chromosomes	Haplotype frequency	Intron 6 965–21	Exon 9 1212	Exon 13 2292	Intron 13 2416–37	Exon 16 3199
<i>SCN1A</i> -1	154	0.65	T	G	C	C	A
<i>SCN1A</i> -2	80	0.34	C	A	T	A	G
<i>SCN1A</i> -3	2	0.01	C	A	C	C	A
<i>SCN3A</i> haplotype	Number of chromosomes	Haplotype frequency	Intron 2 383+81	Intron 5 692–46	Intron 6 965–12		
<i>SCN3A</i> -1	59	0.92	C	G	T		
<i>SCN3A</i> -2	5	0.08	T	A	Δ T		

The markers in *SCN1A* span 14 kb of genomic DNA. Haplotypes 1 and 2 were described previously.¹⁵ The markers in *SCN3A* span 8 kb of genomic DNA. *SCN1A* haplotype 3 was observed in two unrelated autistic individuals.

identified in a yeast 2-hybrid screen.²⁵ The sodium channel/calmodulin complex assumes 2 alternative conformational states depending on the Ca(2+) concentration. The amino-acid substitution R1902C caused a two-fold reduction in K_d for Ca(2+)CaM with no change in binding of apoCaM. This small difference might affect subcellular localization or modulation of channel properties. R1902C was observed in 1/117 AGRE families and 0/296 controls. One affected sib did not carry the mutation. Mutation

of a residue in the cardiac sodium channel that is located closely to the corresponding arginine alters channel inactivation and causes disease.²⁹ Additional screening for R1902C in patients and controls would be worthwhile.

The 5 coding variants that were only detected in affected individuals may contribute to autism: R542Q, I1034T, F1038L, I1955T and R1902C (Table 1). The fact that I1034T and R1902C were not inherited by an affected sib does not rule them out as candidate

mutations, since affected individuals in the same family may carry a different combination of predisposing mutations for a polygenic disorder. Follow-up experiments that could provide evidence for pathogenicity of these variants include screening more autism patients to find additional examples of each variant, functional analysis of channel kinetics, and generation of transgenic mice carrying the mutations. Although the variants are not located within transmembrane protein domains of known function, the >95% conservation of the cytoplasmic loops in human and rodent orthologs suggests that they contain as yet uncharacterized functional elements.¹⁸

Mutations affecting protein sequence or splice sites of the sodium channel genes were found in 6/117 AGRE families and are clearly not a major cause of autism in this group. Transcriptional regulatory elements were not included in the survey, and remain a potential source of quantitative variation and disease.

Sodium channel mutations and neurological disease

Small alterations in sodium channel properties have been associated with neurological disability *in vivo*. For example, a 10 mV shift in the voltage dependence of activation of mouse *SCN8A* alters the repetitive firing pattern of cerebellar Purkinje neurons and produces ataxia.^{30,31} A small increase in persistent current in *SCN2A* produces a seizure disorder in transgenic mice.²¹ *SCN1A* mutations responsible for GEFS+ cause only minor changes in channel kinetics.^{23,32} Polymorphic sodium channel variants, such as those described here, may contribute to the range of cognitive and emotional function in normal individuals. The combined effect of variants in multiple ion channel genes could also predispose to psychiatric disorders such as autism. This hypothesis is consistent with recent genetic models indicating that segregation of multiple rare alleles may account for common polygenic disorders in the human population.³³ Because of the low frequency of each variant allele, large-scale comparisons of thousands of patients and controls will be required to obtain statistically significant association data for such variants. It will also be important to test the effect of each putative disease variant in functional assays of channel activity.

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