



Heterozygosity for a protein truncation mutation of sodium channel SCN8A in a patient with cerebellar atrophy, ataxia and mental retardation

Michelle M Trudeau, Joline C Dalton, John W Day, Laura PW Ranum and Miriam H Meisler

J. Med. Genet. published online 19 Oct 2005;
doi:10.1136/jmg.2005.035667

Updated information and services can be found at:
<http://jmg.bmjournals.com/cgi/content/abstract/jmg.2005.035667v1>

These include:

Rapid responses

You can respond to this article at:
<http://jmg.bmjournals.com/cgi/eletter-submit/jmg.2005.035667v1>

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article

Notes

Online First contains unedited articles in manuscript form that have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Online First articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Online First articles must include the digital object identifier (DOIs) and date of initial publication.

To order reprints of this article go to:
<http://www.bmjournals.com/cgi/reprintform>

To subscribe to *Journal of Medical Genetics* go to:
<http://www.bmjournals.com/subscriptions/>

Revised September 26, 2005

Heterozygosity for a protein truncation mutation of sodium channel *scn8a*
in a patient with cerebellar atrophy, ataxia and mental retardation

Michelle M. Trudeau¹, Joline C. Dalton^{2,4}, John W. Day^{2,3},
Laura P. W. Ranum^{2,4}, Miriam H. Meisler¹

¹Departments of Human Genetics, University of Michigan, Ann Arbor, MI 48109-0618, USA; and
²Institute of Human Genetics, ³Department of Neurology and ⁴Department of Genetics, Cell Biology,
and Development, University of Minnesota, Minneapolis, MN 55455, USA

Key words: sodium channel, haploinsufficiency, retardation, ataxia

Word count: 2385 (excluding references)

Corresponding author:

Miriam H. Meisler, PhD

Professor of Human Genetics

4909 Buhl Box 0618

University of Michigan Medical School

Ann Arbor, MI 48109-0618

Email: meislerm@umich.edu

Tel: (734) 763-5546; Fax: (734) 763-9691

ABSTRACT

Introduction: The *SCN8A* gene on chromosome 12q13 encodes the voltage-gated sodium channel $Na_v1.6$, which is widely expressed in neurons of the CNS and PNS. Mutations of the mouse ortholog of *SCN8A* result in ataxia and other movement disorders.

Methods: The 26 coding exons of *SCN8A* were screened by CSGE in DNA from 151 patients with inherited or sporadic ataxia, and variants were sequenced.

Results: A 2 bp deletion in exon 24 was identified in a 9 year old boy with mental retardation, pancerebellar atrophy, and ataxia. The mutation, Pro1719ArgfsX6, introduces a translation termination codon into the pore loop of domain 4, resulting in truncation of the C-terminal cytoplasmic domain and predicted loss of channel function. Three additional heterozygotes in the family exhibit less severe cognitive and behavioral deficits including ADHD. No additional occurrences of this mutation were observed in 625 unrelated DNA samples (1250 chromosomes).

Discussion: The phenotypes of the heterozygous individuals suggest that mutations in *SCN8A* may result in motor and cognitive deficits of variable expressivity. Due to the small size of the pedigree and incomplete phenotype information, analysis of additional families will be required to define the contribution of the *SCN8A* mutation to the observed clinical features.

INTRODUCTION

Voltage-gated sodium channels play an essential role in the initial phase of the action potential during neuronal firing. The human genes *SCN1A* through *SCN11A* encode a small family of sodium channel α subunits, large pore-forming transmembrane proteins [1,2]. Association with small β subunits modifies the channel activity and trafficking of the α subunits. *SCN8A* on chromosome 12q13 encodes the neuronal channel $\text{Na}_v1.6$ that is widely distributed in neurons of the central and peripheral nervous systems. In the mouse, mutations of *SCN8A* result in ataxia, tremor, muscle weakness, and dystonia [3-6]. Homozygosity for a null allele of mouse *SCN8A* results in progressive paralysis during postnatal week 3 [5], the period when $\text{Na}_v1.6$ becomes localized at the nodes of Ranvier [7,8]. Electrophysiological recordings from *SCN8A* homozygous null mice have detected altered neuronal firing patterns, including reduced repetitive firing in cerebellar Purkinje cells, and reduced resurgent current in cerebellar Purkinje cells, prefrontal cortex pyramidal neurons and spinal sensory neurons [9-12].

Mutations in the sodium channels *SCN1A* and *SCN2A* are responsible for inherited and sporadic epilepsies [4, 13, 14]. Severe myoclonic epilepsy of infancy (OMIM 607208) results from haploinsufficiency of *SCN1A* in patients who are heterozygous for null mutations [15-17]. Many of the observed null mutations of *SCN1A* cause protein truncation [16]. Deletion of only the C-terminal cytoplasmic domain of *SCN1A* results in disease of comparable severity to truncations near the N-terminus of the protein, indicating that the C-terminus is essential for channel function [4]. Haploinsufficiency has also been observed for the cardiac sodium channel *SCN5A* in a patient with a conduction disorder [18].

Recent studies have investigated the contribution of mutations in neuronal sodium channels to cognition and psychiatric disorders. Several potentially causal heterozygous missense mutations were found in the channels *SCN1A*, *SCN2A* and *SCN3A* in families with autism [19]. In a study of suicide, transmission disequilibrium was observed for a single nucleotide polymorphism in an intron of *SCN8A* [20]. Long-term potentiation, a cellular component of learning, is accompanied by changes in sodium channel kinetics [21]. Most recently, inhibitors of sodium channels were found to have a therapeutic effect in a rat model of anxiety [22].

In order to identify disease-associated mutations of human *SCN8A*, we screened 151 unrelated patients with inherited or sporadic ataxia [23]. We identified four members of a family who are heterozygous for a protein truncation allele of human *SCN8A*. The data suggest that loss-of-function mutations of *SCN8A* may result in both cognitive and motor deficits.

RESULTS

Detection of a frameshift mutation of *SCN8A* causing protein truncation. Mutation screening was carried out for 151 patients with inherited or sporadic ataxia. These patients did not carry mutations in the known ataxia genes *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7*, *SCA8*, *SCA10*, *SCA12* and *SCA14* [23, 24, 25]. Twenty-six coding exons of *SCN8A*, exons 1-24 plus 10a and 10b, were amplified by PCR from genomic DNA using the primers described in Table 1. Heteroduplex analysis was carried out by conformation sensitive gel electrophoresis [26]. The PCR product containing the first 519 bp of exon 24 from one individual contained two slowly migrating conformers in addition to the wildtype product, indicative of heterozygosity (Fig 1A). The exon 24 PCR product from this individual was cloned and 10

individual clones were sequenced from both strands. Two different alleles were recovered, one with wildtype sequence and one with a 2 bp deletion of nucleotides 5156 and 5157 of the coding sequence (Fig 1B). The deletion shifts the reading frame and causes a premature stop codon shortly downstream of the deletion. This mutation, Pro1719ArgfsX6, is predicted to truncate the protein in the pore region of transmembrane domain 4, resulting in deletion of the entire cytoplasmic C-terminal domain (Fig. 1C). Similar mutations in other voltage-gated sodium channels result in complete loss of channel activity [4].

The proband was a 9-year-old boy with marked delay of cognitive and motor development and stimulant-responsive attention deficit disorder. His MRI showed moderate pancerebellar atrophy that was accentuated in the vermal and parasagittal regions, as well as optic nerve hypoplasia, but no cerebral abnormalities. Neurological examination revealed bilateral esophoria, strabismic amblyopia and unsustained gaze-evoked nystagmus on horizontal gaze. The proband had normal strength, tone and reflexes, but notable ataxia of speech, dysmetria in the upper extremities resulting in nearly illegible handwriting, and mildly ataxic gait with wide base and *en bloc* turning. The motor abnormalities are consistent with the cerebellar malformation. Karyotype data was not available.

Inheritance of the sodium channel mutation. DNA from four additional family members was analyzed (Fig. 2). The father (I-1) is homozygous for the wildtype allele (Fig. 2) and exhibits no clinical abnormalities; he is the custodial parent. The proband's mother (I-2) is heterozygous for Pro1719ArgfsX6, as are the maternal aunt (I-3) and first cousin (II-5) (Fig. 2). The mother has a history of emotional instability with mild cognitive impairment, as does the maternal aunt. The first cousin has been diagnosed with ADHD. None of these family members was available for formal clinical evaluation including MRI to assess possible subclinical cerebellar atrophy. Due to the incomplete information about the other heterozygotes, it is unclear whether they exhibit a milder version of the abnormalities seen in the proband, resulting from haploinsufficiency of SCN8A, or if the proband's symptoms are caused by an unrelated developmental disorder.

DNA was unavailable from other family members, but anecdotal evidence of behavioral deficits was reported for the maternal grandmother and her two brothers, now in their 60s, and her son, aged 35 years. These individuals attended special education classes as children and require assisted living situations as adults. The family is of Norwegian and Swedish background.

Haplotype analysis. Family members were genotyped for 3 microsatellite markers and one SNP from chromosome 12q13.3. The Pro1719ArgfsX6 mutation is carried on a chromosome segment with haplotype D12S1663-1, D12S368-3, 12S83-5, and the A allele of rs303802 (Figure 2). The allele frequencies for these markers are 0.30, 0.07, 0.30 and 0.8, respectively.

Exon 24 of SCN8A does not contain a hotspot for mutation. The CpT dinucleotide deletion in Pro1719ArgfsX6 removed the final C in a C₆ repeat. To determine whether this is a site of genome instability, we screened a total of 625 DNA samples (1250 chromosomes). The samples included 151 ataxia patients from the current study, 262 additional ataxia patients, 137 patients with other neurological disorders, and 75 CEPH parents. No additional occurrence of Pro1719ArgfsX6 was identified, indicating that this is a rare allele in the Northern European population represented by our samples.

DISCUSSION

P1719fsX1724 is the first reported mutation in human SCN8A. Several lines of evidence indicate that the loss of the C-terminal domain in the SCN8A mutant allele would result in loss-of-function. This domain of the voltage-gated sodium channels has been highly conserved during evolution and is thought to play a role in channel inactivation. An interaction site for the sodium channel β 1 subunit has been localized within the C-terminal domain [27]. Biophysical studies of similarly-located mutations in the channels SCN1A and SCN5A have been carried out in transfected mammalian cells; the

results demonstrate that truncation of the C-terminal domain greatly reduces or eliminates channel activity [28, 29]. Many additional mutations in *SCN1A* that result in Severe Myoclonic Epilepsy of Infancy are deletions of the C-terminal domain [4]. The reduced level of Na_v1.6 in heterozygous individuals is expected to reduce neuronal excitability, resulting in altered firing patterns. Electrophysiological studies of neurons from heterozygous null mice would be of great value for understanding the consequences of reduced Na_v1.6 in different types of neurons. Preliminary studies have detected impaired learning and increased anxiety in mice heterozygous for an *SCN8A* null mice (B. McKinney, M. Meisler and G. Murphy, unpublished observations).

The tissue-specific expression of *SCN8A* in the nervous system, the predicted loss of channel activity by the mutant allele, and the low population frequency of the mutant allele, are all consistent with a causal role in the neurological deficits of the proband. It is not unexpected that reduction in the amount of the Na_v1.6 sodium channel, due to heterozygosity for a loss-of-function allele, would have clinical consequences. Haploinsufficiency for the closely related channel *SCN1A* (Na_v1.1) results in a severe seizure disorder [4], demonstrating that levels of expression in a null heterozygote can be insufficient for normal function. The threshold for uncompromised function in the mouse is between 10% and 50% of normal levels of Na_v1.6 [6].

The severity of cognitive defects in family members who are heterozygous for the *SCN8A* mutation varies from mental retardation in the proband to ADHD in his first cousin. If 50% is close to the threshold for normal *SCN8A* function, then variation in genetic background or environment may be expected to influence the cognitive outcome. *SCN8A* expression is particularly high in the cerebellum, and targeted inactivation of *Scn8a* in Purkinje cells is sufficient to produce ataxia [30]. On the other hand, cerebellar malformation has not been observed in *SCN8A* null or heterozygous mice. The ataxic features of the proband are consistent with the moderate pancerebellar atrophy revealed by MRI, but the lack of MRIs for the other heterozygotes in the family preclude firm conclusions regarding the role of the *SCN8A* mutation in the cerebellar atrophy.

The CpT dinucleotide deletion in the *SCN8A* mutation P1719fsX1724 occurs in the context of a C₆ nucleotide sequence. Deletion of one or two nucleotides from C₄ to C₆ repeats are responsible for the common deafness allele of connexin 26 and *RAI1* mutations in patients with Smith-Magenis syndrome. However, no other mutations in this C₆ run were observed among 625 unrelated individuals, indicating that this is not a highly unstable site.

Followup studies will be required to assess the prevalence of *SCN8A* mutations and their significance in disorders of cognition and behavior. In future screening, it will be worthwhile to include the recently described 5' noncoding exons and promoter region of the gene [31], which was not included in the present study. Families with both motor and cognitive features will be of highest priority for future studies.

MATERIALS AND METHODS

Mutation detection. DNA from 151 patients with inherited or sporadic ataxia was screened for mutations in all of the exons and splice sites of *SCN8A* by conformation sensitive gel electrophoresis as previously described [26]. Primer sequences are provided in Table 1. These patients had previously been found to lack mutations in the known ataxia genes *SCA1*, *SCA2*, *SCA3*, *SCA6*, *SCA7*, *SCA8*, *SCA10*, *SCA12* and *SCA14* [23, 24, 25 and L. Ranum, personal communication]. The abnormally migrating PCR product from exon 24 was purified with the Qiaquick gel extraction kit (Qiagen) and sequenced by the University of Michigan DNA Sequencing Core. To separate the two alleles carried by the proband, the PCR product was cloned into the TOPO TA vector (Invitrogen) and DNA from 10 independent clones was sequenced. Exon 24 was also screened by CSGE in 262 additional ataxia

patients of Northern European origin and 137 patients with other neurological disorders, also of Northern European origin. All experiments were carried out with appropriate institutional IRB approval.

Genotyping assay for *Pro1719ArgfsX6* in exon 24. To detect the 2 bp deletion in exon 24, a 318 bp genomic fragment containing the mutated sequence was amplified from genomic DNA using a forward primer in intron 23 (TTG CCT TAA TGA TGT CCT TGC CTG) and a reverse primer in the coding sequence of exon 24 (TAC AAA GAA GAA GAT GCC CAC TGA). Product length was determined on an ABI sequencer using GeneMapper software. DNA from 75 CEPH parents was genotyped.

Acknowledgements:

We are grateful to the family for their cooperation. Supported by NIH grants NS34509 (MHM) and NS040389 (LPWR), The Wilson Family Medical Foundation (MHM), and the National Ataxia Foundation (LPWR). We thank Drs. Jeffrey Innis and Donna Martin for helpful comments on the manuscript.

Ethics approval: Blood samples were obtained with Internal Review Board approved consent from subjects at the University of Minnesota.

Competing interest: The authors have no competing interests to declare.

Copyright: The corresponding author has the right to grant on behalf of all authors and does grant on behalf of all authors, an exclusive license (or nonexclusive license for government employees) on a worldwide basis to the BMJ Publishing Group Ltd and its licensees, to permit this article (if accepted) to be published in JMG and any other BMJPG products and to exploit all subsidiary rights, as set out in our license (<http://jmg.bmjournals.com/misc/ifora/licenceform.shtml>)

REFERENCES

1. Plummer NW, Meisler MH. Evolution and Diversity of Mammalian Sodium Channel Genes. *Genomics* 1999; **57**: 323-331
2. Goldin AL. Resurgence of sodium channel research. *Annu Rev Physiol* 2001; **63**: 871-894
3. Meisler MH, Plummer NW, Burgess DL, Buchner DA, Sprunger LK. Allelic mutations of the sodium channel *SCN8A* reveal multiple cellular and physiological functions. *Genetica* 2004; **122**:37-45
4. Meisler MH, Kearney JA. Sodium channel mutations in epilepsy and other neurological disorders. *J. Clin. Invest.* 2005; in press
5. Burgess DL, Kohrman DC, Galt J, Plummer NW, Jones JM, Spear B, Meisler MH. Mutation of a new sodium channel gene, *SCN8A*, in the mouse mutant 'motor endplate disease'. *Nat Genet* 1995; **10**: 461-465
6. Kearney JA, Buchner DA, de Haan G, Adamska M, Levin SI, Furay AR, Albin RL, Jones JM, Montal M, Stevens MJ, Sprunger LK, Meisler MH. Molecular and pathological effects of a modifier gene on deficiency of the sodium channel *SCN8A* (Na_v 1.6). *Hum Mol Genet* 2002; **11**: 2765-2775
7. Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR. Sodium channel $Na(v)1.6$ is localized at nodes of ranvier, dendrites, and synapses. *Proc. Natl. Acad. Sci. U.S.A.*, 2000; **97**: 5616-5620
8. Boiko T, Rasband MN, Levinson SR, Caldwell JH, Mandel G, Trimmer JS, Matthews G. Compact myelin dictates the differential targeting of two sodium channel isoforms in the same axon. *Neuron* 2001; **30**: 91-104
9. Raman IM, Sprunger LK, Meisler MH, Bean BP. Altered subthreshold sodium currents and disrupted firing patterns in Purkinje neurons of *SCN8A* mutant mice. *Neuron* 1997; **19**: 881-891
10. Maurice N, Tkatch T, Meisler M, Sprunger LK, Surmeier DJ. D1/D5 dopamine receptor activation differentially modulates rapidly inactivating and persistent sodium currents in prefrontal cortex pyramidal neurons. *J Neurosci* 2001; **21**: 2268-2277
11. Do MT, Bean BP. Sodium currents in subthalamic nucleus neurons from $Na_v1.6$ -null mice. *J Neurophysiol* 2004; **92**: 726-733
12. Cummins TR, Dib-Hajj SD, Herzog RI, Waxman SG. $Na_v1.6$ channels generate resurgent sodium currents in spinal sensory neurons. *FEBS Lett.* 2005; **579**: 2166-2170
13. Escayg A, MacDonald B, Meisler M, Baulac S, Huberfeld G, An-Gourfinkel I, Brice A, LeGuern E, Moulard B, Chaigne D, Buresi C, Malafosse A. Mutations of *SCN1A*, encoding a neuronal sodium channel, in two families with GEFS+2. *Nat Genet* 2000; **24**: 343-345
14. Meisler MH, Kearney J, Ottman R, Escayg A. Identification of epilepsy genes in human and mouse. *Annu Rev Genet* 2001; **35**: 567-588
15. Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. De novo mutations in the sodium channel gene *SCN1A* cause severe myoclonic epilepsy of infancy. *Am J Hum Genet* 2001; **68**: 1327-1332
16. Wallace RH, Hodgson BL, Grinton BE, Gardiner RM, Robinson R, Rodriguez-Casero V, Sadleir L, Morgan J, Harkin LA, Dibbens LM, Yamamoto T, Andermann E, Mulley JC, Berkovic SF, Sheffer IE. Sodium channel alpha-1 subunit mutations in severe myoclonic epilepsy of infancy and infantile spasms. *Neurology* 2003; **61**: 765-769
17. Fujiwara T, Sugawara T, Mazaki-Miyazaki E, Takahashi Y, Fukushima K, Watanabe M, Hara K, Morikawa T, Yagi K, Yamakawa K, Inoue Y. Mutations of sodium channel {alpha} subunit type 1 (*SCN1A*) in intractable childhood epilepsies with frequent generalized tonic-clonic seizures. *Brain* 2003; **126**: 531-546

18. Schott JJ, Alshinawi C, Kyndt F, Probst V, Hoorntje TM, Hulsbeek M, Wilde AA, Escande D, Mannens MM, Le Marec H. Cardiac conduction defects associate with mutations in *SCN5A*. *Nat Genet* 1999; **23**: 20-21
19. Weiss LA, Escayg A, Kearney JA, Trudeau M, MacDonald BT, Mori M, Reichert J, The Autism Genetic Resource Exchange Consortium, Buxbaum JD, Meisler MH. Sodium channels *SCN1A*, *SCN2A* and *SCN3A* in familial autism. *Molec Psychiatry* 2003; **8**: 186-194
20. Wasserman D, Geijer T, Rozanov V, Wasseran J. Suicide attempt and basic mechanisms in neural conduction: Relationships to the *SCN8A* and *VAMP4* genes. *Am J Med Genet B Neuropsychiatr Gene* 2005; **133**: 116-119
21. Xu J, Kang N, Jiang L, Nedergaard M, Kang J. Activity-dependent long-term potentiation of intrinsic excitability in hippocampal CA1 pyramidal neurons. *J Neurosci* 2005; **25**: 1750-1760
22. Mirza NR, Bright JL, Stanhope KJ, Wyatt A, Harrington NR. Lamotrigine has an axiolytic-like profile I the rat conditioned emotional response test of anxiety: a potential role for sodium channels? *Psychopharmacology (Berl)* 2005 Jan 29 [Epub]
23. Moseley ML, Benzow KA, Schut LJ, Bird TD, Gomez CM, Barkhaus PE, Blindauer KA, Labuda M, Pandolfo M, Koob MD, Ranum LP Incidence of dominant spinocerebellar and Friedreich triplet repeats among 361 ataxia families. *Neurology* 1998; **51**:1666-1671
24. Matsuura T, Ranum LPW, Volpini V, Pandolfo M, Sasaki H, Tashiro K, Watase K, Zoghbi HY, Ashizawa T. Spinocerebellar ataxia type 10 is rare in populations other than Mexicans. *Neurology* 2002; **58**: 983-984
25. Chen DH, Cimino PJ, Ranum L, Yabe I, Saasaki H, Matsushita M, Bird TD, Raskind WH, Prevalence of SCA14 and spectrum of PKCy mutations in large panel of ataxia patients. *Am J Hum Genet* 2003; **73S**: Abstr. 2215
26. Plummer NW, Galt J, Jones JM, Burgess DL, Sprunger LK, Kohrman DC, Meisler MH. Exon organization, physical mapping, and polymorphic intragenic markers for the human neuronal sodium channel gene *SCN8A*. *Genomics* 1998; **54**: 287-296
27. Spanpanato J, Kearney JA, de Haan G, McEwen D, Escayg A, Aradi I, MacDonald BT, Levin SI, Soltesz I, Benna P, Montalenti E, Isom L, Goldin A, Meisler MH. A novel GEFS+ mutation in the sodium channel *SCN1A* identifies a cytoplasmic domain for beta subunit interaction. *J Neurosci* 2004; **24**: 10022-10034
28. Sugawara T, Tsurubuchi Y, Fujiwara T, Mazaki-Miyazaki E, Nagata K, Montal M, Inoue Y, Yamakawa K. $Na_v1.1$ channels with mutations of severe myoclonic epilepsy in infancy display attenuated currents. *Epilepsy Res* 2003; **54**: 201-207
29. Cormier JW, Rivolta I, Tateyama M, Yang AS, Kass RS. Secondary structure of the human cardiac Na^+ channel C terminus: evidence for a role of helical structures in modulation of channel inactivation. *J Biol Chem* 2002; **277**: 9233-41
30. Levin SI, Meisler MH. Conditional inactivation of the voltage-gated sodium channel *SCN8A* ($Na_v1.6$) in cerebellar Purkinje and granule cells. 2004 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience
31. Drews VL, Lieberman AP, Meisler MH. Multiple transcripts of sodium channel *SCN8A* ($Na_v1.6$) with alternative 5' and 3' UTRs and initial characterization of the *SCN8A* promoter. *Genomics* 2005; **85**:2145-257.

Table 1. PCR primers for amplification of *SCN8A* exons from genomic DNA.

Primer	Sequence, 5' to 3'	Product (bp)
Exon 1 F	GGA CGC AGC ATA ACT AAC GA	350
Exon 1 R	TGC TCC TCC TCC TCC AAC TC	
Exon 2 F	GTG GTG ACT CAT ACC CAT GC	200
Exon 2 R	CAC TCA CTC CAC ATT TGC CG	
Exon 3 F	GAT ACC CAA TGG AAA TGT GTT TG	350
Exon 3 R	TCC TTG ATG GGT AAA CTG TG	
Exon 4 F	CCC AGT ACA ACA TTC CAA AGG TCT	500
Exon 4 R	GAA TCA TAG ACA TAA GCC CAC AGG	
Exon 5 F	CTG ACA GTA AAG CCA TTC TGA TTG	514
Exon 5 R	TCT GTA GTA AGG GAG GTC ACA CAC	
Exon 6 F	TTC CGG GGA TTC TGT CTC CT	280
Exon 6 R	TCC TAT TCC TCT TGC AGA GA	
Exon 7 F	TTT CTG CTG GGG CTA GTT AG	250
Exon 7 R	GTA AAC ATA TTG CCT ATT TTG ACC	
Exon 8 F	GCT GGG GTG GAA TTA TGT TG	250
Exon 8 R	CTC ATG CAA ACT CTA TGG CC	
Exon 9 F	GTA CTG CAC AAG GAA ACA GTC C	400
Exon 9 R	GTC ACC ATG ATC TGG CAG GAA GG	
Exon 10a F	TTC CTC CTT TCC CTT CAG TAG	350
Exon 10a R	CTG ACT TAA ACA CCT TCT CGG	
Exon 10b F	GGA ACC TAA CCACTT TGC TCA GTA	618
Exon 10b R	ACA CAG AAA TGG CTG GGG TGT TAA	
Exon 10c F	ACT GGA CTG TGT ATC AAG TAA GGC	297
Exon 10c R	CCA AGG GAC AGG GAT TAT ATT TAC	
Exon 11 F	CTG AGA GTG AGT AGT GTG TC	350
Exon 11 R	CCT AAT ATC GGG AGA CAG TG	
Exon 12 F	ATT TCT TTT TCT TAC CCC CTG C	280
Exon 12 R	GTA GCT GCA AGA TAG GAA ATG	
Exon 13 F	GCC ATG TGG TGA GAA AAT TGA TTG AG	500
Exon 13 R	CTG GGC CAG GGG TAG AAT AT	
Exon 14 F	CAA GAG CCT CTT TGA GTC TGT CAC G	650
Exon 14 R	ACA AGC ACC CTG TTT GCT CTC AC	
Exon 15 F	CTC AGA GCT GCC TGA TCT CC	210
Exon 15 R	GTC TGT GAG CAA CAG GAG TG	
Exon 16 F	GAT GGA GGA GAG GGC AGG TC	280
Exon 16 R	GCC AAT CCC TCC TCA CAC TC	
Exon 17 F	AAG CCT GTG TAG TCG GGG AGT AAA	459
Exon 17 R	AGG AAC TTT ACC CAC ACC TAC ATC	

Exon 18 F	GTT TTT CTC TTC TGT TTC TGT G	190
Exon 18 R	CAT ACA GAA GGA TCA GCT GC	
Exon 19 F	GTG CTT GCT CTC ATT TCC ACC C	400
Exon 19 R	GCC TGA ATT TCA CAG AAG TG	
Exon 20 F	TAG ACT CCC ATC CAC TCC C	180
Exon 20 R	CTT TCC AGC CAT CTG AGA AG	
Exon 21 F	ACT AGG AGC TGA TTC TCT TC	250
Exon 21 R	GGC TGA CTT CCA ACC ACT GG	
Exon 22 F	AGG TCC AAA CCC ATA GCA TG	200
Exon 22 R	GTT CTC ACC AAG GCC TCA GC	
Exon 23 F	CAT AGG TTG GCT TGG AAA GG	400
Exon 23 R	TCC TCA CAA TCA GTC AGG TC	
Exon 24 F1	CTA AGG GTT CCA CAA TGC CAG GTA	538
Exon 24 R1	GCT TAC AAA GAA GAA GAT GCC CAC	
Exon 24 F2	GCT TTA AGG GAG ATT GTG GGA ACC	503
Exon 24 R2	AGA TAC CTC CTC CTG CTT GCG ACG	
Exon 24 F3	CTT CCA AAG TGT CTT ACG AGC CAA	424
Exon 24 R3	TCT TCA GGT TAG AGT CTC CAC AGC	

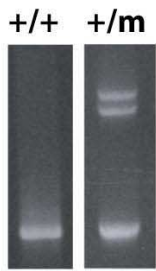
FIGURE LEGENDS

Figure 1. Detection of the mutation Pro1719ArgfsX6 in a patient with ataxia and mental retardation. (A) In addition to the wildtype PCR product obtained by amplification of exon 24, the proband's sample contains two slowly-migrating conformers on the CSGE gel, indicative of heterozygosity. (B) Sequences of PCR products representing the wild-type and deleted allele cloned from the proband's genomic DNA. Dashed lines mark the 2-bp deletion which removed nucleotides 5157 and 5158 of the coding sequence. (C) Location of the mutation in the pore region of domain 4 of sodium channel Na_v1.6.

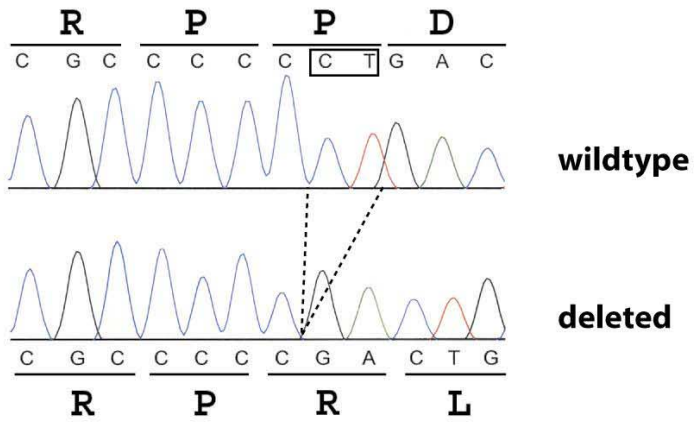
Figure 2. Inheritance of the *SCN8A* mutation Pro1719ArgfsX6. The haplotype for markers on chromosome 12q13.3 associated with the mutation is boxed. +/+, wildtype homozygote; +/-, heterozygote.

Fig. 1

A



B



C

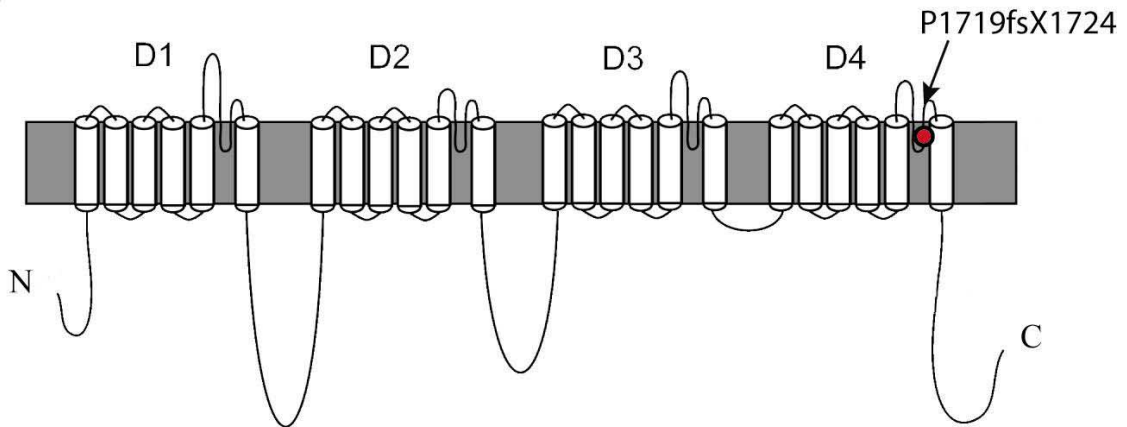
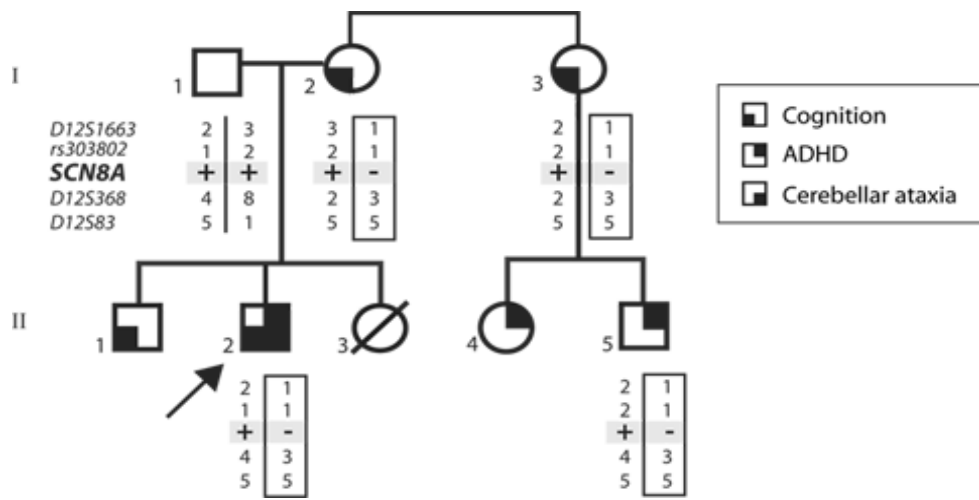
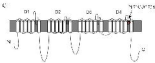
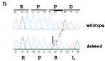


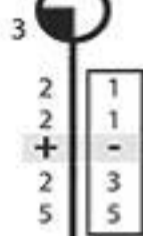
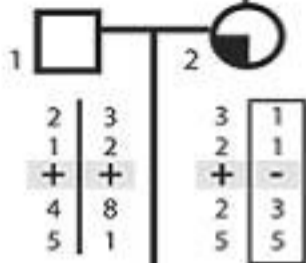
Fig. 2








I

D12S1663
rs303802
SCN8A
D12S368
D12S83



-  Cognition
-  ADHD
-  Cerebellar ataxia

II

