Channelopathy-related *SCN10A* gene variants predict cerebellar dysfunction in multiple sclerosis

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ABSTRACT

Objective: To determine the motor-behavioral and neural correlates of putative functional common variants in the sodium-channel $\text{Na}_v1.8$ encoding gene (*SCN10A*) in vivo in patients with multiple sclerosis (MS).

Methods: We recruited 161 patients with relapsing-onset MS and 94 demographically comparable healthy participants. All patients with MS underwent structural MRI and clinical examinations (Expanded Disability Status Scale [EDSS] and Multiple Sclerosis Functional Composite [MSFC]). Whole-brain voxel-wise and cerebellar volumetry were performed to assess differences in regional brain volumes between genotype groups. Resting-state fMRI was acquired from 62 patients with MS to evaluate differences in cerebellar functional connectivity. All participants were genotyped for 4 potentially functional *SCN10A* polymorphisms.

Results: Two *SCN10A* polymorphisms in high linkage disequilibrium ($r^2 = 0.95$) showed significant association with MSFC performance in patients with MS (rs6795970: $p = 6.2 \times 10^{-4}$; rs6801957: $p = 0.0025$). Patients with MS with rs6795970^{AA} genotype performed significantly worse than rs6795970^G carriers in MSFC ($p = 1.8 \times 10^{-4}$) and all of its subscores. This association was independent of EDSS and cerebellar atrophy. Although the genotype groups showed no difference in regional brain volumes, rs6795970^{AA} carriers demonstrated significantly diminished cerebellar functional connectivity with the thalami and midbrain. No significant *SCN10A*-genotype effect was observed on MSFC performance in healthy participants.

Conclusions: Our data suggest that *SCN10A* variation substantially influences functional status, including prominent effects on motor coordination in patients with MS. These findings were supported by the effects of this variant on a neural system important for motor coordination, namely cerebello-thalamic circuitry. Overall, our findings add to the emerging evidence that suggests that sodium channel $\text{Na}_v1.8$ could serve as a target for future drug-based interventions to treat cerebellar dysfunction in MS. *Neurology*® 2016;86:410-417

GLOSSARY

BOLD = blood oxygenation level-dependent; **CRIMSON** = Cross-Modal Research Initiative for Multiple Sclerosis and Optic Neuritis; **EAE** = experimental autoimmune encephalomyelitis; **EDSS** = Expanded Disability Status Scale; **FWE** = familywise error; **MAF** = minor allele frequency; **MS** = multiple sclerosis; **MSFC** = Multiple Sclerosis Functional Composite; **PASAT** = Paced Auditory Serial Addition Test; **ROI** = region of interest; **SARA** = Scale for the Assessment and Rating of Ataxia; **SNP** = single nucleotide polymorphism.

Symptoms associated with cerebellar dysfunction are one of the major contributors to disability in multiple sclerosis (MS).¹ Unlike other clinical abnormalities in MS that tend to be remitting, cerebellar dysfunction tends to become persistent early in the course of the illness and is refractory to either disease-modifying or symptomatic therapy.^{2,3} It is commonly assumed that axonal

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degeneration and demyelination are the prime causes of permanent disability in people with MS.⁴ Nevertheless, emerging evidence suggests that neuronal channel dysfunction (i.e., channelopathy) may be an independent contributor to cerebellar disability in MS.⁵

Nav1.8 is a voltage-gated sodium channel encoded by the sodium channel, voltage-gated, type X, α -subunit (*SCN10A*) gene, which has been recently implicated in the pathophysiology of cerebellar deficits in MS. It has been shown that Nav1.8 channels are expressed ectopically in the cerebellar Purkinje neurons of patients with MS and mice with experimental autoimmune encephalomyelitis (EAE).^{6,7} This channelopathy causes abnormal firing patterns in the cerebellar Purkinje cells^{8,9} and motor coordination deficits in the absence of obvious signs of ataxia in mice models.⁵

In this study, we aimed to examine the effects of potentially functional *SCN10A* genetic variations on clinical and imaging outcomes in patients with MS. We hypothesized that consistent with results from animal studies *SCN10A* genotype would affect performance in motor coordination tasks in MS, irrespective of gross clinical ataxia. We further hypothesized that change in cerebellar neuronal function due to *SCN10A* variations would reflect on resting-state cerebellar functional connectivity with other brain regions.

METHODS **Participants.** Participants from the Cross-Modal Research Initiative for Multiple Sclerosis and Optic Neuritis (CRIMSON) study were recruited at the MS Research Center in Tehran, Iran, via referrals and advertisements. CRIMSON is a longitudinal observational study designed to identify genetic and environmental determinants of MS progression and to develop biomarkers with prognostic or disease monitoring values. The study enrolled participants with a diagnosis of relapsing-onset MS based on the 2010 McDonald criteria¹⁰ with Expanded Disability Status Scale (EDSS)¹¹ score ≤ 6 and an age range of 18–59 years who did not meet the exclusion criteria: (1) less than 12 weeks from the last relapse or corticosteroid therapy, (2) history of previous head trauma and loss of consciousness or other neurologic disorders, (3) history of psychotic disorders or current substance abuse, (4) chronic systemic medical illness, uncontrolled thyroid dysfunction, or a positive history of malignancy. All patients with MS underwent clinical and neurologic examination (EDSS, Multiple Sclerosis Functional Composite [MSFC],¹² and Scale for the Assessment and Rating of Ataxia [SARA]¹³).

We also recruited 94 demographically comparable healthy control participants who did not meet our exclusion criteria (see e-Methods on the *Neurology*® Web site at Neurology.org for further details). Healthy controls also performed MSFC and

were genotyped for variants showing significant effect on MSFC in patients with MS.

Standard protocol approvals, registrations, and patient consents. The ethics review board of the Tehran University of Medical Sciences approved this study. Written informed consent was obtained from all participants.

Genetics. We followed 2 strategies to identify potentially functional common variants in the *SCN10A* gene (minor allele frequency >0.2). First, we searched the PubMed database using the search term “(SCN10A OR Nav1.8) AND (polymorphism OR variant OR SNP)” for articles describing functional validation of variants within the *SCN10A* gene (published before April 10, 2015). This approach yielded 2 single nucleotide polymorphisms (SNPs) that were functionally validated (rs6801957¹⁴ and rs6795970¹⁵). Next, we selected common nonsynonymous genetic variants in the *SCN10A* gene (rs6795970 [also functionally validated], rs57326399, and rs12632942). All of these variants were genotyped using TaqMan SNP Genotyping Assays (Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions. Genotyping calls were made using SNPman software.¹⁶

MRI. Each patient with MS underwent whole-brain structural imaging at 1 of the 2 imaging sites of the study (Shariati Hospital: $n = 68$, Sina Hospital: $n = 93$) using 1.5T Siemens MRI systems (see e-Methods for more details). Participants at the Shariati Hospital also underwent resting-state whole-brain blood oxygenation level–dependent (BOLD) fMRI with an echoplanar imaging acquisition with the following parameters: repetition time = 2,510 ms, echo time = 40 ms, 230 volumes, voxel dimension of $3 \times 3 \times 4$ mm³, 27 slices, distance factor = 30%. Participants were instructed to remain still, stay awake, and have their eyes closed.

Lesion segmentation, voxel-based morphometry, and cerebellar volumetry. For each individual, total brain lesion loads were calculated after manual segmentation of T2 hyperintense lesions by an expert rater (Arash Nazeri) according to an MRI atlas of MS lesions.¹⁷ For voxel-based morphometry,¹⁸ all T1 images were preprocessed using SPM8 software (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) and the VBM8 toolbox (<http://dbm.neuro.uni-jena.de/vbm.html>) with default parameters. Nonparametric statistical analysis was performed using FSL-Randomise (fsl.fmrib.ox.ac.uk/fsl/fslwiki/Randomise; see e-Methods for further details).

Cerebellar volumetry was performed using the MAGEt brain algorithm,^{19,20} a modified multi-atlas segmentation framework that performs bootstrapping based on the dataset being studied to refine automatic segmentations. Volumes were estimated for 4 regions of interest (vermis, central cerebellar white matter, and right and left cerebellar hemisphere gray matter). All segmentations were visually inspected by an expert rater (M.T.M.P.) to ensure accuracy.

Resting-state cerebellar functional connectivity analysis. Resting-state functional image preprocessing and connectivity analysis were performed using FSL v5.0.6 (see e-Methods for further details on fMRI preprocessing). The dual-regression approach (implemented in FSL) was used for estimating resting-state cerebellar functional connectivity maps.²¹ A cerebellar mask was created using the probabilistic atlas of the human cerebellum in Montreal Neurological Institute space.²² BOLD signal temporal dynamics within the cerebellar mask for each individual subject was determined using spatial regression. The resulting time courses were then regressed into the same 4D dataset using temporal regression to obtain subject-specific sets of spatial connectivity maps indexing voxel-wise functional synchrony with cerebellum. To test for significant differences between *SCN10A* genotype

groups in resting-state cerebellar functional connectivity (while accounting for age, sex, and handedness), a voxel-wise general linear model was applied using permutation-based nonparametric testing (5,000 permutations), with threshold-free cluster enhancement as implemented in FSL-Randomise and familywise error (FWE)-corrected $p < 0.05$. Clusters that showed significant difference in their connectivity with cerebellum between the genotype groups ($p_{\text{FWE}} < 0.05$) were extracted for follow-up region of interest (ROI) analysis. Correlation coefficients (Pearson r) between the individual participant time courses of BOLD signal variation in the cerebellum and the significant clusters were computed using the FSLNets toolbox v0.5 (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets>) and Fisher z transformed before submitting to statistical analysis.

Statistical analysis. We conducted analyses of covariance with a Bonferroni-corrected threshold p value for 8 comparisons at $p = 0.0062$ to determine the significance of genotype effects on MSFC performance or EDSS among patients with MS, while accounting for age and sex. All other statistical analyses in this study were conducted using general linear models, while accounting for the effects of age and sex in the R v3.02 environment (<http://cran.r-project.org/>). Robust regression was implemented to minimize the impact of influential observations using *rlm* function, which is part of the MASS robust statistics library in R .

RESULTS Clinical phenotypes. Demographic and clinical characteristics of the participants are summarized in table 1. Two of the *SCN10A* polymorphisms demonstrated significant effects on MSFC performance in patients with MS (rs6795970: $F_{143,2} = 7.8$, $p = 6.2 \times 10^{-4}$, rs6801957: $F_{143,2} = 6.2$, $p = 0.0025$). However, we did not observe any association between rs57326399 ($F_{143,2} = 0.13$, $p = 0.88$) and rs12632942 ($F_{143,2} = 0.75$, $p = 0.47$) genotypes and MSFC score. None of the *SCN10A* genetic variants had a significant effect on EDSS (rs6795970: $F_{149,2} = 2.8$, $p = 0.059$, rs6801957: $F_{149,2} = 2.3$, $p = 0.10$, rs57326399: $F_{149,2} = 0.85$, $p = 0.43$, rs1263294: $F_{149,2} = 0.87$, $p = 0.42$). Given that rs6795970 showed the strongest effect on MSFC and was in high linkage disequilibrium with rs6801957 ($r^2 = 0.95$), we report further analyses on rs6795970 genotype (we summarize the results for rs6801957 in table e-1).

There was no significant association between rs6795970 variation and SARA total score ($F_{107,2} = 0.67$, $p = 0.51$), cerebellar Kurtzke functional system score¹¹ ($F_{150,2} = 0.43$, $p = 0.65$), or other functional system scores ($p_{\text{Ambulation}} = 0.09$, $p_{\text{Pyramidal}} = 0.43$, $p_{\text{Visual}} = 0.68$, $p_{\text{Brainstem}} = 0.35$, $p_{\text{Sensory}} = 0.42$, $p_{\text{Sphincter}} = 0.61$). Follow-up analyses demonstrated that individuals with rs6795970^{AA} genotype were worse in MSFC performance in comparison to both the rs6795970^{GG} ($t = -3.22$, $p = 0.0029$) and the heterozygote groups ($t = -2.63$, $p = 0.013$), while the difference between the rs6795970^{GG} and the heterozygote patients was not significant ($t = -1.15$, $p = 0.26$). Hence, a recessive mode of action for the minor A allele was assumed for further analyses.

The effect of rs6795970^{AA} genotype on MSFC performance ($t = -3.85$, $p = 1.8 \times 10^{-4}$, partial $\eta^2 = 0.093$, figure 1) remained stable after adjusting for the effects of influential observations using robust regression ($t = -3.30$, $p = 0.0013$). Further analysis on the functional subtests of MSFC revealed similar significant associations between rs6795970^{AA} genotype and worse performance on the Timed 25-Foot Walk Test ($t = 3.70$, $p = 3.4 \times 10^{-4}$), 9-Hole Peg Test ($t = -2.75$, $p = 0.0067$), and Paced Auditory Serial Addition Test (PASAT) ($t = -2.35$, $p = 0.02$). Moreover, accounting for EDSS as a measure of MS-related disability in the model demonstrated an independent effect of rs6795970 genotype on performance in MSFC ($t = -3.82$, $p = 2 \times 10^{-4}$), Timed 25-Foot Walk Test ($t = 3.73$, $p = 4 \times 10^{-4}$), 9-Hole Peg Test ($t = -2.14$, $p = 0.034$), and PASAT ($t = -2.39$, $p = 0.018$).

In the healthy participants, rs6795970 and rs6801957 were in perfect linkage disequilibrium ($r^2 = 1$). *SCN10A* genotype did not demonstrate any effect on performance in MSFC (recessive model: $p = 0.65$) or its subtests in healthy controls.

Table 1 Demographic and clinical characteristics

	Patients with multiple sclerosis			Healthy participants		
	All (n = 161)	rs6795970 ^G (n = 134)	rs6795970 ^{AA} (n = 27)	All (n = 94)	rs6795970 ^G (n = 83)	rs6795970 ^{AA} (n = 11)
Age, y, mean (SD) [range]	31.8 (8.1) [18-57]	31.6 (7.9)	33.2 (9.1)	33.8 (9.4) [21-59]	33.1 (8.9)	38.4 (12)
Sex, F/M	126/35	105/29	21/6	71/23	63/20	8/3
Handedness, R/L	147/14	122/12	25/2	87/7	76/7	11/0
MSFC, mean (SD)	0.0 (0.7)	0.1 (0.6)	-0.5 (0.9)	1.8 (0.5)	1.8 (0.4)	1.7 (0.6)
EDSS, mean (SD) [range]	2.8 (1.4) [0-6]	2.6 (1.3)	3.3 (1.9)			
Age at onset, y, mean (SD)	24.8 (7.3)	25.0 (7.2)	23.7 (7.7)			
Cerebellar mode of onset, %	11.6	10.7	16.7			

Abbreviations: EDSS = Expanded Disability Status Scale; MSFC = Multiple Sclerosis Functional Composite scale.

Figure 1 Effect of *SCN10A* rs6795970 (recessive model) on MSFC among patients with MS and HC

HC = healthy controls; MS = multiple sclerosis; MSFC = Multiple Sclerosis Functional Composite; n.s. = nonsignificant.

In addition, we observed a significant *SCN10A*-genotype by diagnosis interaction effect on MSFC score ($p = 0.019$, figure 1). In our sample, no significant difference was found in the minor allele frequency (MAF) of *SCN10A* variants between the healthy controls and patients with MS (rs6795970: $MAF_{\text{Healthy controls}} = 0.24$, $MAF_{\text{MS}} = 0.54$, $p = 0.21$). In addition, we retrieved the publicly available genome-wide association study results from the database of Genotypes and Phenotypes (<http://www.ncbi.nlm.nih.gov/gap>; study accession: phs000171.v1.p1). Similarly, no significant difference was observed in the allelic frequency of rs6795970 between the healthy controls and patients with MS ($p = 0.42$; $n_{\text{Healthy controls}} = 883$; $n_{\text{MS}} = 978$).

Structural imaging and lesion burden. Among patients with MS, no significant effect was detected for rs6795970 genotype ($p_{\text{FWE}} < 0.05$) on regional brain volumes ($n = 155$). In line with the voxel-wise comparisons, the ROI analysis of cerebellar volumes did not reveal any significant effect for the *SCN10A* polymorphism (table e-2). Among the cerebellar volumes, central cerebellar white matter was the best predictor of performance in MSFC²³ (table e-2).

However, the effect of *SCN10A*-rs6795970 genotype on MSFC score was independent of the central cerebellar white matter volume ($t = -3.90$, $p = 1.6 \times 10^{-4}$, partial $\eta^2 = 0.098$).

SCN10A-rs6795970 genotype was not associated with lesion burden (whole brain lesion volume: $p = 0.09$, presence of cerebellar lesions: $p = 0.38$). Further, a significant rs6795970 genotype by lesion volume interaction effect was observed on MSFC performance ($t = 2.18$, $p = 0.031$) (figure 2). A significant negative correlation between lesion volume and MSFC score was detected in participants carrying the rs6795970^G allele ($r = -0.38$, $p = 1.94 \times 10^{-5}$). However, this association was not observed in individuals with the rs6795970^{AA} genotype ($r = 0.02$, $p = 0.92$). The interaction effect became more prominent after accounting for EDSS in the model ($t = 3.27$, $p = 0.0022$).

Resting-state functional connectivity. Dual regression analysis revealed a significant difference ($p_{\text{FWE}} < 0.05$) in cerebellar resting-state functional connectivity between rs6795970^{AA} ($n = 12$) and rs6795970^G allele carriers ($n = 50$). Patients with MS with the rs6795970^{AA} genotype demonstrated diminished cerebellar functional connectivity with clusters mainly within the thalami, along with voxels in the midbrain and right lateral occipital cortex (figure 3, table 2). The post hoc ROI analysis revealed a 1.36 standard deviation difference in mean cerebello-thalamic functional connectivity between the 2 groups ($p = 7.42 \times 10^{-6}$, Mann-Whitney U test). Cerebello-thalamic connectivity was significantly associated with MSFC ($t = 1.98$, $p_{\text{one-tailed}} = 0.026$) and PASAT ($t = 2.24$, $p_{\text{one-tailed}} = 0.014$) performance.

DISCUSSION Our findings provide in vivo evidence that variations in the *SCN10A* gene, which encodes Nav1.8, have a sizable role in determining heterogeneity in functional status (measured by MSFC), as well as in disruption of cerebello-thalamic functional connectivity among people with relapsing-onset MS. However, *SCN10A* genotype did not have any effect on MSFC performance in the healthy participants and did not show any difference in genotype frequency between healthy individuals and patients with MS, suggesting that these effects are MS-specific and *SCN10A* variants act primarily as disease modifiers.

The 2 *SCN10A* variants that showed a significant effect on functional status of patients with MS have been previously functionally validated.^{14,15} These variants are located in a locus previously linked to Brugada syndrome²⁴ and variations in ECG²⁵ in various genome-wide association studies. While rs6801957 appears to alter Nav1.8 expression by affecting a T-box binding element in an *SCN10A* enhancer region,¹⁴ rs6795970^G-Nav1.8 (missense variation 1073^{Ala}) has

Figure 2 *SCN10A* rs6795970 genotype by lesion volume interaction effect on MSFC among patients with multiple sclerosis

Multiple Sclerosis Functional Composite (MSFC) is adjusted for age, sex, and Expanded Disability Status Scale score (EDSS).

shown decelerated time-dependent recovery from inactivation, but larger peak current and accelerated time to peak with respect to rs6795970^A Na_v1.8 (1073^{Val}).¹⁵ Therefore, fine-mapping studies will be required to identify the actual causal variant, and functional studies in animal models of MS will be necessary to determine how these variants affect cerebellar dysfunction in MS.

Purkinje neurons receive integrated inputs indirectly from a variety of regions in the brain and spinal cord.²⁶ They form the output layer of the cerebellar cortex and play a key role in cerebellar neural circuitry and function.²⁶ Cerebellar circuitry has been traditionally viewed to be involved in motor control, and while cerebellar dysfunction does not lead to paralysis, it interferes with adaptive modification and precise execution of fine coordinated movements.²⁶ Animal studies have demonstrated a selective motor coordination deficit in transgenic mice with ectopic expression of the Na_v1.8 in their Purkinje neurons, in the absence of paresis, gross observable ataxia, and sensory dysfunction.⁵ Interestingly, we observed a similar motor deficit profile in patients with MS with *SCN10A*-rs6795970^{AA} genotype (motor coordination deficits measured using 9-Hole Peg Test and Timed 25-Foot-Walk Test, without significant gross ataxia as indexed by SARA and

cerebellar functional system score). Motor subtests of MSFC provide objective quantitative measures of gait and fine hand movements, which may not be readily observable in clinical examination.²⁷ Moreover, recently there has been increasing recognition of the cerebellum's role in cognitive functions.²⁸ In line with the literature, our findings demonstrate that patients with MS with the rs6795970^{AA} genotype perform worse than rs6795970^G allele carriers in the PASAT test. Taken together, these findings suggest that genetic variations in *SCN10A* may alter Na_v1.8 channelopathy effects on MS-related cerebellar dysfunction.

Na_v1.8 expression in Purkinje neurons is suggested to cause its behavioral consequences through altering neuronal activity in these cerebellar cells. Na_v1.8 is a voltage-gated sodium channel that supports repetitive firing in unmyelinated sensory neurons of the dorsal root ganglia and peripheral nerve axons via rapid recovery from inactivation.²⁹ Transgenic mice with ectopic expression of Na_v1.8 in Purkinje neurons and mice with EAE demonstrate similar alterations in electrophysiologic properties of their Purkinje cells.^{5,8} Purkinje neurons from these mice are hyperexcitable compared to those from wild-type mice in vitro, show increased rates of both simple and complex spikes in vivo, and have an irregular complex spike pattern, unlike the highly stereotyped complex spike pattern observed in the wild type.⁵ Moreover, human Na_v1.8 channel displays a larger persistent current, more depolarized inactivation, and slower inactivation than previously studied rodent Na_v1.8 channels.³⁰ Thus, it could be hypothesized that the effects of ectopic Na_v1.8 expression within Purkinje neurons in human should be at least as large as, and perhaps even larger than, the effects previously demonstrated in rodent models. The axons of Purkinje cells travel into the deep cerebellar nuclei, and the deep nuclei from the outputs of the cerebellum.²⁶ The main cerebellar efferents are the cerebello-thalamo-cortical pathways along with the pathways to the brainstem.²⁶ The electrophysiologic alterations in Purkinje neurons could result in abnormalities in the cerebellar output and its functional coupling with other brain regions that in turn could indirectly be measured by change in BOLD signals in resting-state fMRI.³¹ Genetic variations significantly enriched for ion channels are related to the strength of the resting-state functional networks.³² In this study, we have shown that Na_v1.8-related rs6795970^{AA} variant causes marked disruption in cerebello-thalamic resting-state functional connectivity along with cerebellar connectivity with areas within the midbrain. It has been shown that cerebellar functional connectivity with thalamus and other brain regions could play an adaptive and compensatory role in MS.³³ Diminished cerebellar functional connectivity has been

Figure 3 Effect of *SCN10A* rs6795970 genotype on cerebellar functional connectivity

(A) Clusters with significantly reduced resting-state cerebellar functional connectivity in patients with multiple sclerosis with rs6795970^{AA} genotype vs patients with MS carrying the rs6795970^G allele are shown in red (thresholded at a familywise error corrected $p < 0.05$). (B) Boxplots depict standardized cerebello-thalamic functional connectivity for rs6795970^G vs rs6795970^{AA} genotype groups ($p = 7.42 \times 10^{-6}$). In this subsample ($n = 62$), rs6795970 and rs6801957 variants were in perfect linkage disequilibrium ($r^2 = 1$).

associated with chronicity of illness and severity of cognitive and motor deficits in the disease.^{34,35}

Traditionally, it is assumed that clinical abnormalities in MS stem from structural deficits in the CNS (i.e., demyelination and neurodegeneration).⁴ However, these mechanisms do not necessarily explain all of the clinical heterogeneity in cerebellar dysfunction in MS.² We also assessed the effect of the *SCN10A* genotype on functional status in patients with MS in relation to neurodegeneration and inflammatory demyelination using brain atrophy measures and lesion volume, respectively. We showed that there is no significant *SCN10A* genotype effect on the regional brain or cerebellar volumes, and the *SCN10A* genotype's impact on functional status in MS is independent of cerebellar atrophy. However, we observed a significant genotype by lesion volume interaction

effect on MSFC performance, so that the difference in MSFC performance between the 2 genotype groups was more evident in individuals with lower lesion burden. Interestingly, this seems similar to the findings from studies on *SCN10A* knockout and wild-type mice induced with EAE that suggest that $Na_v1.8$ is a major contributor to the symptom manifestation in earlier stages of the disease, and $Na_v1.8$ effect is masked by inflammatory demyelination in the later stages.⁵

These findings must be interpreted within the limitations of this study. We only assessed the impact of *SCN10A* genotypes on standard tests of motor coordination and ataxia for MS. A more comprehensive battery of motor and cognitive tests is necessary to obtain a more detailed picture of motor and cognitive deficits that accompany *SCN10A* variation. Longitudinal

Table 2 Clusters demonstrating significant reduction in resting-state functional connectivity with cerebellum in rs6795970^{AA} carriers vs rs6795970^G allele carriers

Cluster	Volume, mm ³	Minimum p value	MNI coordinates			Hemisphere	Brain regions
			x	y	z		
1	4,224	0.027	14	-26	4	Right	Thalamus, midbrain
2	1,984	0.034	-6	-30	-12	Left	Midbrain, thalamus
3	768	0.035	50	-66	44	Right	Lateral occipital cortex
4	256	0.045	-6	-18	12	Left	Thalamus

Abbreviation: MNI = Montreal Neurological Institute.

Results from dual-regression analysis are thresholded at a familywise error corrected $p < 0.05$ while controlling for the effects of age, sex, and handedness. In this subsample of patients with multiple sclerosis ($n = 62$), rs6795970 and rs6801957 variants were in perfect linkage disequilibrium ($r^2 = 1$).

studies may also shed light on the effect of *SCN10A* variation on the disease course of MS and its cerebellar-related signs. Notably, we examined cerebellar functional connectivity only in the resting condition. Additional studies are required to replicate our findings in the resting state and also evaluate potential effects of *SCN10A* genotype on cerebellar functional dynamics during motor or behavioral tasks. Other acquired channelopathies have also been implicated in MS pathophysiology.³⁶ Future studies are required to determine the combined effects of these channelopathies on MS-related cerebellar dysfunction. Finally, MAFs of the *SCN10A* genotypes were slightly higher in patients with MS (albeit nonsignificant). If replicated in larger and independent samples, this may suggest a contributory role for the *SCN10A* gene in susceptibility to MS.

Our data suggest that the *SCN10A* genotype may predict an MS subphenotype with significantly greater deficit in motor coordination and cognition and compromised cerebello-thalamic functional integrity. This study further supports previous evidence that Na_v1.8 channelopathy is involved in the pathophysiology of cerebellar dysfunction in MS. Sodium channel blockers have been used in treating paroxysmal episodes of ataxia in MS.^{37,38} Moreover, drugs that specifically target and block the Na_v1.8 channel have been developed and used in animal models of MS.³⁹ If such agents become available for use in humans, they may prove beneficial in the treatment of cerebellar dysfunction in MS.

AUTHOR CONTRIBUTIONS

T.R., S.S., R.M., Aria Nazeri, S.N., M.J.S., M.N., A.N.M., M.O., R.D., A.P.H.T., A.S.R., A.A., Arash Nazeri, and M.A.S. designed and conducted the study. T.R., S.S., M.T.M.P., Aria Nazeri, S.N., and Arash Nazeri conducted the statistical analysis. All authors participated in drafting or revising the manuscript.

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DISCLOSURE

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