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Clinical/Scientific Notes

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VARIANT SCREENING OF THE CODING REGIONS OF *MEIS1* IN PATIENTS WITH RESTLESS LEGS SYNDROME

Restless legs syndrome (RLS) is a common and genetically complex neurologic disease presenting with an urge to move the legs and dysesthesias in the evening and at times of rest. Genome-wide association studies have linked single nucleotide polymorphisms in *MEIS1* and 3 other loci to an increased susceptibility to RLS.[1-3] However, to date, only one potentially causal variant has been reported.[4] Therefore, we screened the coding regions and exon-intron boundaries of *MEIS1* for variants, which by exerting a strong phenotypic effect could provide a basis for assessing the function of the gene in RLS.

Methods. Using Idaho LightScanner high-resolution melting curve analysis, we screened DNA of 188 patients with RLS of a first discovery sample (72.8% female, mean age 60.0 ± 11.2 years), all harboring RLS risk alleles of *MEIS1* (G/T or G/G for rs2300478), for aberrant melting patterns (e-Methods on the *Neurology*® Web site at www.neurology.org). Exons showing changes suggestive of variants were sequenced on an ABI Prism 3730 sequencer, and variants identified were subsequently genotyped in an independent German sample (henceforth termed "second sample") consisting of 735 patients with RLS (70.8% female, mean age 61.5 ± 14.2 years) and 735 unrelated control subjects (74.5% female, mean age 59.8 ± 11.3 years) by matrix-assisted laser desorption ionization/time-of-flight mass spectrometry. Disease segregation was evaluated in one family with the p.R272H mutation of exon 8 of *MEIS1*, previously related to RLS.[4] RLS in all patients was diagnosed in accordance with standard diagnostic criteria[5] (e-Methods).

Standard protocol approvals, registrations, and patient consents. Ethics review board approval and participants' written informed consent were obtained.

Results. In the discovery sample, we identified 3 novel nonsynonymous nucleotide substitutions in exons 3 (p.H81Q) and 6 (p.S204T) and in one transcript containing exon 13 (p.M453T) of *MEIS1* in one patient each. In addition, 2 patients showed the p.R272H variant.

Genotyping in the second sample revealed p.H81Q in 2 control subjects and p.M453T in 2 case patients and 3 control subjects, whereas p.S204T was observed in one case patient. p.R272H was not found in any additional case patients or control subjects (figure, A).

Segregation analysis performed on 7 family members revealed 4 affected individuals who presented the p.R272H variant (figure, B). One family member (III-1) and one married-in individual (III-3) were affected but did not show the p.R272H variant. p.R272H was not found in one unaffected individual (II-1).

Because all p.R272H patients were of Czech heritage, we further evaluated the presence of a putative p.R272H founder mutation in 279 Czech patients with RLS (63.1% female, mean age 55.8 ± 14.9 years), but did not find any additional carriers.

Discussion. Screening of the coding regions of *MEIS1* in patients with RLS revealed 3 novel variants. In all cases, patients reported one or more relatives with at least a suspicion of RLS; however, families were small and individuals in many instances not available for further study so that pathogenicity of the variants was not evaluated. We also confirmed the p.R272H variant of *MEIS1*[4] in 2 patients with RLS, one belonging to a family in which the RLS trait seems to be inherited in an autosomal dominant fashion. As observed previously, the p.R272H variant is located within the highly conserved TALE homeobox domain, which is essential for dimerization and transcription activation and disruption of which is known to be detrimental.[4] Therefore, this variant is considered the most likely candidate for an RLS-linked pathogenic mutation to date. In our sample, it was only present in RLS-affected individuals. However, segregation analysis also revealed affected individuals without the variant, suggesting that because RLS is a common disease, these cases could represent phenocopies, that is, similar phenotypes due to different genetic alterations. As opposed to the phenotype described in the North American p.R272H family, disease representation was rather homogeneous in our family with severe and early onset of symptoms. The second patient with p.R272H reported only a daughter

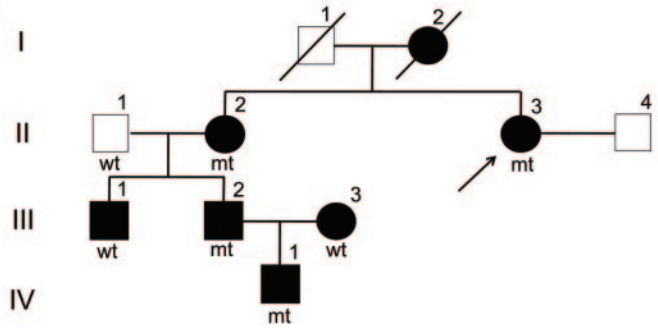
Supplemental data at www.neurology.org

Figure Variants of the coding regions of *MEIS1*

A

Region		Genomic position (chr. 2; NCBI 36/hg18)	Nucleotide/amino acid substitution		First sample	Second sample		
						Cases	Controls	MAF
MEIS1 exon 3	novel	66,520,482	CA[C/G] - His>Gln	H81Q	1/186	0/0/722	0/2/717	0.001
MEIS1 exon 6	novel	66,523,664	[T/A]CA - Ser>Thr	S204T	1/188	0/1/723	0/0/723	<0.001
MEIS1 exon 8	Ref. (4)	66,592,857	C[G/A]T - Arg>His	R272H	2/365	0/1/723	0/0/725	<0.001
MEIS1 exon 13	novel	66,652,131	A[T/C]G - Met>Thr	M453T	1/178	0/2/716	0/3/722	0.002

B



(A) Table illustrating the nucleotide and amino acid substitutions of novel coding variants and p.R272H of *MEIS1* as well as their frequency in the first (mutation carriers/total number of patients with restless legs syndrome [RLS] tested) and the second sample (homozygotes/heterozygotes/homozygotes in case patients and control subjects). (B) Segregation analysis of the p.R272H variant of *MEIS1* in an RLS family. Men are represented by squares and women by circles; a diagonal line indicates a deceased individual. For individuals sequenced, mt indicates those carrying the p.R272H variant and wt indicates noncarriers. The arrow denotes the index patient. MAF = minor allele frequency.

with possible RLS during pregnancy, which could be suggestive of variable expressivity of the p.R272H variant as well as the presence of additional modifying factors. Overall, segregation analyses remain inconclusive because of the small size of pedigrees, yet support the notion that p.R272H *MEIS1* could be causally related to RLS.

All additional variants affect amino acids highly conserved in vertebrates. Although not located within a known functional protein domain, bioinformatics algorithms[6] predict p.S204T and p.H81Q to be disease-causing, whereas p.M453T is likely to be functionally neutral. However, the fact that, as opposed to p.H81Q and p.M453T, p.S204T was only found in RLS-affected individuals renders this variant a second potential candidate for a disease-causing genetic alteration.

However, one limitation of this study is the fact that the control subjects used are general population control subjects and thus we cannot exclude the possibility that variants also found in control subjects (p.H81Q and p.M453T) are not related to RLS. Accordingly, these warrant further replication in an independent dataset.

Our results show that exonic variants in *MEIS1* are not common in RLS. However, it is still possible that rare exonic variants of strong effect could play a causative role in RLS in rare cases, as is known for other complex diseases,[7] and their study is important because they could provide significant clues toward understanding of the disease mechanism.

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CSF COMPLEMENTS SERUM FOR EVALUATING PARANEOPLASTIC ANTIBODIES AND NMO-IgG

The detection of neural-reactive immunoglobulin G (IgG) autoantibodies aids the diagnosis of organ-specific autoimmune neurologic disorders. Many paraneoplastic autoantibodies reliably predict a particular cancer type and are accompanied by varied neurologic presentations of subacute onset.[1] The detection of neuromyelitis optica (NMO)–IgG predicts a relapsing inflammatory demyelinating disorder predominated by optic neuritis and transverse myelitis.[2] When an autoimmune neurologic disorder is suspected, serologic testing of serum is frequently undertaken before more invasive CSF evaluation. However, CSF evaluation can complement testing of serum when suspicion for an autoimmune etiology persists despite a negative serum result. Here we report, for a 25-year period of testing by standardized indirect immunofluorescence protocols, the frequency of neural autoantibody detection in serum and CSF.

Methods. The immunofluorescence protocols we used were validated in this laboratory for detection of paraneoplastic antibodies (anti-neuronal nuclear antibody [ANNA]-1; ANNA-2; ANNA-3; Purkinje cell cytoplasmic antibody [PCA]-1; PCA-2; PCA-Tr; collapsin response-mediator protein [CRMP]-5-IgG; amphiphysin antibody; antiglial/neuronal nuclear antibody [AGNA]-1; NMDA receptor antibody) and NMO-IgG. We searched the Mayo Clinic Neuroimmunology Laboratory database (January 1986 to March 2010) for all patient samples submitted for service evaluation of paraneoplastic or NMO-IgG. We included both Mayo Clinic and non-Mayo patients for whom both serum and CSF were submitted, and reviewed available oncologic data for patients with antibodies identified by CSF testing.

Results. Testing was performed on a clinical service basis for a median of 12 years (range 2–25 years). The antibody detection rate in all specimens ranged from 0.01% for PCA-Tr to 7% for NMO-IgG (table).

In patients for whom paired serum and CSF samples were tested, the antibody detection rate ranged from 0.08% (PCA-Tr) to 9% (NMDA receptor antibody). One or more neural autoantibodies were detected in 462 patients (497 antibodies detected). In 405 of those 462 patients, both serum and CSF yielded a positive result (88%). In 57 patients, serum or CSF alone was positive (12%). Among those patients, serum alone yielded a positive result in 31 (54%) and CSF alone in 26 (46%). For classic paraneoplastic antibodies, CSF alone yielded a positive result in 20 patients, twice as commonly as serum alone (10 patients). For NMO-IgG, serum alone yielded a positive result in 21 patients 3.5 times more commonly than CSF alone (6 patients).

Discussion. From our review of a 25-year experience with immunofluorescence testing on a service basis in the Mayo Clinic Neuroimmunology Laboratory, we found that the rate of clinically pertinent autoantibody detection was highest when both serum and CSF were tested. It is plausible that this finding may reflect a greater likelihood of physicians deciding to test both serum and CSF in patients with the highest index of clinical suspicion.

When both serum and CSF were tested, CSF was more commonly informative than serum for paraneoplastic antibody detection. This raises concern that clinically important neural antibodies may be missed when only serum is tested. This finding was most prominent for NMDA receptor-specific IgG. Consistent with this finding, Kumar et al.[3] recently reported 3 patients, each of whom had NMDA receptor IgG detected in CSF but not in serum. Where there is a high suspicion for

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