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MEIS1 p.R272H IN FAMILIAL RESTLESS LEGS SYNDROME

Restless legs syndrome (RLS, OMIM 102300) is a neurologic condition characterized by a distressing urge to move the legs, usually accompanied by an uncomfortable sensation described as a crawling, muscle ache, or tension. It is usually brought on by rest, worse in the evening or night, and relieved by movement.¹ Recently, *MEIS1* and *BTBD9* have been identified and confirmed as RLS susceptibility genes; however, all the variants found to be associated with disease are located deep in intronic regions and are not thought to be functional.^{2–4} To date, sequencing of *MEIS1* and *BTBD9* in RLS samples has not been reported and therefore functional variants responsible for the increased disease risk have not been identified. Here we report sequencing of all *MEIS1* and *BTBD9* coding exons and exon-intron boundaries in RLS familial probands followed by assessment of segregation of novel variants with disease within families and evaluation of prevalence in patients with RLS and the general population.

Methods. Genomic DNA was extracted from peripheral blood lymphocytes using standard protocols. Primer pairs for *MEIS1* (NM 002398) and *BTBD9* (NM 052893) were used to sequence all coding exons by PCR in a discovery group consisting of 71 RLS familial probands. PCR products were purified using Agencourt bead technology (Beverly, MA) with Biomek FX automation (Beckman Coulter, Fullerton, CA). Prevalence of novel mutations was examined by TaqMan probe in a RLS case-control series consisting of 378 RLS cases (mean age 64.5 ± 13.4 years; onset age 45.3 ± 19.2 years; range 2–86 years) and 853 unrelated controls without evidence of RLS from North America and Europe. In addition, disease segregation was evaluated in affected and unaffected family members. All patients were examined and observed longitudinally and diagnosed with RLS as previously described.⁴ The ethical review board involved approved the study and all participants provided informed consent.

Results. Sequencing analysis of *BTBD9* in RLS probands did not identify any novel variant in coding regions or within 10 bases of exon-intron boundaries.

One known synonymous variant (rs41303370; p.V239V) and one variant in the 3' untranslated region were observed (rs6906771). Sequencing of *MEIS1* identified one sample with a novel arginine to histidine (p.R272H; ss107795884) mutation in the first amino acid of the highly conserved homeobox three-amino acid loop extension (TALE) domain (figure, A). Genotyping of this mutation in our RLS case-control series from North America (378 cases and 528 controls) failed to identify p.R272H in any additional individuals. Further genotyping in 325 controls from European populations identified one male mutation carrier from Ireland not diagnosed with a neurologic disease at collection (age 64 years).

Six family members, 3 diagnosed with RLS, were available for segregation analysis of *MEIS1* p.R272H; all samples diagnosed with RLS for which DNA was available (II-4, II-7, III-2) and one obligate carrier (II-1; unaffected parent of affected offspring) presented the p.R272H mutation (figure, B).

Discussion. Sequencing analysis of RLS probands identified one novel *MEIS1* nonsynonymous mutation in the highly conserved TALE domain, essential for dimerization and transcription activation.⁵ Evaluation of the frequency of this mutation in the case-control series identified only one control from Ireland carrying the mutation; therefore, if pathogenic, p.R272H does not appear to be fully penetrant, nor a common cause of disease (1/378 cases).

Segregation analysis of *MEIS1* p.R272H is inconclusive due to the small size of the pedigree and having DNA available only for 3 affected family members. However, the presence of the mutation in all affected individuals supports a pathogenic role for this variant in RLS. The existence of an unaffected mutation carrier (II-1; died at 92 years of age) further supports the reduced penetrance of this mutation. Clinically, all affected family members present considerable differences in ages at onset, ranging from 15 (II-4) to 60 (II-7, III-2) years, and disease severity, from 14 (II-7) to 25 (II-4, III-2) points on the IRLSS scale, indicating the high heterogeneity of disease and potentially the presence of modifying factors.

(A) Protein homologues were aligned using ClustalW; p.R272H position is highlighted in black, nonconserved amino acid positions are highlighted in gray. (B) Males are represented by squares, females by circles, diamonds with a number represent the number of additional sibs. A diagonal line indicates deceased subjects, filled symbols indicate affected individuals. p.R272H mutation carriers are indicated with mt beneath the symbol, noncarriers with wt.

This study reports sequencing of *MEIS1* and *BTBD9* in patients with RLS. These analyses revealed that nonsynonymous mutations in these genes are not a common cause of RLS in the US population. However, a novel nonsynonymous mutation, p.R272H, in the highly conserved TALE domain of *MEIS1* was identified. The importance of homeobox consensus sequences and the detrimental effect of arginine to histidine mutations in this domain have been described in several studies.^{6,7} However, cosegregation of this mutation and disease within a small pedigree is inconclusive and replication in independent datasets is warranted to evaluate p.R272H as a pathogenic cause of familial RLS.

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CALCIFIC EMBOLUS CAUSING SUBARACHNOID HEMORRHAGE

Nonaneurysmal subarachnoid hemorrhage (SAH) accounts for about 15% of spontaneous, nontraumatic SAH and is often characterized by bleeding in distinctive anatomic locations. For example, perimesencephalic SAH is associated with an excellent prognosis and no significant risk of rebleeding, and has been hypothesized to have a venous origin.^{1,2} SAH in the superficial cortical sulci without blood in proximity to the circle of Willis also suggests a nonaneurysmal source, as most aneurysms develop at circle of Willis branch points. A number of causes of nonaneurysmal nonperimesencephalic SAH have been described, including vascular malformations, various forms of vasculitis, cerebral venous thrombosis, amyloid angiopathy, Moyamoya syndrome, and coagulopathies.^{1,3} We present a case of a nonaneurysmal nonperimesencephalic SAH following a calcific embolus to the middle cerebral artery, an unusual association.

Case report. A 51-year-old left-handed man without significant past medical history or recent trauma presented with insidious onset of headache, progressing over 2 days to the worst headache of his life. On the second day, he developed aphasia and mild left-sided weakness and numbness and presented to the hospital. On arrival, neurologic examination showed left-sided sensory loss and subtle left arm weakness. The focal neurologic symptoms resolved after 3 hours, though his headache persisted. Initial head CT demonstrated SAH over the right frontal convexity (figure). CT angiography showed no evidence of aneurysm, but revealed a probable calcific embolus in the posterior division of the right middle cerebral artery (MCA) without obvious flow limitation. Brain MRI showed scattered punctate areas of acute infarction in the right frontal, parietal, and temporal lobes, and SAH in right hemispheric superficial cortical sulci. Catheter angiography demonstrated a nonocclusive calcific embolus in the posterior division of the right MCA just beyond the MCA bifurcation. Vasospasm was not present, nor was there any evidence of vasculitis. Retrospective review of his initial head CT showed that the embolus was demonstrated on this study, but missed due to its proximity to the

sphenoid bone. Echocardiography revealed a thickened and highly calcified bicuspid aortic valve felt to be a high-risk source of calcific emboli.

During the first 5 days of hospitalization, he had several episodes of transient left-sided numbness lasting 15–30 minutes which resolved with flat head positioning and fluid boluses. Several possible explanations exist for these transient neurologic episodes. They may have been simple partial seizures as the result of cortical irritation, or vasospasm, although vasospasm was not seen during the angiogram. The positional nature of these episodes seems to argue for TIAs related to decreased perfusion. He was treated with aspirin, started on midodrine to increase his systemic blood pressure and cerebral perfusion, and slowly mobilized without further episodes. On follow-up 3 months later, he had no recurrent symptoms and a normal neurologic examination. Repeat CT angiography was unchanged, with persistent right MCA calcific embolus.

Discussion. Calcific emboli are not commonly thought to cause SAH. Clues to the nonaneurysmal nature of this SAH were the superficial location of the subarachnoid blood, the gradual onset of headache, and the presence of early transient focal neurologic symptoms that would be atypical of aneurysmal SAH.

The mechanism by which a calcific embolus resulted in SAH in our patient is uncertain, although several possibilities exist. The nonocclusive embolus seen in the right MCA may have been accompanied by small satellite emboli to distal superficial vessels, producing the small punctate infarctions, and damaging small superficial cortical arteries, leading to hemorrhage. While there is no evidence on the MRI of additional calcific emboli closer to the hemorrhage, these may have been too small to be detected. It is also possible that the calcific embolus caused dissection of the right MCA, although SAH associated with intracranial arterial dissection is typically more severe than seen in this case. Furthermore, SAH was absent in the sylvian fissure, which would be unusual in an MCA dissection. Finally, it is possible that rupture of small superficial leptomeningeal collateral vessels could have occurred with compensatory dilatation of these vessels after the proximal MCA partial occlusion. Our patient showed clear ev-

(A) Noncontrast head CT shows subarachnoid hemorrhage (arrow) in the right frontal convexity. (B) Noncontrast head CT shows calcific embolus (arrow) overlying the right middle cerebral artery adjacent to the sphenoid bone. (C) Diffusion-weighted MRI demonstrates small areas of acute infarction in the territory of the right middle cerebral artery. CT angiography (D) and catheter angiography (E) demonstrate the calcific embolus in the posterior division of the right middle cerebral artery (arrowheads).

idence of hemodynamic symptoms, with multiple hemispheric TIAs responsive to measures designed to increase cerebral blood flow. However, embolic MCA occlusions are a common cause of stroke, while concurrent SAH in ischemic stroke is extremely rare, arguing against this possibility.

This case adds to the potential specific causes of nonaneurysmal nonperimesencephalic SAH. Prompt identification of such causes is imperative to limit morbidity from unnecessary invasive diagnostic studies and so that treatment can be appropriately targeted.

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