Mutations in the TRPV4 Gene Are Not Associated With Sporadic Progressive Muscular Atrophy

Progressive muscular atrophy (PMA) is an adult-onset neurodegenerative disease characterized by progressive loss of lower motor neurons (LMNs). Its disease course ranges from slowly progressive in many years to rapid progression rates similar to those observed in patients with amyotrophic lateral sclerosis (ALS). Whether PMA is a distinct disease identity separate from ALS remains the question, especially because upper motor neuron signs may develop over time and mutations in the superoxide dismutase 1 gene, which are known causes of ALS, have also been identified in patients with familial PMA. Moreover, mutations in the charged multivesicular protein 2B gene have been reported in 3 patients with sporadic LMN-predominant ALS. Nonsynonymous mutations in the transient receptor potential vanilloid 4 (TRPV4) gene, which encodes a calcium permeable protein channel, are known causes of ALS, have also been identified in patients with familial PMA. Furthermore, mutations in the charged multivesicular protein 2B gene have been reported in 3 patients with sporadic LMN-predominant ALS. Nonsynonymous mutations in the transient receptor potential vanilloid 4 (TRPV4) gene, which encodes a calcium permeable protein channel, have recently been identified in patients with LMN disorders such as congenital distal spinal muscular atrophy, scapuloperoneal spinal muscular atrophy, and Charcot-Marie-Tooth/hereditary motor and sensory neuropathy type 2c. These disorders are characterized by predominant LMN degeneration. The TRPV4 gene is of special interest because mutations may lead to neurotoxicity induced by intracellular hypercalcemia and therefore, pharmacological blockade of TRPV4 channels may offer a target for therapy of TRPV4-associated disorders. We hypothesized that TRPV4 may be a candidate gene for susceptibility to PMA and screened a Dutch cohort of patients with sporadic PMA and control subjects for nonsynonymous mutations.

Methods. A total of 264 patients with sporadic PMA and 768 healthy control subjects, all Caucasian and of Dutch descent, were included in the study. Patients were seen by experienced neurologists at the Dutch national ALS referral center. Diagnosis was based on LMN involvement on neurologic and electrophysiological examination. Exclusion criteria included a history of acute poliomyelitis, spinal radiculopathy, diabetic amyotrophy, thyrotoxicosis, or hyperparathyroidism; sensory signs on neurologic examination; structural lesions on magnetic resonance imaging or computed tomography of the head and spine; motor conduction block on extensive standardized nerve conduction studies; and clinical signs of upper motor neuron involvement—that is, pseudobulbar symptoms, clonus of the masseter reflex, hyperreflexia (including brisk reflexes in weakened muscles), or extensor plantar response. The medical ethical committee of the University Medical Centre Utrecht, the Netherlands, approved the study protocol, and all patients gave informed consent. Genomic DNA was extracted from peripheral blood using standard methods. Coding regions of TRPV4 were screened for mutations by direct sequencing. Primers for polymerase chain reaction amplification were designed using LIMSTILL (http://limstill.nibb.knaw.nl) and are available on request. The polymerase chain reaction amplification fragments were sequenced with BigDye Terminator 3.1 sequencing kit (Applied Biosystems) and analyzed on a 3730XL DNA Analyzer. Each identified mutation was confirmed by an independent polymerase chain reaction and sequencing reaction. The impact of the mutation on the structure and function of the protein was predicted with PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) and PMut (http://mmb.pcb.ub.es/PMut/PMut.jsp).

Results. Patient characteristics and the results of the TRPV4 gene analysis are shown in the Table. None of the previously described mutations (R232C, R269H, R269C, R315T, R316C, and V620D) were identified in our patients with PMA. However, we did identify a novel heterozygous c.2337G>A mutation in exon 14, changing valine to isoleucine at position 750 (V750I). Nonetheless, the estimated impact of this mutation was neutral or benign, and it was also present in 2 control subjects.

Comment. Recent studies have demonstrated that mutations in the TRPV4 gene are associated with a heterogeneous group of diseases characterized by LMN or axonal degeneration. We evaluated genetic variance in this

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients With Sporadic PMA</th>
<th>Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>264</td>
<td>768</td>
</tr>
<tr>
<td>Median age, y</td>
<td>63</td>
<td>62</td>
</tr>
<tr>
<td>Sex, male/female, No.</td>
<td>189/75</td>
<td>428/340</td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
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<tr>
<td>Mutation V750I, No. (%)</td>
<td>2 (0.8)</td>
<td>2 (0.3)</td>
</tr>
<tr>
<td>PolyPhen-2</td>
<td>Benign</td>
<td></td>
</tr>
<tr>
<td>PMut</td>
<td>Neutral</td>
<td></td>
</tr>
<tr>
<td>P value*</td>
<td>.27</td>
<td></td>
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</tbody>
</table>

Abbreviation: PMA, progressive muscular atrophy.

*Calculated using Fisher exact test.
gene as a possible risk factor for PMA, but none of the previously described mutations could be identified. One novel mutation was detected at similar frequencies in patients and control subjects (P = .27); thus, it most likely represents a rare benign polymorphism. Hence, we conclude that genetic heterogeneity of TRPV4 is not associated with PMA.

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COMMENTS AND OPINIONS

Superficial Siderosis, Traumatic Tap, and Xanthochromia

We read the article by Vale et al1 about idiopathic superficial siderosis with great interest. The authors pointed out that this syndrome may still be missed on magnetic resonance imaging as the imaging abnormalities follow the contours of the brain and can be overlooked. They also stated that “xanthochromia or the presence of red blood cells (RBC) in the [cerebrospinal fluid] CSF is a common finding and may be due to damage to a small blood vessel during the procedure (known as a ‘traumatic tap’).” Whereas the presence of red blood cells may indeed be the consequence of a traumatic spinal tap, xanthochromia necessarily witnesses a bleeding that occurred earlier than the spinal tap.

In clinical practice, xanthochromia is detected by the eyeball test.2 When present at the time of the spinal tap, it cannot be attributed to a traumatic procedure because the source of xanthochromia is the late consequence of red blood cell lysis. Xanthochromia appears 12 hours after a hemorrhage, at the latest. The yellow color of the supernatant has its source in the presence of bilirubin, a breakdown product of oxyhemoglobin.3 From our point of view, the presence of xanthochromia on simple visual inspection is a good sign to differentiate acute or chronic subarachnoid hemorrhage from traumatic tap and should prompt the clinician to engage further investigations.4

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