A Missense Mutation in the Coiled-Coil Domain of the KIF5A Gene and Late-Onset Hereditary Spastic Paraplegia

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Background: To our knowledge, up to now, only 2 mutations in the KIF5A gene, a member of the kinesin superfamily, have been identified as the molecular cause of early-onset autosomal dominant hereditary spastic paraparesis (ADHSP).

Objective: To assess the genetic defect in a family with late-onset ADHSP.

Patients and Methods: Only the proband agreed to undergo complete neurological testing and mutational analysis. The proband was screened for mutations in the spastin, atlastin, NIPA1, and KIF5A genes, either by denaturing high-performance liquid chromatography or sequence analysis.

Results: The history of the family was consistent with ADHSP characterized by late onset of the disease. Mutational analysis results were negative for the spastin, atlastin, and NIPA1 genes but identified a missense mutation (c.1082C>T) in the coiled-coil coding region of the KIF5A gene.

Conclusions: This finding enlarges the phenotypic spectrum of ADHSP linked to KIF5A and enhances the role of that gene in the epidemiology of this disease. We propose that the KIF5A gene should be routinely analyzed in patients with hereditary spastic paraplegia negative for spastin and atlastin mutations.

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Hereditary spastic paraplegia (HSP) is a heterogeneous group of neurodegenerative diseases characterized by progressive weakness, spasticity, and loss of vibratory sense in the lower limbs. Clinically, HSPs are divided into “uncomplicated” (symptoms confined to lower extremity weakness, bladder disturbance, and, to a lesser extent, impaired position sense in the legs) or “complicated” (when additional neurological deficits are present).

Recent genetic studies revealed that at least 28 loci of HSP have been associated with autosomal dominant (ADHSP), recessive, or X chromosome–linked transmission, but only 9 of the responsible genes have been identified. Among them, spastin (SPG4), atlastin (SPG3A), KIF5A (SPG10), HSP60 (SPG13), and, more recently, NIPA1 (SPG6) have been associated with pure ADHSP. The functions of several of these genes are related to axonal transport or intracellular trafficking. Herein we report on a new mutation in the KIF5A gene found in the index case of a pedigree with ADHSP.

Methods

Patients

This Italian family (Figure 1) was identified through the neurological examination of the proband (3-9). Nine additional relatives were reported as having difficulties in gait, but only the proband agreed to give DNA for genetic testing.

Genetic Studies

Genomic DNA was extracted from peripheral blood samples by standard protocols after written informed consent. Mutational analysis was performed on the spastin and KIF5A genes by denaturing high-performance liquid chromatography (DHPLC) as previously described while the atlastin and NIPA1 genes were analyzed by direct sequencing using polymerase chain reaction (PCR) primers and protocols either previously described or available on request (NIPA1). The PCR products were purified using the QiAquick PCR purification kit (Qiagen, Hilden, Germany) and bi-directionally sequenced using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif). Samples were analyzed on an ABI PRISM 310 automated sequencer (Applied Biosystems).
BIOINFORMATIC ANALYSIS

The effect of the A361V mutation on the coiled-coil structure of KIF5A was predicted using the COILS program. The normal and A361V mutant sequences were analyzed using the MTIDK matrix, with window lengths of 14, 21, and 28. The hypothetical activation by the mutation of a cryptic splice site was tested using SpliceView software.

GENETIC ANALYSIS

DNA analysis was only performed on the proband (3-9) because of the unavailability of the other pedigree members. No mutations were found in the SPG4, SPG3A, and NIPA1 genes, although we cannot exclude either the presence of cryptic intrinsic mutations in these genes or that mutations may have been missed by DHPLC screening, despite its very high sensitivity. Further DHPLC analysis of the KIF5A gene revealed a clear heterozygous elution pattern of the exon 11-related PCR product.

PHENOTYPIC DESCRIPTION

The proband (3-9) was a 48-year-old man who experienced difficulty in gait and urinary urgency since the age of 35 years. Neurological examination revealed corticospinal tract deficits affecting the lower extremities with an asymmetry in the physiological range. Major findings were spastic gait and marked hyperreflexia, and extensor plantar response muscle tone was increased during gait as well as at passive mobilization, associated with weakness and pes cavus. No sensory loss or decreased vibration sense were recorded. There was no upper limb involvement. Negative findings in the clinical evaluation were absence of extrapyramidal signs, ataxia, retinal involvement, amytrophy, mental retardation, dementia, deafness, or epilepsy.

Neurophysiological examination revealed slight abnormalities in evoked motor potential of the lower limbs in the presence of a normal central conduction time. The upper extremities' responses were in the normal range. The evoked sensitive potentials in the lower and upper extremities and the examination results of the optic tract were normal. Peripheral conduction velocities and F waves of the lower extremities were in the normal range, excluding a neuromuscular involvement. Normal magnetic resonance imaging results of the brain and spinal cord excluded structural abnormalities. The differential diagnosis with multiple sclerosis was supported by normal results of cytochemical analysis of cerebrospinal fluid and by the absence of oligoclonal bands. All hematoclinical analysis results were in the normal range, in particular the inflammatory index and the folate and vitamin B12 levels.

The familial tree (Figure 1) showed recurrence of gait disturbance in this family in 4 generations. The precise age of disease onset was reported only for the father of our proband, who had clinical symptoms at the age of 50 years, while other affected members were reported to have had an adult onset of spastic gait. No clinical data were available for other family members.

Figure 1. Pedigree of the studied family. Arrow indicates the proband. Filled symbols represent affected individuals and slash marks, deceased individuals.

RESULTS

Successive sequence analysis (Figure 2) identified a heterozygous single-nucleotide variant at position 1082 (c.1082C>T) predicted to result in alanine-to-valine substitution at codon 361 (A361V) in the coiled-coil tail region of KIF5A. The affected amino acid is conserved in the KIF5A family of several vertebrate species (Figure 3); moreover, this mutation was not found in a group of 750 unrelated chromosomes from healthy individuals of the same ethnic origin tested by DHPLC, indicating that this was unlikely to be a rare polymorphism and most probably represented the mutation underlying the phenotype observed in the patient studied.

Figure 2. Schematic representation of the KIF5A gene. The affected amino acid is conserved in the KIF5A family of several vertebrate species. The mutation was not found in a group of 750 unrelated chromosomes from healthy individuals of the same ethnic origin tested by DHPLC.

BIOINFORMATIC ANALYSIS

The mutation did not show any significant effect either on the coiled-coil structure or on the activation of cryptic splice sites (data not shown).

COMMENT

Neurons are an example of extremely polarized cells with axons that reach up to 1 m in length; because axons have little or no protein synthesis machinery, axonal proteins must be synthesized in the cell body. Neurons require an appropriate system of intracellular traffic and an intracellular transport system, and this requirement could be the “Achilles heel” of these large, complex cells.

The KIF5A protein belongs to the superfamily of kinesins, which are molecular motor proteins responsible for many of the major microtubule-dependent transport pathways in neuronal and nonneuronal cells. They typically consist of 2 identical, approximately 110- to 120-kD heavy chains and 2 identical, approximately 60- to 70-kD light chains. The heavy chains have a 3-domain structure. The globular N-terminal motor domain, which contains the adenosine triphosphate and microtubule-binding sites, is about 340 amino acids long. The heavy chains of kinesin dimerize through an α-helical coiled-coil region, the so-called stalk domain. The third domain consists of a C-terminal globular tail that is thought to be involved in light-chain and cargo binding.
To our knowledge, to date, only 2 disease-causing mutations in the KIF5A gene have been described in the literature, localized in exons 10 (R280C) and 8 (N256S), respectively. These 2 lesions belong to the region of the kinesin devoted to microtubule binding, known to affect the adenosine triphosphatase activity of the motor domain.

In this study, we identified a novel missense mutation in the KIF5A gene in the proband of a large pedigree with ADHSP. The absence of this mutation was tested in 375 normal controls. Nevertheless, because we were unable to analyze the other members of the family, we cannot exclude that it is a very rare polymorphism that segregates independently from the disease.

This defect occurs in a region adjacent to the globular motor domain (termed the neck region) that permits dimerization and contains a sequence that is predicted to form an α-helical coiled-coil domain. Furthermore, this domain appears to be structurally important for coordinating the activities of the 2 kinesin heads during processive movement along microtubules.

The heptad repeat motif (a, b, c, d, e, f, and g) forms the structural basis of the α-helical coiled coil and is found in a wide variety of proteins. The “a” and “d” positions are usually represented by small nonpolar residues that stabilize the coil. Therefore, substitution of amino acids at these positions may destabilize the protein. In addition, the coiled coil may be further stabilized by salt bridges formed between charged side chains in the “g” and “e” positions of opposing helices. The mechanism by which the A361V mutation results in a pathogenic alteration of KIF5A is not clear because this mutation occurs in the “b” position of the heptad repeat in a residue that is exposed at the surface of the coiled-coil domain, and the replacement of the alanine by the valine does not change the hydrophobic character of the structure.

Interestingly, at least the propositus and his father in this family are characterized by a late onset of disease, whereas the patients previously described showed a prevalent juvenile onset of the symptoms, probably reflecting a different pathologic mechanism of this mutation.

Recent reports suggest that about 50% of mutations involved in ADHSP are related to the spastin (40%) and atlastin (10%) genes. Among 10 families showing a pure ADHSP who were recently screened for the spastin, atlastin, and KIF5A genes in our laboratory, we found 3, 1 (unpublished result), and 2 mutations, respectively. Although these figures are too small to be significant, we propose that, in the absence of linkage information, the KIF5A gene should be routinely tested in ADHSP.
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REFERENCES


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