CASE REPORT

Hereditary spastic paraplegia: identification of an *SPG3A* gene mutation in a Chinese family

KY Chan 陳國燕
CK Ching 程楚君
Chloe M Mak 麥　苗
CW Lam 林青雲
Albert YW Chan 陳恩和

Hereditary spastic paraplegias are a heterogeneous group of chronic central motor system disorders, characterised by progressive lower limb spasticity. Hereditary spastic paraplegia is clinically classified into pure and complicated forms, by the absence or presence of additional neurological or extra-neurological features. Hereditary spastic paraplegias follow all modes of inheritance and the pure-form autosomal dominant type is the one most commonly reported. Spastic paraplegia 4, autosomal dominant (SPG4, MIM#182601) and spastic paraplegia 3, autosomal dominant (SPG3A, MIM#182600), account for most autosomal dominant hereditary spastic paraplegias. Using DNA mutation analysis, the authors identified an *SPG3A* missense mutation (p.R239C) in a Chinese family where three members have early-onset pure spastic paraplegia. To our knowledge, this is the first report of a gene mutation in hereditary spastic paraplegias in our locality. DNA-based diagnosis plays a key role in the early diagnosis of familial hereditary spastic paraplegias.

Introduction

Hereditary spastic paraplegias (HSP) are a group of clinically and genetically diverse diseases characterised by progressive and severe lower limb spasticity per se or in combination with other neurological features. *SPG3A* mutation is the most frequent cause of autosomal-dominant hereditary spastic paraplegia (ADHSP) with an onset before the age of 10 years.[1] This gene, located at chromosome 14q, was identified in 2001. It is responsible for the encoding of atlastin, a 558-amino acid protein, which is a member of the dynamin family of large guanosine triphosphatases.[2,3] It is unclear how the lack of atlastin causes the symptoms of HSP. Recent research suggests that atlastin is found predominantly in the brain, particularly in the pyramidal neurones in the cerebral cortex and hippocampus, and is an integral membrane protein involved in Golgi membrane dynamics or vesicle trafficking in brain-specific axonal growth.[3,4] Over 30 different mutations, mainly missense mutations, have been reported in the *SPG3A* gene,[4] and family studies have failed to reveal any genotype-phenotype correlation in cases of ADHSP.[5] We report a family where three members have a clinical diagnosis of idiopathic spastic paraplegia caused by a missense mutation in the *SPG3A* gene.

Case report

The proband (III-2) is a 6-year-old boy who was referred to a paediatric clinic at the age of 3 years for developmental delay and a tiptoe gait. He is the second child of non-consanguineous Chinese parents. His antenatal and perinatal histories were unremarkable. Family history of neurological diseases was not stated. He presented to us at the age of 3 years with walking difficulty and was noticed to have tight hip adductors, hamstrings and tendo achilles. He was initially diagnosed with spastic diplegic cerebral palsy. Magnetic resonance imaging (MRI) of his brain and thoraco-lumbo-sacral spine revealed no pathology. Nerve conduction studies showed normal sensory and motor conduction velocities. The spasticity in his lower limbs increased and was accompanied by brisk reflexes, bilateral ankle clonus and Babinski signs despite physiotherapy and orthotic treatment. His upper limb functions were normal, as was his bulbar system. He had no cerebellar ataxia, and had normal bladder and bowel control, normal intelligence and language function, and normal vibration sensation. At the age of 6 years, he was diagnosed as having early-onset pure spastic paraplegia. He could walk indoors and outdoors and climb stairs holding onto a railing. His functional mobility was classified as gross motor function classification system (GMFCS) level 2. On further investigation of the family history, it was found that the father, aged 40 years (II-2) and elder sister, aged 9 years (III-1) had similar symptoms with onsets at age 3 to 4 years (Fig a). Both of them showed neurological abnormalities similar to those of the proband and were being managed at another regional hospital. They could walk with spastic gaits, with no significant functional limitations, and did not require supportive devices. The elder sister was a community walker capable of going up and down stairs with

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Princess Margaret Hospital, Laichikok, Hong Kong:
Department of Paediatrics and Adolescent Medicine
KY Chan, FRCPCH, FHKAM (Paediatrics)
Department of Pathology
CK Ching, MSc, MB, ChB
CM Mak, PhD, FHKAM (Pathology)
AYW Chan, MD, FHKAM (Pathology)
Department of Chemical Pathology, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong
CW Lam, PhD, FHKAM (Pathology)

Correspondence to: Dr KY Chan
E-mail: chanky1@ha.org.hk

no limitations. Her functional mobility was classified as GMFCS level 1. There had been no regression in her motor ability to date. The father had tendon release surgery during childhood and, aside from low back pain, did not have significant orthopaedic problems or joint contractures. A mild regression in his motor ability had been noticed since early adulthood. He could no longer walk up and down stairs without assistance.

Mutation analysis of the *SPG3A* gene was performed. Peripheral blood samples were collected from the index patient (III-2), his father (II-2), and his elder sister (III-1) after informed consent had been obtained. Genomic DNA was extracted using a QIAamp Blood Kit (Qiagen, Hilden, Germany). The coding exons and the flanking introns of the *SPG3A* gene were amplified using polymerase chain reaction (PCR). Primer sequences are available upon request. Amplification was performed using the touch-down method: 94ºC for 12 min, 10 cycles of 94ºC for 30 s, 59ºC for 30 s (the temperature was lowered 1ºC for each cycle), 72ºC for 30 s; 30 cycles of 94ºC for 45 s, 53ºC for 30s, 72ºC for 30 s and a final extension at 72ºC for 8 min. The reaction mixture (final volume 20 mL) contained 10X PCR buffer (Qiagen, Hilden, Germany), 5X Q-solution (Qiagen, Hilden, Germany), 2 mM magnesium chloride, 200 μM dNTP, 1 μM of each primer, 0.625 units HotStarTaq polymerase and 50 ng DNA template. The PCR products were sequenced directly using the BigDye Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, US).

A known disease-causing mutation of *SPG3A,* arginine-to-cysteine substitution at codon 239 (p.R239C),[2] was identified in the proband (III-2), his father (II-2), and his elder sister (III-1) [Fig b].

Discussion

Hereditary spastic paraplegia is a group of neurodegenerative disorders characterised by progressive spasticity of the lower limbs caused by pyramidal tract dysfunction. It is classified clinically according to the family history, mode of inheritance, age of onset, and the extent and features of the neurological involvement, into pure and complicated types. In the pure type, spasticity is limited to the lower limbs. In the complicated type, spasticity is associated with other neurological signs, including ataxia, mental retardation, extrapyramidal signs, visual dysfunction, or epilepsy. With the recent discovery of multiple genes and loci involved in HSP, the clinical variability is further complicated by the extended genetic heterogeneity. Therefore, the clinicians need to make a meticulous enquiry into the age of onset and clinical and family history, elicit well-defined neurological patterns of involvement, and perform cerebral and spinal MRI to detect associated abnormalities such as cerebellar or corpus callosum atrophy or to exclude leukodystrophy, to guide prioritisation of molecular testing. Depienne et al[6] have proposed a work-up for molecular studies for different types of HSP (Table).

遺傳痙攣性截癱：一華籍家庭的致病基因突變研究

遺傳痙攣性截癱是集合多種慢性中樞運動神經系統病變的疾病，患者會逐漸出現下肢痙攣。遺傳痙攣性截癱可分為單純型和複雜型兩類，視乎有否出現額外的神經性或外神經性症狀。這種疾病會按遺傳種類表現；自體顯性是最普遍的單純型，而痙攣性截癱4（SPG4，MIM#182601）及痙攣性截癱3（SPG3A，MIM#182600）是最廣泛的自體顯性病。本案例利用DNA突變分析，在同一個華籍家庭裏出現早期單純型痙攣性截癱的三位成員中分辨出*SPG3A*錯義突變（p.R239C）。據我們所知，這是本地遺傳痙攣性截癱首個基因突變的案例。DNA研究分析對於遺傳痙攣性截癱的早期診斷非常重要。

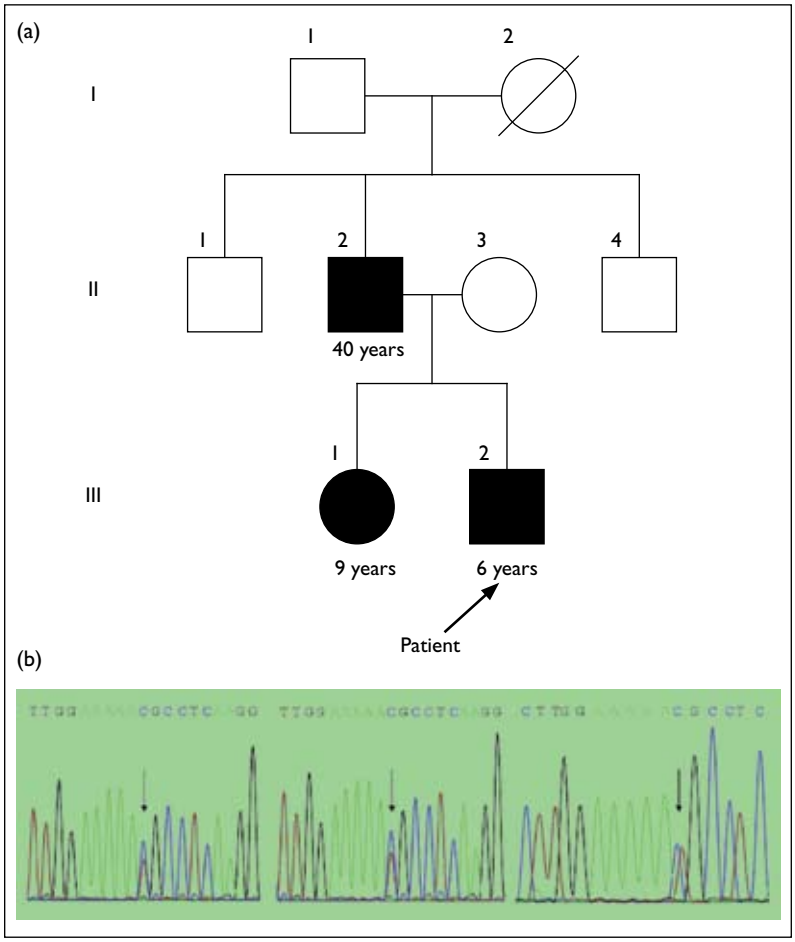

FIG. (a) Pedigree of the reported family with spastic paraplegia 3, autosomal dominant (SPG3A). (b) The DNA sequences (in sense direction) of III-2 (left), II-2 (middle), and III-1 (right) show p.R239C (arrows)

TABLE. Prioritising molecular testing in hereditary spastic paraplegia (HSP)[6]

Types of HSP	Molecular study		
	Familial cases in multiple generations	Familial cases in one generation	Isolated cases
Pure > complicated	*SPG4*	*SPG4*	*SPG4*
Normal MRI*	*SPG3A*†	*SPG3A*†	*SPG3A*†
	SPG31, *SPG10*		
	SPG6, *SPG8*		
Complicated > pure			
Abnormal MRI brain			
Thin corpus callosum		*SPG11*	*SPG11*
Cerebellar atrophy		*SPG7*	
Ataxia		*ARSACS*	

* MRI denotes magnetic resonance imaging

† For early-onset cases

Mutations in the *SPG4* gene (spastin protein) are responsible for about 40% of cases of ADHSP, while SPG3A accounts for about 10% of cases.[7] In early-onset ADHSP, the *SPG3A* gene mutation is the most common.[1] Other forms, such as SPG6, SPG8, SPG10, SPG12, SPG13, SPG19, SPG31, SPG33, and SPG37, have also been reported to be associated with the uncomplicated form of ADHSP.[6,7] The identification of a known mutation in the *SPG3A* gene in the proband and other family members is important for providing a prognosis and guiding genetic counselling. Dürr et al[8] studied the clinical features of 34 HSP patients with *SPG3A* mutation and found that: the mean age of symptom onset was 4.6±3.9 years; none had upper limb spasticity; 13% had decreased vibration sense in the ankles; 25% had bladder disturbances; and none had cerebellar and bulbar signs. After a mean duration of 32 years, less than 25% of these patients required a walking aid or a wheelchair. Dürr et al[8] also compared the clinical characteristics of SPG3A with those of SPG4 and non-SPG3A phenotypes, and it was found that SPG3A patients had an earlier age of symptom onset, pyramidal signs restricted to the lower limbs, fewer sphincter disturbances, and less reduction in vibration sensation.

More than 30 different mutations in the *SPG3A* gene have been reported and the genotype-phenotype correlation has been widely studied but is not well established.[4,6] The missense mutation, p.R239C, detected in our cases, is a known mutation which has been identified in other ethnic groups.[2,5,9,10] Li et al[11] found one novel mutation (p.H189D) and p.R239C mutation in two families when screening for *SPG3A* mutation in 20 families and 23 sporadic cases in the Han Chinese population.

Children with early-onset and non-progressive spasticity of the lower limbs are commonly diagnosed with spastic diplegic cerebral palsy. One must be cautious when making this diagnosis, particularly in cases with no apparent underlying causes, minimal hand dysfunction, and normal intelligence. It is important to investigate the family history in detail and search for consanguineous marriage if HSP is suspected. In our case, family history of neurological disorders was not stated initially. Information about the other family members was given when all investigations proved negative and when consent for genetic testing was requested. It has also been reported that the diagnosis of ADHSP is sometimes difficult to make because a family history is often absent. A negative family history may be caused by a number of factors. The age of symptom onset may vary within the same family, hence individuals with affected children may not have developed symptoms at the time of evaluation. Mildly affected family members may not report any problems despite the presence of definite spasticity. Causative genes can have incomplete penetrance, masking the vertical transmission of mutation.[12] Hereditary spastic paraplegia should also be considered in cases of idiopathic infantile- or childhood-onset spastic paraplegia with a negative family history as de-novo mutations in causative genes are not uncommon.[12,13]

In summary, we describe a family with childhood onset of HSP due to mutation in the *SPG3A* gene. To the best of our knowledge, this is the first such family to have genetic confirmation of this disorder in our locality. DNA-based evaluation plays a key role in the early diagnosis of HSP.

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