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Microarray analysis unmasked two siblings with pure hereditary spastic paraplegia shared a run of homozygosity region on chromosome 3q28–q29

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ABSTRACT

Hereditary spastic paraplegia (HSP) is a clinical and genetic heterogeneity group of neurodegenerative disorders which is characterized by progressive weakness and spasticity of the lower limbs. More than 70 genetic types of HSP have been described so far. Here we describe a Chinese non-consanguineous family with two affected siblings manifesting early-onset autosomal recessive HSP in pure forms. To identify genotype and characterize phenotype, CytoScan HD array analysis was performed on the two siblings. A run of homozygosity (ROH) shared by the two patients was detected on chromosome 3q28–q29. The ROH region, about 7.7 Mb on the chromosome 3:190172058-197851260 partially overlapped with the ROH region of SPG14 previously reported. Subsequently, microsatellite analysis confirmed this ROH and whole-exome sequencing was carried out while no causative mutations were found in the exons of known HSP genes and 68 candidate genes in that region. In conclusion, our data suggest the ROH in this region may play a pivotal role in SPG14 pathogenesis. This is the first clinical description of a pure form spastic paraplegia in a non-consanguineous family associated with the SPG14 locus.

1. Introduction

Hereditary spastic paraplegia (HSP) is one of the most clinically and genetically heterogeneous groups of inherited neurodegenerative disorders mainly characterized by slowly progressive lower-limb spasticity which worsens over time. The neuropathologic finding is the result of the long motor axons of the corticospinal-tract degeneration [1–3]. The modes of inheritance include autosomal dominant (AD-HSP), autosomal recessive (AR-HSP), X-linked or mitochondrial trait [4,5]. Clinically, HSPs are classified as pure and complicated forms. The complicated HSPs are combined with further neurological and systemic abnormalities, whereas the pure forms essentially exhibited lower limb weakness and spasticity. To date, more than 70 different loci have been mapped and 55 spastic paraplegia genes (SPGs) were identified. The corresponding proteins of SPGs play key roles in the regulation of intracellular membrane trafficking, endoplasmic reticulum membrane shaping, DNA repair, autophagy, mitochondrial function, lipid metabolism and myelin formation [6–8].

SPG14 is an autosomal recessive form of HSP which has been mapped to a candidate disease locus on chromosome 3q27–q28 between markers D3S1580 (chr3:188542793–188543136) and D3S3669 (chr3:192501965–192502314) [9]. By now only a single consanguineous Italian family with three affected subjects presenting a complex phenotype including mild mental retardation and mild distal motor neuropathy has been described.

Herein, we reported two siblings born in a non-consanguineous Chinese family with spastic paraplegia. The clinical manifestations of our patients present a pure form of HSP. SNP microarray confirmed an ROH on chromosome 3q28–q29 of the two affected siblings that partially coincided with the region of SPG14 in the previous report.

2. Patients and methods

2.1. Patients

The present study involved two siblings from a family with pure spastic paraplegia. The family pedigree is shown in Fig. 1. Their parents are non-consanguineous marriage, and their father died of cirrhosis

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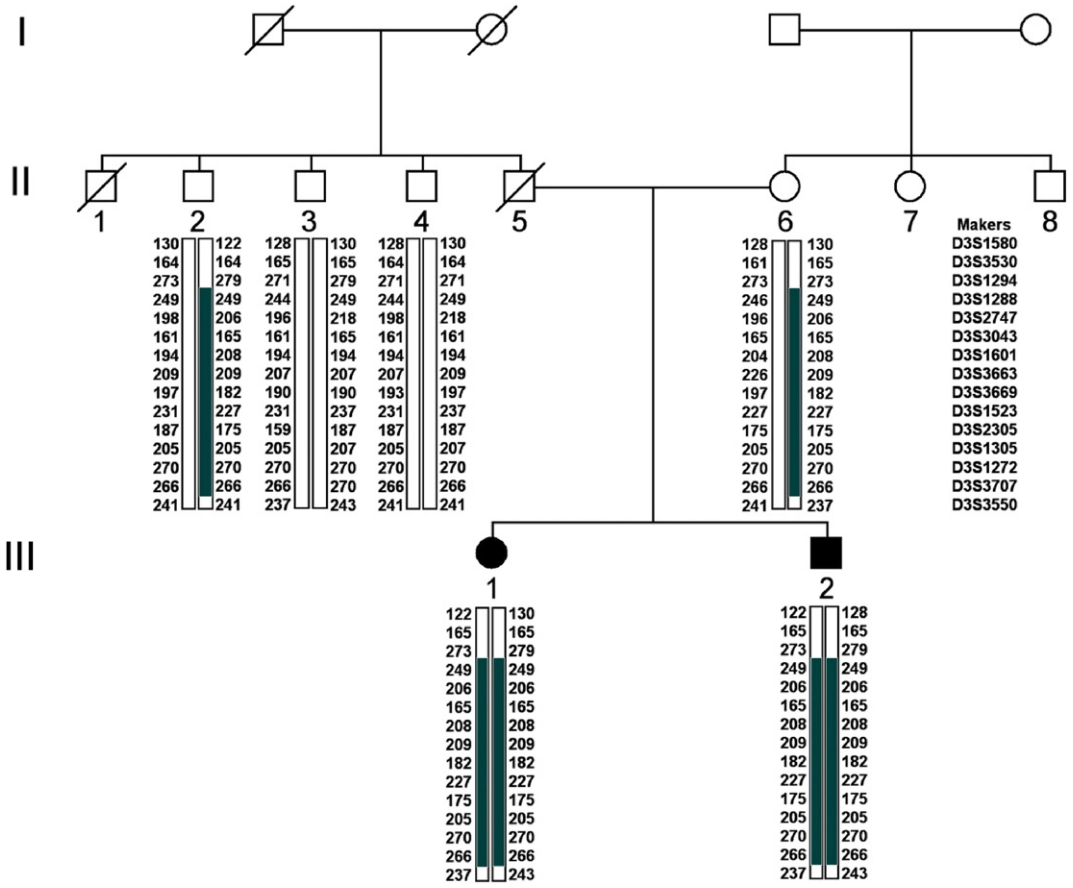

Fig. 1. Pedigree of the AR-HSP family. The haplotypes are composed of fifteen microsatellite markers from chromosome 3q28–3q29. Long green bars indicate the homozygosity region that is shared by the two patients (between D3S1288 to D3S3707).

7 years ago. Two affected (III-1 and III-2) and six unaffected members (II-2–4 and II-6–8) were examined by two experienced neurologists (Dr. Q-L Song and Dr. J Su). Additionally, the affected individuals took the examination of magnetic resonance imaging (MRI) and electroencephalogram (EEG) analysis.

2.2. Homozygosity mapping

Total genomic DNA of the two siblings and their mother were isolated and purified from peripheral blood using the QIAamp DNA blood mini kit (Qiagen, Valencia, CA, USA). Then, the DNA samples were analyzed by CytoScan HD arrays according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). Briefly, 250 ng DNA was digested with NspI and ligated to adapters for subsequent PCR amplification. The amplification products were purified using purification beads, fragmented, labeled, and hybridized for 16–18 h in the GeneChip Hybridization Oven, The arrays were then washed with a GeneChip Fluidics Station 450, and scanned with a GeneChip Scanner 3000 7G (Affymetrix). Copy number and ROH analysis were performed using the Affymetrix Chromosome Analysis Suite software.

Fifteen microsatellite markers on 3q28–q29 included D3S1580, D3S3530, D3S1294, D3S1288, D3S2747, D3S3043, D3S1601, D3S3663, D3S3669, D3S1523, D3S2305, D3S1305, D3S1272, D3S3707 and D3S3550, nine of which were consistent with G. Vazza et al. [9] previously reported. Genomic DNA of the probands, their mother and uncles were extracted and used for amplifications. Fragments were analyzed using an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Forster City, CA, USA) with the denaturing POP7 polymer. Electropherograms were analyzed using GeneMapper software.

2.3. Exome sequencing

Paired-end sequencing was performed on Illumina GAIIx/HiSeq 2000 instruments (Illumina, San Diego, CA, USA) available at Shanghai Biotechnology Co., Ltd. Exon capture was conducted using Agilent Sure Select Technology (Agilent, Santa Clara, CA, USA). For sequence alignment, variant calling and annotation, the sequences were mapped to their location with the human genome reference sequence (hg19 build) using a Burrows-Wheeler Aligner. Local realignment of the potential insertion/deletion sites was carried out with a Genome analysis tool (GATK). SNP and indel variants were performed against reference dbSNP 138. All variants were annotated with reference to consensus coding sequences (CCDS) (NCBI release 20090902) and RefSeq (UCSC dumped 20101004). The novel variants were checked with the Integrative Genomics Viewer (IGV), and then further investigated in the family and normal Chinese population.

3. Results

3.1. Clinical data

The patients are two siblings from a Han Chinese family. The elder sister (III-1) is currently 18 years old. She was born at term with an uneventful delivery. She experienced progressive weakness of the legs and tumbled several times since nine. Over the next few years, gait impairment progressed continuously; foot drop and foot inversion were present in her right side. At the time of admission to our hospital, she was bedridden with very little mobility in her legs. The younger brother (III-2) is currently ten years old. He was noticed slowly progressive weakness of legs and gait disturbance at 7 years old. With the passage