Clinical research

Exome sequencing released a case of X-linked adrenoleukodystrophy mimicking recessive hereditary spastic paraplegia

Zi-xiong Zhan a, Xin-xin Liao a, Juan Du a,b, Ying-ying Luo a, Zhao-ting Hu a, Jun-ling Wang a,b, Xin-xiang Yan a,b, Jian-guo Zhang c, Mei-zhi Dai c, Peng Zhang c, Kun Xia d, Bei-sha Tang a,b,d, Lu Shen a,b,*

a Department of Neurology, Xiangya Hospital, Central South University, Changsha 410008, China
b Neurodegenerative Disorders Research Center, Central South University, Changsha 410008, China
c BGI-Shenzhen, Shenzhen, Guangdong Province 518083, China
d State Key Laboratory of Medical Genetics, Central South University, Changsha 410008, China

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A B S T R A C T

Genetic heterogeneity is common in many Mendelian disorders such as hereditary spastic paraplegia (HSP), which makes the genetic diagnosis more complicated. The goal of this study was to investigate a Chinese family with recessive hereditary spastic paraplegia, of which causative mutations could not be identified using the conventional PCR-based direct sequencing. Next-generation sequencing of all the transcripts of whole genome exome, after on-array hybrid capture, was performed on two affected male subjects (the proband and his brother). A missense mutation (c.1661G>A, p.R554H) was identified in ABCD1. Subsequently, PCR-based direct sequencing of other family members revealed that the mutation was co-segregating with the disease, indicating that ABCD1 mutation was the pathogenic event for this family. Very long-chain fatty acids (VLCFA) assay in the two affected cases confirmed X-ALD. Our study suggests exome sequencing can be used not only to find a novel causative gene, but also to quickly identify mutations of known genes when the clinical elements are etiologically misleading.

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1. Introduction

Genetic heterogeneity is one of the problems in genetic diagnosis. In order to make a molecular diagnosis of a disease, candidate genes have to be targeted step by step by Sanger sequencing. This strategy is not only time-consuming, but also costly. In the worst case, none responsible variant is found even though all candidate genes have been sequenced. This situation may be solved by whole-exome sequencing (WES). WES is able to re-sequence the whole human genomic exons within days and with unprecedented accuracy[1]. Recently, WES has been demonstrated to be effective in identifying rare Mendelian disorders in clinical practice[2].

Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of disorders characterized by a slowly progressive spastic paraplegia. Complicated and pure HSP are distinguished by the presence of associated clinical features. To date, more than thirty HSP genes and twenty additional loci have been identified.

X-linked adrenoleukodystrophy (X-ALD) is caused by a defect in the gene ABCD1 which codes for a peroxisomal membrane protein[3]. The alternations in the gene result in defective peroxisomal β-oxidation and the accumulation of VLCFAs in all tissues[3]. ABCD1 mutations have variable penetrance in males, and several clinical presentations are distinguished: childhood and adolescent cerebral ALD, adrenomyeloneuropathy (AMN), olio-pontocerebellar form, adult cerebral ALD, Addison-only form, and asymptomatic[4]; female heterozygotes may exhibit AMN. Mild phenotype may progress to a serious one in the duration of the disease, and phenotypes can vary within a family[5,6]. AMN, the second most common subtype, represents 27% of cases in Spanish[7], 40.6% in France, 32% in American, 25.3% in Japanese[8], but only 7.9% in Chinese[9]. The main complaint of AMN being slowly progressive spastic gait similar to HSP, misdiagnose is possible when the apparent inheritance pattern do not fit X linked inheritance[10], indicating that AMN might be rare or misdiagnosed in China. Inheritance pattern of X-ALD is X linked, but variable penetrance in male and female may result in pseudo-AD or AR patterns.
We report here how WES allowed to rescue a wrong diagnosis of recessive “pure” HSP in two sibs erroneously diagnosed as pure recessive HSP.

2. Subjects and methods

2.1. A Chinese family presenting as recessive hereditary spastic paraplegia

This Chinese family had 11 members over three generations including two affected males (Fig. 1A). The perinatal course and early development of both patients were uneventful, and walking appeared at around one year of age. The proband (III:1) was a 41-year-old Han male, who came to our clinic with a chief complaint of abnormal gait. He had suffered from progressive gait unsteadiness since age 26, and the symptom became evident in last few years. He experienced progressive urinary dysfunction and mild hypesthesia in his distal legs ten years after onset. He also noticed a slight decrease in his auditory discrimination nearly 2 years ago. The disease progressed slowly and the symptoms were not severe enough to require a walking aid, but he could no longer run or walk long distance at age 41. The upper limbs were intact, and no skin or mucous membrane hyperpigmentation, cognitive impairment, febrile seizures, muscle atrophy or extrapyramidal disturbances were observed.

Neurological examination at age 41 revealed spastic paresis of the lower extremities and talipes cavus deformities. Proximal muscle strength of bilateral lower limbs was mildly decreased. Vibratory sensation, light touch, pain, temperature and position sense were normal. He had hyperreflexia in his four limbs, and bilateral ankle clonuses were elicited. Hoffmann and Babinski signs were present bilaterally. His gait was wide-based and ataxic, and he had trouble standing with both feet together. He had truncal ataxia, but there was no difficulty in accomplishing finger-to-nose test and heel-to-knee test. The rest of the neurological examination was normal. The protocol used in our department for investigating chronic myelopathies was applied. The results of routine screening tests including complete blood count, blood biochemical indices, the level of serum copper, ceruloplasmin, vitamin B12 and folate were within normal ranges. Laboratory examinations showed normal levels of urine 17-hydroxyl corticosteroids and 17-ketone steroids, and plasma cortisol and adrenocorticotropic hormone (ACTH). Cerebral and whole spine magnetic resonance imaging were normal. Nerve conduction studies (motor, sensory) of the median, ulnar, radial, peroneal and tibial nerves were within normal limits. Needle electromyography (EMG) revealed positive sharp waves, as well as fibrillations and high amplitude polyphasic potentials with an incomplete recruitment pattern in muscles, suggesting a neurogenic damage. Auditory brain stem response (ABR) showed a moderate conduction impairment of bilateral brainstem hearing paths and auditory nerves.

His brother (III:2) was a 40 year-old man with almost identical clinical course and EMG findings as him. His grandparents (I:1, I:2) died at seventies without any symptoms of movement disorder. Physical examination was carried out among all of the other family members and the results turned out to be normal. Informed consent was obtained from all of the patient’s family members in accordance with human study protocols.

According to the Harding criteria[11], a clinical diagnosis of recessive HSP was suggested by by two neurologists for this family. Hence, the complete coding region (both sense and antisense chains) of SPG11, SPG15, SPG4, SPG7 and SPG2 were sequenced[12–16]. However no responsible variant was found.

2.2. Whole-exome sequencing

We performed WES on the two affected individuals (III:1 and III:2) using the SureSelect Human All Exon Kit (Agilent). This kit targets approximately 38 Mb of the genome in a single tube, covering all RefSeq and Ensembl genes and genes in the 2008 Consensus Coding Sequence Region database. Captured exons were then submitted to pair end sequencing on a HiSeq 2000 platform, and reads were aligned to the human genome reference (UCSC hg 18 version) using SOAPaligner[17]. We estimated the consensus genotype and quality with SOAPsnp (v1.03)[18]. For insertions or deletions (insdel) in the targeted exome regions, we aligned the reads to the reference genome using Burrows-Wheeler Aligner (BWA – http://bio-bwa.sourceforge.net/) and then identified the breakpoints by Genome Analysis Toolkit (GATK – http://www.broadinstitute.org/gatk/). Finally, we annotated the genotypes of insertions and deletions[19].

2.3. Capillary sequencing

Possible pathological variants were confirmed by Sanger sequencing using ABI 3730 DNA sequencer (PE Applied...

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Fig. 1. Partial pedigree and causative sequence of a Chinese family presented as “recessive HSP”. Note: Partial pedigree (A). All sampled subjects were identified by their Roman numerals below the symbol. Arabic numerals denoted each individual in a generation. Open symbols – unaffected; filled symbols – affected; symbols with a diagonal line – deceased subjects; symbols with a point – carrier; squares – male; circles – female; arrow – the proband; minus – the wild-type allele; plus – the mutation allele, c.1661G→A, p.R554H in exon 7 of ABCD1. Electropherograms of a normal control, a carrier (mother) and the affected proband (B).
Biosystems), following the manufacturer’s protocol. Sequencing data was analyzed by DNASTar software (DNASTAR, Inc. Madison, Wisconsin, USA).

2.4. Very long chain fatty acids (VLCFAs) assay

Ten milliliter of whole EDTA fasting blood of the two affected cases was extracted respectively. VLCFAs in the plasma of each case was analyzed by the method previous reported [20] by Micromole Lab (Beijing, China).

3. Results

3.1. Exome sequencing and bioinformatics analysis identified a candidate gene

Exome was sequenced in the two siblings. An average of 6.5 gigabases (Gb) of sequence was generated per affected individual as single-end, 76 bp reads. 4.9 billion bases (76.05%) passed the quality assessment and aligned to the human reference sequence. 52.5% of the total bases mapped to the targeted bases with a mean coverage of ~90 folds (Fig. 1A and B). At this depth of coverage, about 95.45% of targeted bases were sufficiently covered to pass our thresholds for variant calling. Using de novo assembly of exon sequences, we also detected 1798 indels in sample III:1 and 1754 indels in sample III:2. To distinguish potentially pathogenic mutations from other variants, we focused only on nonsynonymous (NS) variants, splice acceptor and donor site mutations (SS) and coding indels (I), anticipating that synonymous variants were far less likely to be pathogenic. Given that this is a rare disorder, it is likely that causative variants will be novel. A novel variant was defined as one that did not exist in the datasets used for comparison, including dbSNP129, eight previously exome-sequenced HapMap samples (HapMap 8), and the SNP release of the 1000 Genome Project (20100208 release). After filtering, the candidate gene pool was reduced by ~20-fold compared to the whole NS/SS/Indel variant set. Considering that most pathogenic variants either affected highly conserved sequences and/or were predicted to be deleterious, we used SIFT to assess the non-synonymous variants for a likely functional impact[21]. This further reduced the candidate gene pool by ~30-fold compared to the whole NS/SS/Indel variants. As a result of the filtering steps above, the candidate pool was reduced to 17 genes (Fig. 1C). Then the function of these genes was reviewed by the internet resource of OMIM and AceView. Finally, ABCD1, the causative gene for X-ALD, which contained a mutation of c.1661G>A, p.R554H in exon 7, was suggested to be the causative gene of the family.

3.2. Variants of the ABCD1 gene

To identify the variant c.1661G>A as the causative mutation for the kindred, specific primers were designed and directly DNA sequencing were performed among the genomic DNA of all the family members. Segregation analysis proved that the variants were present and segregated with the phenotype (Fig. 2B).

3.3. Very long-chain fatty acids (VLCFA) assay

Measurement of VLCFA in the plasma of both brothers revealed that the values of C24:0, C26:0, C24/C22 and C26/C22 were all at higher-level when compared with normal, which was consistent with the biochemical defect of X-ALD (Table 1).

4. Discussion

The two affected siblings of our HSP family had suffered from slowly progressive spastic gait since their 20s, and physical examination revealed spinal cord involvement, normal MRI imaging and absence of endocrine dysfunction. According to the Harding criteria [11], the two patients were considered as “pure” form HSP (despite progressive hearing loss in the proband). The protocol used in our department for the diagnosis of chronic myelopathy includes several biochemical assays, but VLCFA was not considered for this adult onset case with pure spasticity and without leukodystrophic...
changes on brain MRI. Common genes for recessive HSP were directly sequenced but no causal mutation was found. Subsequently, WES uncovered the mutation c.1661G>A, p.R554H in ABCD1 in both sibs. This variant was confirmed by Sanger sequencing. VLCFA assay supported the diagnosis of X-ALD. This variant was confirmed by Sanger sequencing.

**Conflict of interest**

The authors have no current or potential conflicts of interest to report.

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