ORIGINAL ARTICLE

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Diagnosis of Alexander disease in a Japanese patient by molecular genetic analysis

Received: June 4, 2001 / Accepted: July 13, 2001

Abstract Alexander disease is a leukodystrophy that is neuropathologically characterized by the presence of numerous Rosenthal fibers in astrocytes. Recently, mutations in the gene encoding glial fibrillary acidic protein (GFAP) were identified in patients with Alexander disease. We sequenced the *GFAP* gene of a Japanese girl who presented with typical symptoms of Alexander disease but in whom the diagnosis was not proven by histopathology. We identified a missense mutation, R239C, which is identical to the mutation previously reported to be most frequent. As was the case in previously described patients, our patient was also heterozygous for the de novo mutation. Interestingly, despite the fact that this is a de novo mutation, R239C was found to be common in different ethnic groups, implying that the site is a “hot spot” for mutagenesis. Molecular genetic analysis now makes the antemortem diagnosis of Alexander disease possible.

Key words Alexander disease · GFAP · Mutation · Antemortem diagnosis · Japanese

Introduction

Alexander disease is a rare type of leukodystrophy characterized clinically by macrocephaly, psychomotor retardation, spasticity, and seizures, and histopathologically by the

presence of numerous Rosenthal fibers (RF), which are cytoplasmic inclusion bodies in astrocytes (Russo et al. 1976; Borrett and Becker 1985; Arend et al. 1991; Pridmore et al. 1993; Springer et al. 2000). It has been known that glial fibrillary acidic protein (GFAP), one of the intermediate-filament proteins, exists in RF associated with α B-crystallin and a small heat-shock protein, HSP27 (Iwaki et al. 1989; Head et al. 1993), and that RF is generated in situations of GFAP excess, such as chronic marked gliosis in humans or in transgenic mice that overexpress wild-type Gfap (Messing et al. 1998). The gene and cDNA encoding human GFAP was cloned and the coding region was 1299bp (Brenner et al. 1990). Recently, six mutations were reported in the *GFAP* gene of patients with Alexander disease, and all mutations were missense mutations involving arginines. All patients were heterozygous; the parents of these patients that were tested were all negative for the respective mutation, suggesting that these mutations arose de novo, and that the inheritance of the disease is autosomal dominant (Brenner et al. 2001). Based on this report, we sequenced the *GFAP* gene of a Japanese patient who had typical symptoms and magnetic resonance imaging (MRI) findings of Alexander disease.

Subject and methods

Patient

A 1-year, 4-month-old girl was admitted to our hospital in August 2000 for investigation of seizure and psychomotor regression. She is the first child of healthy, nonconsanguineous parents and had been born at 40 weeks gestation without prenatal or perinatal difficulties. Birth weight was 3656g, length was 51cm (+1.1SD) and head circumference was 36cm (+2.2SD). Early developmental milestones were normal, including rolling over, sitting alone, standing with help, and speaking a word. At the age of 1 year, she had the first febrile seizure, and after 2 weeks

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Fig. 1. **a** Axial computed tomographic image shows symmetrical abnormal low density in the white matter of the frontal lobes. There is a high-density area in the white matter adjacent to the frontal horns. **b, c** Axial T1- and T2-weighted spin echo images, respectively, show T1 and T2 prolongation of bilateral frontal lobes including arcuate fibers. Also seen are high-intensity signals in the white matter adjacent to the frontal horns, putamen, and thalamic nuclei on a T2-weighted image

she had status epilepticus with fever. Thereafter, she lost the ability to stand with help and to speak words.

Physical examination showed a height of 79 cm (+0.4SD), a weight of 9.7 kg (+0.1SD), and a head circumference of 49 cm (+2.3SD). No skin lesions or hepatosplenomegaly were noted. She was alert and had a good social response. The cranial nerves were intact. Funduscopic examination findings were normal. She could sit without help, but muscle hypotonia was present. She had brisk deep tendon reflexes. Planter responses were bilaterally extensor. No involuntary movement, cerebellar signs, or abnormal sensation were observed.

A plane computed tomographic scan on admission showed symmetrical low density in the frontal white matter with high-density areas adjacent to the anterior horns of lateral ventricles (Fig. 1a). MRI revealed a bilateral frontal low-intensity signal and a high-intensity signal in the white matter adjacent to the frontal horns, with contrast enhancement in T1-weighted (T1W) images. T2W images showed a high-intensity signal in the bilateral frontal white matter that included arcuate fibers, putamen, and thalamic nuclei (Fig. 1b,c). Proton magnetic resonance spectroscopy of frontal white matter showed a low N-acetyl-aspartate/creatine ratio (0.90).

The following laboratory investigations were normal: complete blood count; routine blood chemistry; serum lactate, pyruvate, and amino acids; urinalysis; urinary amino and organic acids; examination of cerebrospinal fluid (CSF) including cell count, protein, glucose, lactate, pyruvate, and myelin basic protein; and enzyme activities of arylsulfatase A, β -galactosidase, and hexosaminidase A in white blood cells. α B-crystallin and HSP28 in the CSF were not elevated (α B-crystallin <0.1 ng/ml; HSP28 2.37 ng/ml; normal 0.2–3.0 ng/ml). Electroencephalograms showed some spikes over the left frontal area. Brainstem auditory evoked potentials revealed an increase in the I–V interval, and the absolute latencies of waves III, IV, and V were prolonged. Somatosensory evoked potentials showed a prolonged central conduction time between cervical cord and cortex. Motor nerve conduction velocity was normal. Informed consent for this study was obtained from the parents of the patient.

PCR amplification of genomic DNA and sequencing

Genomic DNA was isolated from peripheral leukocytes from the patient, her parents, and a control individual. Primers for polymerase chain reaction (PCR) were designed in introns or untranslated regions of the *GFAP* gene to amplify exonic fragments containing the entire coding region. Sequences of primers and PCR conditions used in this study are available on request. The PCR products were electrophoresed through 1.5% agarose gels, purified by GeneElute Agarose Spin Column (Sigma, St Louis, MO, USA) or QIAquick PCR Purification kit (Qiagen, Hilden, Germany), and directly sequenced using the Dye Terminator Cycle Sequencing FS Ready Reaction kit (PE Biosystems, Foster City, CA, USA) and ABI Prism 310 Genetic Analyzer (PE Biosystems), according to manufacturer's instructions.

Digestion of PCR fragment with *Hpy*CH4V

To simplify the method of detection of the missense mutation (279C $\rightarrow$ T), a PCR fragment containing exon 4 was digested with the restriction endonuclease *Hpy*CH4V (New England Biolabs, Beverly, MA, USA). In the presence of the mutation, the PCR product was cleaved by *Hpy*CH4V at two sites, whereas the wild-type PCR product was cleaved at one site (Fig. 2a). Restriction digests were analyzed in 3% NuSieve agarose (BMA, Rockland, ME, USA). A DNA size marker 100-bp ladder was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England).

Results and discussion

None of the PCR fragments of the patient were different in size from the corresponding fragments of a control. Direct sequencing of all PCR fragments revealed a C-to-T transition at nucleotide 729 (279C $\rightarrow$ T) in exon 4 (Fig. 2c), resulting in the substitution of an arginine for a cysteine at codon 239 (R239C). The nucleotide change was detected in

Fig. 2. **a** Schematic representation of the wild-type and mutant polymerase chain reaction (PCR) fragments digested with *Hpy*CH4V. Numbers indicate length (in base pairs) of segments cleaved by *Hpy*CH4V. **b** Pedigree of the patient and an electrophoresis on 3% NuSieve agarose of PCR products digested with *Hpy*CH4V. **c** Sequence of the PCR fragment including exon 4 of the patient, showing heterozygosity of a C-to-T transition (arrow)

both strands. No other nucleotide difference from the reported sequence or the sequence of our control was observed. PCR-restriction fragment length polymorphism using the restriction endonuclease *Hpy*CH4V confirmed this nucleotide change, and showed that the patient was heterozygous for 279C → T and that neither parent had this mutation (Fig. 2b), suggesting that the mutation was de novo. This mutation was identical to the most frequent mutation reported in American patients (Brenner et al. 2001). As was the case for the previously described patients (Brenner et al. 2001), our patient was heterozygous for this de novo mutation. Interestingly, despite the fact that this is a de novo mutation, R239C appears to be common in different ethnic groups, implying that this site is a “hot spot” for mutagenesis.

Our patient had typical manifestations (macrocephaly, psychomotor retardation, spasticity and seizures) and radiological findings of Alexander disease, i.e., a dominant

low-intense signal in the frontal lobe, both in the deep white matter and the arcuate fibers, and a high-intensity signal in the white matter adjacent to the frontal horns, with contrast-enhancement in T1W (Russo et al. 1976; Arend et al. 1991; Pridmore et al. 1993; Springer et al. 2000). Although the clinical diagnosis of Alexander disease might not be difficult in this particular case, definitive diagnosis of Alexander disease usually requires histological findings in brain tissue. Before the recent report that showed mutations in the *GFAP* gene associated with Alexander disease (Brenner et al. 2001), the presence of numerous RF was necessary for a definitive diagnosis of this disorder. Although it is not yet clear how mutations in the *GFAP* gene lead to the formation of RF, mutations may affect the degradation or solubility of GFAP, and, as a result, GFAP may form RF by interacting with α B-crystallin and small heat-shock proteins (Iwaki et al. 1989; Johnson and Bettica 1989; Head et al. 1993), as was shown in transgenic animals overexpressing wild-type Gfap (Messing et al. 1998).

In our case, the identification of the R239C mutation strongly supported the diagnosis of Alexander disease without histopathological findings in the brain tissue. Although reports of additional cases may be necessary to confirm the diagnostic value of molecular genetic analysis, we are convinced that this will be an important method for the antemortem diagnosis of Alexander disease. This will be especially relevant should therapy become available, in which case accurate antemortem diagnosis using molecular genetic analysis will be crucial.

Acknowledgments We thank Dr. Toyokazu Saito (Kitasato University) for his support, Dr. Nobuhiko Ochi (2nd Aoitōri-Gakuen) for measuring α B-crystallin and HSP28, Ms. Kazuko Endate for technical assistance, and Dr. Sara Shanske (Columbia University) for reviewing the manuscript.

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