Introduction

Alexander disease [1] (MIM 203450) is a rare neurological disease, distinguished in three subtypes according to the age of onset: infantile (before 2 years of age), juvenile (until the middle teens), and adult [2, 3, 15, 21]. The infantile form, the most common subtype, is usually fatal within the first decade and megalencephaly, seizures, psychomotor retardation, and progressive spastic quadriparesis are typical. MRI of the brain shows signal abnormalities in the cerebral white matter, mainly in frontal regions [29]. Patients with the juvenile form of Alexander disease usually have slower progression with bulbar or pseudo-bulbar palsy, ataxia and spasticity. They often do not have megalencephaly, are mentally in-
tact, and show less white matter involvement at MRI compared to infantile forms. The adult cases are the least common, can be distinguished in familial [4, 11, 15, 17, 18, 23, 25, 27, 28] or sporadic [2, 9, 14, 15, 22, 26, 32] forms, and display the most variable phenotype.

A common neuropathological feature of all forms of Alexander disease is the diffuse presence of Rosenthal fibers [20], intracytoplasmic aggregates within astrocytes containing glial fibrillary acidic protein (GFAP), the principal intermediate filament of astrocytes [6, 10, 13].

Heterozygous missense mutations of the GFAP gene (MIM 137780) have been identified in neuropathologically proven infantile- and juvenile-onset Alexander disease patients [3], and thereafter confirmed in the adult forms (for a complete list of sequence variants, see http://www.waisman.wisc.edu/alexander/index.html).

Here we describe multiple family members with adult Alexander disease, who show variable clinical and radiological features and are all carriers of a novel GFAP complex molecular defect composed of two mutations in one allele.

**Methods**

**Clinical study**

Detailed medical history and neurological examinations were performed on four members of an Italian family with features of adult Alexander disease.

Family pedigree (Fig. 1) was drawn during interviews with the family members.

Four members agreed to enter the study. Following approval by the local Medical Ethical Committee, all of them gave written consent to the investigations.

**Neuroimaging**

All patients underwent one or more brain MR examinations. In two of them, a paramagnetic contrast medium was injected. MRI results were scored according to the diagnostic criteria proposed by van der Knaap et al. [29].

**Molecular study**

DNA samples from each of the 4 family members under study, prepared according to standard extraction methods, were amplified with specific GFAP primers, designed on genomic regions flanking each of the 9 exons. Oligonucleotide sequences and PCR conditions used have been reported, along with details about the subsequent direct DNA sequencing of the amplification products thus obtained [5].

DNA from 100 unrelated healthy individuals (200 control chromosomes) were checked to confirm that the novel mutations found in the present patients were not representing polymorphisms of the Italian population.

**Results**

**Case reports**

Clinical data are shown in Table 1. Laboratory studies in subject II.4 (proband) revealed normal values of blood and urine chemistry, ceruloplasmine and copper serum levels, thyroid hormones, and VDRL, TPHA, HIV tests. In the same subject the tilt table test and the RR variability tests exhibited mild abnormalities, while polysomnography was normal.

A brain MRI (Fig. 2), completed with the administration of a contrast medium together with the study of the spinal cord, disclosed atrophy of the lower brainstem and upper cervical cord, with light focal point-like hyperintensities in the medulla and along the lateral aspect of both putamina on T2-weighted images. The enhanced structures detected were the inferior olives. The spinal cord showed a reduction of volume at the level of C1 and C2.

In subject III.5, the proband’s son, a brain MRI (Fig. 3) disclosed nodular hyperintensities on T2-weighted images in medulla, pons, and medium cerebellar peduncles bilaterally. These lesions extended to the