

A Novel *GFAP* Mutation and Disseminated White Matter Lesions: Adult Alexander Disease?

Knut Brockmann^a Moritz Meins^b Angelika Taubert^c Ralf Trappe^b
Martin Grond^c Folker Hanefeld^a

Departments of ^aPediatrics and Neuropediatrics and ^bHuman Genetics, University of Göttingen, Göttingen, and
^cDepartment of Neurology, Community Hospital, Siegen, Germany

Key Words

Alexander disease · Glial fibrillary acidic protein · Adult onset · Multiple sclerosis

Abstract

The recent discovery of heterozygous de novo mutations in the glial fibrillary acidic protein (GFAP) gene as the cause of infantile and juvenile Alexander disease has shed new light on the long-standing debate whether the adult subtype has the same etiology as infantile and juvenile Alexander disease. A 40-year-old man presented with subacute left hemiplegia and ataxia. Cranial MRI revealed disseminated patchy white matter changes involving the corpus callosum, basal ganglia and brainstem. CSF investigation demonstrated elevated total protein but was otherwise normal. Mutation analysis of the *GFAP* gene was performed in the patient, his mother and healthy brother. A novel heterozygous mutation in exon 4, 681G→C, predicting an amino acid substitution E223Q in the rod region of *GFAP* was detected in the patient and his mother but not in his healthy brother or 150 control chromosomes. We conclude that the patient

is actually afflicted with Alexander disease. Mutation analysis of *GFAP* should be considered in patients with remitting neurological deficits, disseminated white matter lesions and absence of inflammatory CSF changes.

Copyright © 2003 S. Karger AG, Basel

Introduction

Alexander disease, originally described in 1949 [1], is a rare, fatal disease of the central nervous system due to a dysfunction of astrocytes [2]. Three major subtypes have been delineated based on the age of onset. The most common infantile form presents as a megalencephalic leukoencephalopathy with rapid progression usually leading to death within few years [3]. The juvenile type is characterized by a slowly progressive brainstem dysfunction [4]. Adult-onset Alexander disease has been reported rarely and with the most variable phenotypes. Several familial cases exhibited palatal myoclonus, cerebellar ataxia and ocular motor abnormalities [5, 6]. In some patients, clinical features mimicked the juvenile subtype, though with a later onset and a more protracted course. Others presented with clinical symptoms resembling multiple sclerosis [7, 8].

K.B. and M.M. contributed equally to this work.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 S. Karger AG, Basel
0014-3022/03/0502-0100\$19.50/0

Accessible online at:
www.karger.com/ene

Knut Brockmann
Department of Pediatrics and Neuropediatrics, University of Göttingen
Robert-Koch-Strasse 40
DE-37075 Göttingen (Germany)
Tel. +49 551 39 6210, Fax +49 551 39 6252, E-Mail kbrock@med.uni-goettingen.de

Alexander disease is pathologically characterized by demyelination, astrogliosis and the presence of countless Rosenthal fibers throughout the brain, predominantly in subpial, subependymal and perivascular regions. Rosenthal fibers are cytoplasmic inclusions containing glial fibrillary acidic protein (GFAP) and small heat shock proteins [9]. Messing et al. [10] observed inclusion bodies indistinguishable from Rosenthal fibers in fatal *GFAP* transgenic mice, who showed overexpression of human *GFAP* in astrocytes. This finding pointed to *GFAP* as a candidate gene in Alexander disease.

Recently, de novo heterozygous *GFAP* mutations have been detected in infantile cases with a histopathologically verified diagnosis of Alexander disease [11]. A genotype-phenotype correlation has been demonstrated [12]. Mutations were observed predominantly in exons 1 and 4, clustering around 2 hot spots. Subsequently, *GFAP* mutations have been shown in adult members of a family with autosomal dominant palatal myoclonus and spinal cord atrophy [13] and in patients with juvenile onset of disease [14, 15].

Here, we report an adult male with clinical symptoms resembling multiple sclerosis, disseminated white matter lesions on MRI and a novel *GFAP* mutation.

Case Report

A 40-year-old man presented with subacute left hemiplegia. He was the first of 2 sons of nonconsanguineous parents. His father had died from stroke at the age of 68 years. His 65-year-old mother exhibited symptoms of a mild, chronic impairment of intellectual function along with a change of personality, apparent for 3 years. She declined clinical and neuroradiological examination. His younger brother was healthy. The patient's early motor and mental development was normal but slower than in his brother. He had been stuttering since childhood. He attended a regular school but did not pass the final examination. He had no professional training and was occupied as a road laborer. Arterial hypertension and diabetes mellitus had been treated for 10 years. An intermittent abuse of alcohol was reported by his relatives. The left hemiparesis showed an insidious onset and slowly worsened over a period of 5 weeks. A further deterioration led to admission to the hospital.

On neurological examination, he was fully orientated and friendly, though slowed in his performance. He was stuttering, had a spastic and cerebellar dysarthria and showed weakness of the lower face on the left side. Funduscopy revealed retinal signs of hypertension. Cranial nerves were otherwise normal. No palatal myoclonus was observed. There was spastic hemiplegia of the left side with exaggerated reflexes and clonus of the lower limb. Ataxia with unsteadiness of gait was noted. A general physical examination showed no significant abnormalities except for arterial hypertension. Twenty-four-hour ambulatory blood pressure monitoring revealed systolic values up to 200 mm Hg and diastolic values up to 125 mm Hg.

Investigation of CSF disclosed normal values for cell count and glucose. Total protein was increased to 72 mg/dl (normal, 15–45); no oligoclonal bands were detected. The *Treponema pallidum* hemagglutination assay was nonreactive, and antibodies to *Borrelia burgdorferi* were negative.

EEG, visual-evoked responses and brainstem auditory-evoked responses were normal. Nerve conduction velocities revealed a mild demyelinating motor and sensory neuropathy in the lower limbs, associated with his diabetes. Doppler ultrasonography of carotid and vertebral-basilar arteries showed no abnormalities.

Cranial MRI (fig. 1) revealed disseminated patchy T₂-weighted hyperintensities in supratentorial white matter predominantly in periventricular regions as well as in basal ganglia. A large confluent lesion of the corpus callosum and streaky and patchy lesions of the pons were detected. Virchow-Robin spaces were widened in the white matter and basal ganglia, and the inner and outer CSF spaces were moderately enlarged.

Subsequently, the patient's hemiplegia resolved slowly over a period of several months, leaving him with a mild residual weakness. Since then, his motor impairment as well as his incoordination have followed a fluctuating course.

Methods

EDTA blood samples were collected from the patient, his mother and brother after informed consent. No other relatives were available for mutation analysis. DNA was extracted from blood leukocytes by standard methods. Mutation analysis was performed as described earlier [16]. Basically, the complete coding exons of the *GFAP* gene were amplified by polymerase chain reaction (PCR), which was carried out in 50- μ l reactions with 100 ng of patient DNA, 20 pmol of each primer, 25 μ l of HotStar Taq master mix (Qiagen) in water, using the following PCR protocol (95°C for 15 min; 35 cycles at 95°C for 20 s, 56°C for 30 s, 72°C for 45 s, followed by a final elongation at 72°C for 10 min). Amplification products were tested by agarose gel electrophoresis and purified. PCR products were sequenced directly using the ET dye terminator kit and a MegaBace500 sequencer (Amersham-Pharmacia), according to the manufacturers' instructions. After sequence evaluation, the detected mutation was confirmed by restriction digestion of the amplicon with the restriction enzyme *BsgI* (New England Biolabs).

Results

A novel heterozygous missense mutation in exon 4, 681G→C, was detected in the patient (fig. 2). The mutation creates a new recognition site for the restriction enzyme *BsgI*, thus allowing detection of the mutation by restriction analysis of the respective exon. Cosegregation in the family and prevalence within a control cohort were analyzed by restriction digestion with *BsgI*. The identical mutation 681G→C was found in the mother of the index patient but was not observed in his brother or in more than 150 control chromosomes of an ethnically matched

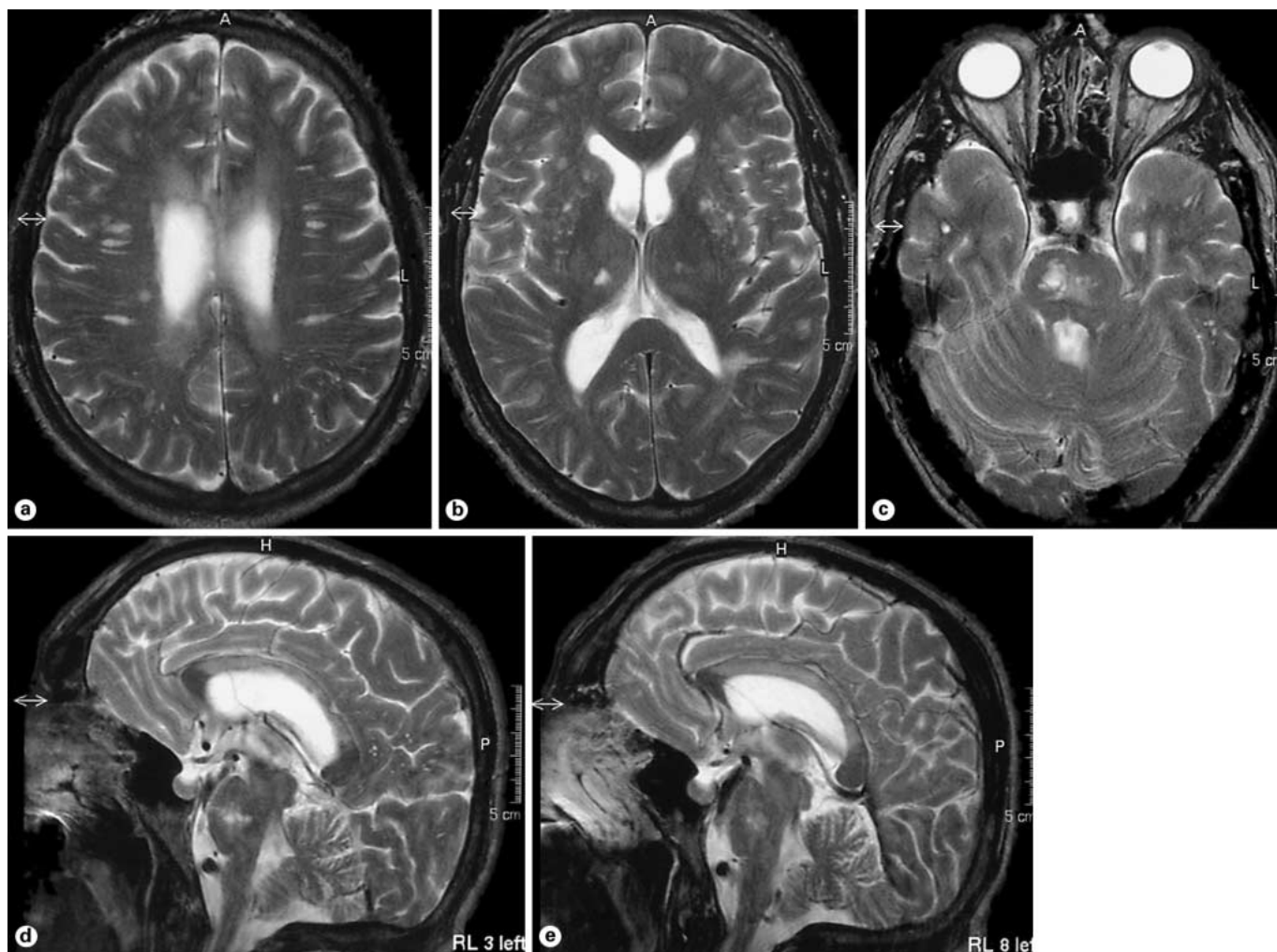


Fig. 1. Axial (**a–c**) and sagittal (**d, e**) T₂-weighted MR images demonstrate disseminated patchy lesions throughout the white matter and basal ganglia along with enlarged Virchow-Robin spaces. The corpus callosum is affected by a large confluent symmetrical lesion (**a, d, e**). Patchy and streaky lesions of the pons are shown in **c, d** and **e**.

control group without neurological diseases (fig. 3). Mutation analysis was not possible in the father, as he had deceased earlier.

Discussion

The recent discovery of mutations in the *GFAP* gene as the cause of infantile and juvenile Alexander disease has shed new light on the long-standing debate whether the presence of excess Rosenthal fibers in adult patients points to an adult subtype of Alexander disease or reflects a nonspecific histopathological pattern [17, 18].

In the patient reported here, the similarities of the clinical course and the brainstem involvement with the cases described by Seil et al. [7] and Spalke and Mennel [8] led to a search for a *GFAP* mutation. A novel heterozygous mutation 681G→C in the *GFAP* gene was found. The same mutation was detected in his mother, who declined a detailed investigation. The comparatively mild clinical course may be adequate for a mutation predicting the exchange of glutamic acid to glutamine (E223Q). Glutamic acid at position 223 is located 16 amino acids from R239 known to be a mutation hot spot in infantile Alexander disease and is perfectly conserved throughout evolution in mammals (human, mouse, rat and cow). Even in

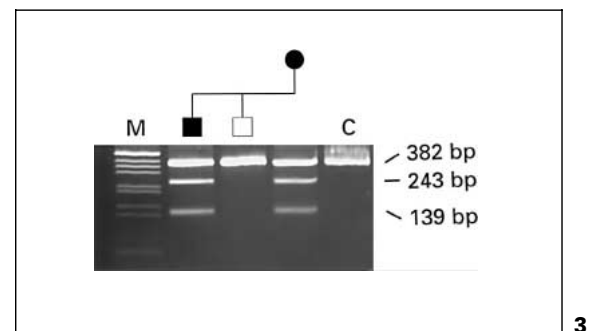
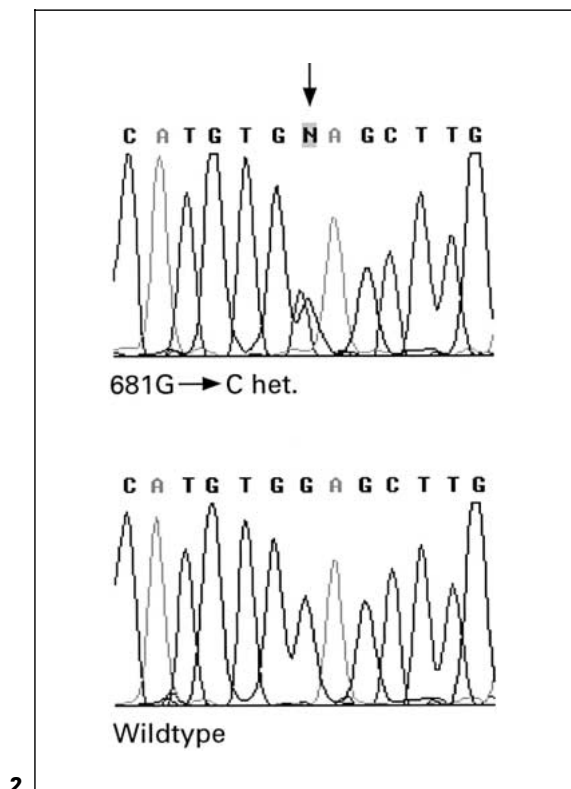


Fig. 2. PCR and direct sequencing of exon 4 for the index patient shows heterozygosity for the mutation 681G→C (upper electropherogram), compared to the normal sequence (wild type, below). Nucleotide numbering refers to the published cDNA sequence, as used by Brenner et al. [11].

Fig. 3. Analysis of exon 4 for the index patient and his family by restriction digestion. The mutation 681G→C creates a new *BsgI* site, 2 fragments of 243 and 139 bp are found instead of the 382-bp fragment. The index patient and his mother carry the mutation (characterized as filled symbols in the overlying pedigree) but not his younger brother (marked with an open symbol). M = Molecular size marker; C = control.

more distant species like the zebrafish and carp, aspartic acid is found in this position, thus preserving acidity and charge at this position. In contrast, the mutation 681G→C leads to replacement of an acidic amino acid by its own neutral amide. Further evidence for the pathogenicity of this mutation derives from the fact that the exchange was neither found in the healthy brother nor in 150 control chromosomes.

To our knowledge, there has been only one report yet of a *GFAP* mutation in a condition compatible with adult Alexander disease. Autosomal dominantly transmitted *GFAP* mutations have been demonstrated in 3 adult members of a family who presented with palatal myoclonus, pyramidal tract signs, cerebellar signs and marked atrophy of the medulla oblongata and spinal cord [13]. These clinical and neuroradiological features were almost identical with those in two families previously reported to have histopathological characteristics of Alexander disease with adult onset [5, 6]. Schwankhaus et al. [6] demonstrated widespread Rosenthal fiber deposition associated with demyelination in 1 adult patient presenting with familial palatal myoclonus, spastic paraparesis and cerebellar ataxia. This female was the daughter of the first

patient reported to have histopathologically proven adult-onset Alexander disease [7]. The 47-year-old man whose symptoms began at the age of 32 had been diagnosed clinically as having multiple sclerosis. Howard et al. [5] distinguished their patients from what had been described as adult Alexander disease, but genetic connection and strong clinical similarities suggest that these patients suffer from the same disease presenting with variable phenotypes within one family.

Only recently has a novel missense mutation of a G-to-T transition at nucleotide 841 in the *GFAP* gene resulting in the substitution of arginine for leucine at amino acid residue 276 (R276L) been detected in 2 brothers with adult-onset spastic paresis without palatal myoclonus and marked atrophy of the medulla oblongata and cervicothoracic cord on MRI. Autopsy in one of these cases showed moderate numbers of Rosenthal fibers in the stratum subcallosum and hippocampal fimbria [19].

Neuroradiological features of our patient comprise (1) disseminated patchy lesions in the white matter and basal ganglia, (2) large confluent lesions of the corpus callosum, (3) patchy and streaky lesions of the pons and (4) enlarged Virchow-Robin spaces in the subcortical white

matter. These findings are ambiguous and may partially relate to the patient's arterial hypertension or, possibly, to his temporary abuse of alcohol.

(1) In a prospective cohort study, white matter lesions in periventricular and subcortical regions have been shown to be associated with hypertension [20]. Sierra et al. [21] found clinically silent white matter lesions in 41% of middle-aged essential hypertensive patients. Focal lesions in basal ganglia, however, correlated with different risk factors including age, a history of ischemic heart disease and carotid artery stenosis [22]. None of these risk factors was present in our patient.

(2) Acquired lesions of the corpus callosum have been reported in a variety of diseases comprising ischemic infarcts, CADASIL, multiple sclerosis, acute disseminated encephalomyelitis and Marchiafava-Bignami disease, but are not amongst the MRI abnormalities detected in patients with arterial hypertension [23]. In our patient, MRI revealed large confluent symmetrical and predominantly centrally located lesions extending from the genu to the splenium. Similar features have been reported in Marchiafava-Bignami disease. Originally described as a histopathological finding in heavy drinkers post mortem, it has later been recognized to occur associated with alcoholism more often and with milder clinical symptoms than initially presumed [24, 25]. Lesions of the corpus callosum are not part of the typical MRI features of Alexander disease. Thus, the callosal lesion in our patient may relate to his alcohol abuse rather than to his *GFAP* mutation.

(3) Pontine lesions detected by MRI in subclinical central pontine myelinolysis have been described as midline, symmetrical and of a triangular to bat wing shape [26]. The pontine lesions found in our patient do not meet these criteria. Pontine ischemic rarefaction is associated with an MRI pattern similar to the features of our case and cannot be ruled out here [27]. However, neuroradiological and neuropathological brainstem abnormalities are highly characteristic of infantile and juvenile Alexander disease [3, 28] as well as of adult cases [6–8].

(4) Enlarged Virchow-Robin spaces present a nonspecific feature and have been observed in association with hypertension, early multiple sclerosis and various other disorders [29].

As most cases of histopathologically proven adult-onset Alexander disease have been reported when MRI was not yet available, the spectrum of MRI abnormalities in the adult form still has to be delineated. In our patient, the clinical and neuroradiological features are complicated by the other risk factors which may contribute to the

white matter lesions. In the presence of hypertension and retinopathy, a cerebrovascular event unrelated with the *GFAP* mutation must be considered. However, the hemiparesis in our patient showed an insidious onset and worsened over a period of several weeks until he was investigated. A cerebrovascular event cannot be ruled out but does not seem to be very likely with this course. On the other hand, a remitting course of histologically proven Alexander disease has been described. The first adult patient with Alexander disease reported [7] was 'a 47-year-old man... who had been diagnosed clinically as multiple sclerosis'. His symptoms began at the age of 32 with 'a paralysis of his left arm', which was 'complete for 5 days, after which he slowly regained function in the arm to the extent that only a mild residual weakness was present'. Later in the course, he had 'intermittent episodes of diplopia' and 'intermittent episodes of a symptom complex of difficulty in walking, impaired position sense of the feet, and feelings of lightheadedness' [7]. Relentless progression of his neurological symptoms followed later.

The MRI pattern in our patient is partially consistent with, but not specific for, hypertensive leukoencephalopathy. The large confluent symmetrical lesions of the corpus callosum present a peculiar finding possibly related to alcohol abuse. However, involvement of the basal ganglia and brainstem is not convincingly explained by these risk factors and strongly points to Alexander disease.

Our observation lends further support to the notion that there is an adult subtype of Alexander disease. The question posed by Messing et al. [30], 'whether adult Alexander disease turns out to have the same etiology as infantile or juvenile Alexander disease' can be affirmed. The clinical and neuroradiological spectrum of adult Alexander disease still needs to be delineated. We recommend to consider mutation analysis of the *GFAP* gene in adult patients with remitting neurological signs and disseminated white matter lesions on MRI involving the basal ganglia and brainstem but a lack of inflammatory CSF abnormalities.

References

- Alexander WS: Progressive fibrinoid degeneration of fibrillary astrocytes associated with mental retardation in a hydrocephalic infant. *Brain* 1949;72:373–381.
- Borrett D, Becker LE: Alexander's disease: A disease of astrocytes. *Brain* 1985;108:367–385.
- Russo LS Jr, Aron A, Anderson PJ: Alexander's disease: A report and reappraisal. *Neurology* 1976;26:607–614.
- Pridmore CL, Baraitser M, Harding B, Boyd SG, Kendall B, Brett EM: Alexander's disease: Clues to diagnosis. *J Child Neurol* 1993;8:134–144.
- Howard RS, Greenwood R, Gawler J, Scaravilli F, Marsden CD, Harding AE: A familial disorder associated with palatal myoclonus, other brainstem signs, tetraparesis, ataxia and Rosenthal fibre formation. *J Neurol Neurosurg Psychiatry* 1993;56:977–981.
- Schwankhaus JD, Parisi JE, Gullledge WR, Chin L, Currier RD: Hereditary adult-onset Alexander's disease with palatal myoclonus, spastic paraparesis, and cerebellar ataxia. *Neurology* 1995;45:2266–2271.
- Seil FJ, Schochet SS Jr, Earle KM: Alexander's disease in an adult: Report of a case. *Arch Neurol* 1968;19:494–502.
- Spalke G, Mennel HD: Alexander's disease in an adult: Clinicopathologic study of a case and review of the literature. *Clin Neuropathol* 1982;1:106–112.
- Head MW, Corbin E, Goldman JE: Overexpression and abnormal modification of the stress proteins alpha B-crystallin and HSP27 in Alexander disease. *Am J Pathol* 1993;143:1743–1753.
- Messing A, Head MW, Galles K, Galbreath EJ, Goldman JE, Brenner M: Fatal encephalopathy with astrocyte inclusions in GFAP transgenic mice. *Am J Pathol* 1998;152:391–398.
- Brenner M, Johnson AB, Boespflug-Tanguy O, Rodriguez D, Goldman JE, Messing A: Mutations in GFAP, encoding glial fibrillary acidic protein, are associated with Alexander disease. *Nat Genet* 2001;27:117–120.
- Rodriguez D, Gauthier F, Bertini E, Bugiani M, Brenner M, N'guyen S, Goizet C, Gelot A, Surtees R, Pedespan JM, Hernandorena X, Troncoso M, Uziel G, Messing A, Ponsot G, Pham-Dinh D, Dautigny A, Boespflug-Tanguy O: Infantile Alexander disease: Spectrum of GFAP mutations and genotype-phenotype correlation. *Am J Hum Genet* 2001;69:1134–1140.
- Okamoto Y, Mitsuyama H, Jonosono M, Hirata K, Arimura K, Osame M, Nakagawa M: Autosomal dominant palatal myoclonus and spinal cord atrophy. *J Neurol Sci* 2002;195:71–76.
- Sawaishi Y, Yano T, Takaku I, Takada G: Juvenile Alexander disease with a novel mutation in glial fibrillary acidic protein gene. *Neurology* 2002;58:1541–1543.
- Gorospa JR, Naidu S, Johnson AB, Puri V, Raymond GV, Jenkins SD, Pedersen RC, Lewis D, Knowles P, Fernandez R, De Vivo D, van der Knaap MS, Messing A, Brenner M, Hoffman EP: Molecular findings in symptomatic and pre-symptomatic Alexander disease patients. *Neurology* 2002;58:1494–1500.
- Meins M, Brockmann K, Yadav S, Haupt M, Sperner J, Stephani U, Hanefeld F: Infantile Alexander disease: A GFAP mutation in monozygotic twins and novel mutations in two other patients. *Neuropediatrics* 2002;33:194–198.
- Herndon RM, Rubinstein LJ, Freeman JM, Mathieson G: Light and electron microscopic observations on Rosenthal fibers in Alexander's disease and in multiple sclerosis. *J Neuropathol Exp Neurol* 1970;29:524–551.
- Riggs JE, Schochet SS Jr, Nelson J: Asymptomatic adult Alexander's disease: Entity or nosological misconception? *Neurology* 1988;38:152–154.
- Namekawa M, Takiyama Y, Aoki Y, Takayashiki N, Sakoe K, Shimazaki H, Taguchi T, Tanaka Y, Nishizawa M, Saito K, Matsubara Y, Nakano I: Identification of *GFAP* gene mutation in hereditary adult-onset Alexander's disease. *Ann Neurol* 2002;52:779–785.
- de Leeuw FE, de Groot JC, Oudkerk M, Witteman JCM, Hofman A, van Gijn J, Breteler MMB: Hypertension and cerebral white matter lesions in a prospective cohort study. *Brain* 2002;125:765–772.
- Sierra C, de la Sierra A, Mercader J, Gomez-Angelats E, Urbano-Marquez A, Coca A: Silent cerebral white matter lesions in middle-aged essential hypertensive patients. *J Hypertens* 2002;20:519–524.
- Uehara T, Tabuchi M, Mori E: Risk factors for silent cerebral infarcts in subcortical white matter and basal ganglia. *Stroke* 1999;30:378–382.
- Friese SA, Bitzer M, Freudenstein D, Voigt K, Küker W: Classification of acquired lesions of the corpus callosum with MRI. *Neuroradiology* 2000;42:795–802.
- Kawamura M, Shiota J, Yagishita T, Hirayama K: Marchiafava-Bignami disease: Computed tomographic scan and magnetic resonance imaging. *Ann Neurol* 1985;18:103–104.
- Ruiz-Martinez J, Martinez Perez-Balsa A, Ruibal M, Urtasun M, Villanua J, Marti Masso JF: Marchiafava-Bignami disease with widespread extracallosal lesions and favourable course. *Neuroradiology* 1999;41:40–43.
- Kleinschmidt-DeMasters BK, Anderson CA, Rubinstein D: Asymptomatic pontine lesions found by magnetic resonance imaging: Are they central pontine myelinolysis? *J Neurol Sci* 1997;149:27–35.
- Pulicino P, Ostrow P, Miller L, Snyder W, Munschauer F: Pontine ischemic rarefaction. *Ann Neurol* 1995;37:460–466.
- van der Knaap MS, Naidu S, Breiter SN, Blaser S, Stroink H, Springer S, Begeer JC, van Coster R, Barth PG, Thomas NH, Valk J, Powers JM: Alexander disease: Diagnosis with MR imaging. *AJNR Am J Neuroradiol* 2001;22:541–552.
- Heier LA, Bauer CJ, Schwartz L, Zimmerman RD, Morgello S, Deck MDF: Large Virchow-Robin spaces: MR-clinical correlation. *AJNR Am J Neuroradiol* 1989;10:929–936.
- Messing A, Goldman JE, Johnson AB, Brenner M: Alexander disease: New insights from genetics. *J Neuropathol Exp Neurol* 2001;60:563–573.

Copyright: S. Karger AG, Basel 2003. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.

Copyright: S. Karger AG, Basel 2003. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.