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Compound Heterozygosity for Metachromatic Leukodystrophy and Arylsulfatase A Pseudodeficiency Alleles Is Not Associated with Progressive Neurological Disease

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Summary

Several allelic mutations at the arylsulfatase A (ASA) locus cause substantial deficiencies of this lysosomal enzyme. Depending on the genetically determined degree of the deficiency, the clinical outcome may be very different—either metachromatic leukodystrophy (MLD), a lethal lysosomal storage disorder affecting the nervous system, or, more frequently, the so-called pseudodeficiency (PD), which has no apparent clinical consequence. Because of compound heterozygosity for MLD and PD, 1/1,000 individuals in the population have low residual enzyme activities, which are intermediate between those of MLD patients and those of PD homozygous normal individuals. In order to assess whether PD/MLD compound heterozygotes bear a health risk, we examined clinically and biochemically 16 individuals with this genotype. Of these subjects, two had neurological symptoms and two showed lesions, without clinical symptoms, in magnetic resonance imaging of the brain. None of these symptoms was progressive, nor did they resemble those of MLD. Nerve conduction velocities were normal in these probands, and they secreted only low amounts of sulfatide in the urine. We conclude that the observed neurological symptoms are unrelated to the ASA genotype and that PD/MLD compound heterozygotes are not at an increased risk for developing progressive nervous system diseases.

Introduction

Metachromatic leukodystrophy (MLD) is an autosomal recessively inherited disorder caused by a deficiency of the enzyme arylsulfatase A (ASA). This defect leads to an accumulation of the enzyme's major substrate cerebroside sulfate (i.e., sulfatide). This glycolipid is mainly located in the myelin membranes of the central and peripheral nervous system. Its storage is accompanied by progressive demyelination leading to neurological symptoms, such as spastic tetraparesis, ataxia, optic atrophy, and dysarthria, as well as to behavioral abnormalities and dementia (for review, see Kolodny 1989).

Various ASA allelic mutations leading to different residual activities of the enzyme have been identified (Polten et al. 1991). In the late-infantile form of MLD, enzyme activity is virtually zero, the onset of the disease is in early childhood, and death ensues after several years. In the juvenile (onset age 3–16 years) and adult (onset age 16 years) forms of MLD, some residual enzyme activity is measurable. The clinical course is more protracted, and the first symptoms often mimic psychiatric disorders. In all forms of MLD, sulfatide is excreted in the urine in large amounts (for review, see Kolodny 1989).

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Table 1

Biochemical and Clinical Data for PD/MLD Compound Heterozygotes

Number	Initials	Sex	Present Age (years)	Length of Observation (years)	Type of MLD in Family	MLD Allele[a]	ASA Activity (% of normal)	Sulfatide Excretion in Urine[b] (μg/mg creatinine)	Sulfatide Loading (% of normal)	Present Status	MRI
1	M.M.	F	38	2	Late infantile	DelEx8	6	Not detectable	Not done	Healthy	Normal
2	H.A.	M	37	9	Late infantile	$S^{96}\rightarrow F$	6; 9[c]	Not done	60	Healthy	Normal
3	G.C.	M	32	1	Late infantile	SDEx2	4	Not done	Not done	Healthy	Normal
4	F.G.	M	5	2	Juvenile	?	6	.008; (not detectable)	75	Healthy	Abnormal
5	F.S.	M	42	2	Juvenile	?	6	Not detectable	Not done	Healthy	Normal
6	E.S.	F	19	3	Juvenile	$I^{179}\rightarrow S$	8; 15[c]	.004	56	Healthy	Normal
7	E.M.	M	56	3	Juvenile	SDEx7	3; 6[c]	.015; (.014)	30	Healthy	Abnormal
8	D.P.	M	40	9	Juvenile	SDEx2	4	.003	Not done	Healthy	Normal
9	D.M.	M	13	9	Juvenile	$P^{426}\rightarrow L$	5	.026	Not done	Soft signs	Normal
10	B.D.	F	22	11	Juvenile	?	8; 12[c]	.007	50	Dementia	Normal
11	B.E.	F	51	11	Juvenile	?	6; 13[c]	Not detectable	57	Healthy	Normal
12	B.H.	M	64	No information	Late infantile	?	11[c]	Not done	46	Healthy ?	Not done
13	S.K.	M	34	No information	Late infantile	SDEx2	10[c]	Not done	23	Healthy ?	Not done
14	K.A.	M	33	15	Adult	$P^{426}\rightarrow L$	8[c]	Not done	59	Healthy	Normal
15	L.M.	M	31	1	Late infantile	SDEx2	10[c]	Not done	32	Healthy	Not done
16	L.E.	M	2	1	Late infantile	SDEx2	7[c]	Not done	76	Healthy	Not done

NOTE.—Families are separated by extra space.

[a] Described in the following references: Bohne et al. (1991), for DelEx8; Gieselmann et al. (1991), for $S^{96}\rightarrow F$; Polten et al. (1991), for SDEx2 and $P^{426}\rightarrow L$; and Fluharty et al. (1991), for $I^{179}\rightarrow S$ and SDEx7. A question mark (?) indicates that the allele could not be identified. Probands 4 and 5 were only screened for the presence of allele SDEx2.

[b] Urine analysis is done from 24-h urine.

[c] Done by laboratory B.

Low ASA activity in urine, leukocytes, or homogenized fibroblasts alone, however, does not establish the diagnosis of MLD: ASA pseudodeficiency (PD) is caused by a particular mutation at the ASA locus, associated with low ASA activity. Synthesis of the enzyme is reduced, and the molecular structure is slightly altered (Gieselmann et al. 1989). The frequency of the PD gene is estimated to be 7.3%–15% in the general population, predicting that 0.5%–2% have low ASA-enzyme activity (Herz and Bach 1984; Hohenschutz et al. 1989). Clinical and biochemical studies have shown that homozygosity for the PD allele (i.e., PD/PD) is a benign condition (Herska et al. 1987; Hohenschutz et al. 1989). Subjects have residual activity that is 10%–20% (measured with artificial and natural substrates) of the activity in normal controls, but they are healthy. The low enzyme activity is apparently sufficient for normal sulfatide catabolism in vivo. There is no sulfatiduria (for review, see Kolodny 1989).

Compound heterozygosity of an MLD allele and a PD allele (i.e., PD/MLD) is expected to occur at a frequency of 0.1%. The residual enzyme activity seems to be intermediate between the activity in benign PD and the activity in lethal adult MLD (Kappler et al. 1991*a*). Residual activity in compound heterozygotes appears to be close to a critical threshold for normal sulfatide turnover (Kihara et al. 1980; Propping et al. 1986; Conzelmann et al. 1987). A matter of speculation has been whether the PD/MLD compound heterozygotes bear any health risk because of their low residual ASA activity. In a large sample of neuropsychiatric patients, the frequency of compound heterozygosity was the same as

Table 2

Summary of Biochemical Data of Groups of Individuals with Different ASA Genotypes

Genotype	ASA Activity [N] (% of normal)	Sulfatide Excretion in Urine[a] [N] (μg/mg creatinine)	Sulfatide Loading [N] (% of normal)
MLD/MLD	.2–4.4; mean = 1.1 [6]	.05–1.12; mean = .22 [6]	
MLD/MLD adult	2.1–5.4; mean = 3.4 [5[b]]		3.4–9.5; mean = 5.6 [5[b]]
PD/MLD	3–8; mean = 5.8 [11]	0–.026; mean = .006 [9]	
	6–15; mean = 10.3 [10[b]]		23–76; mean = 51 [11[b]]
PD/PD	14–21; mean = 17.6 [4]	Not done	
	21–32; mean = 26 [5[b]]		106–124; mean = 114 [5[b]]
No/No	73–143; mean = 100 [30]	Not detectable [>200]	
	57–126; mean = 100 [13[b]]		80–108; mean = 100 [13[b]]

NOTE.—Parts of the control data for the No/No, PD/PD, and MLD/MLD groups have already been published elsewhere (Kappler et al. 1991*a*). For clarity of presentation, they have been included in this table. Urine of PD homozygotes was not available.

[a] Urine analysis is done from 24-h urine.

[b] Done by laboratory B.

that in the general population (Hohenschutz et al. 1989). Thus, the association of that genotype and the neurological disorder in the aforementioned patients was probably coincidental. To examine an unbiased sample of PD/MLD compound heterozygotes, we searched for such individuals among family members of MLD patients. Owing to progress in biochemical and molecular genetic methods (Gieselmann 1991; Polten et al. 1991), the determination of the genotype is now more precise than in previous studies. In this study we try to answer the questions of whether PD/MLD compound heterozygotes exhibit clinical symptoms and whether these symptoms are causally related to their respective ASA genotype.

Subjects and Methods

Selection of Probands

Sixteen PD/MLD compound heterozygotes were identified among relatives of MLD patients. Three criteria had to be fulfilled for an individual to be identified as a PD/MLD compound heterozygote. First, one must be a relative of a patient with MLD. Second, low ASA residual activities (≤15% of activity in normal controls) must be determined in homogenates of cultured fibroblasts. Third, heterozygosity for the PD allele must be demonstrated by allele-specific amplification (Gieselmann 1991). In all 16 individuals allele-specific oligonucleotide hybridization was used to detect known MLD alleles. The procedure has been described by Polten et al. (1991), and references to the specific mutations are given in table 1. Subjects 2 (Tønnesen et al. 1983) and 12–14 (Kappler et al. 1991*a*) have already been described in the literature as being putative compound heterozygotes.

Methods

Skin fibroblast cultures were established from all probands. ASA activity was determined by two different laboratories (Berne = laboratory A, and Bonn = laboratory B) according to methods described elsewhere (Lee-Vaupel and Conzelmann 1987). It was measured in cell homogenates with the artificial substrate p-nitrocatecholsulfate. The PD allele was detected by allele-specific amplification (Gieselmann 1991). Both quantitation of cerebroside sulfate (i.e., sulfatide) in urine (Rossi et al. 1975) and loading experiments in cultured fibroblasts by using radioactively labeled cerebroside sulfate have been described (Kihara et al. 1980; Leinekugel et al. 1992). Loading experiments for case 4 were done by D. Wenger of Philadelphia.

All probands had a thorough general clinical and neurological examination during the past 12 mo (except for subjects 12 and 13, who are lost for follow-up because of moving to an unknown address). We obtained detailed information, from either the probands or their parents, about health history and social integration. They were questioned about age-adequate school attendance and professional life. Probands known for a longer time were additionally followed by telephone interview and by information from their physicians.

Magnetic resonance imaging (MRI) of the brain was performed in different centers always using T2-weighted spin-echo sequences. Images were evaluated in a blind fashion by a neuroradiologist. In cases in which either the clinical status or the MRI findings

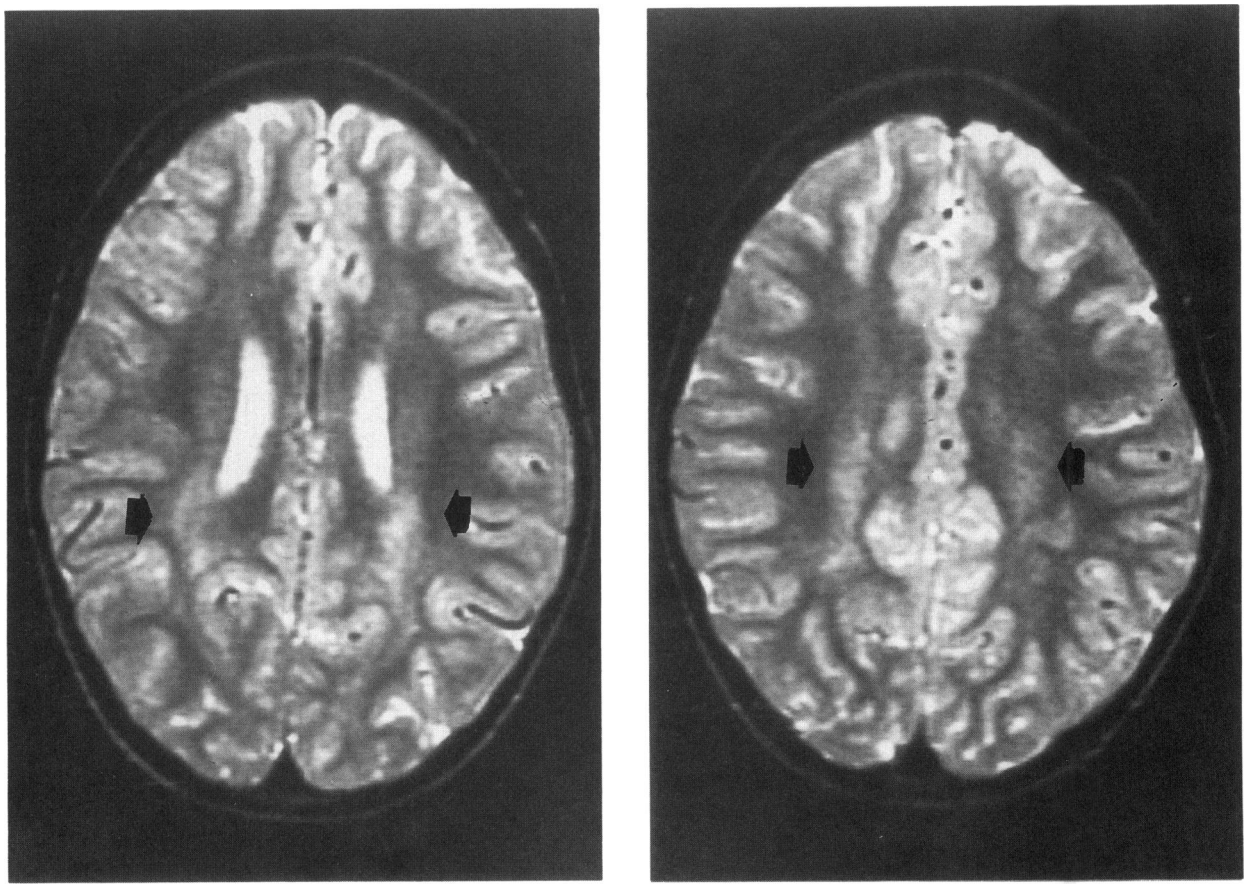

Figure 1 Axial T2-weighted images (TR/TE = 2,500/120 ms) of a 4-year-old boy (proband 4), which demonstrate confluent high signal intensity lesions in parietal white matter. These lesions have also been detected on inversion recovery images. Because of movement artifacts they are not presented.

were not clear, additional neurophysiological studies (nerve conduction velocity and/or determination of visual, acoustic, and somatosensory evoked potentials) were performed.

Results

The probands examined are siblings or parents of MLD patients. All had low residual ASA activity and were shown to be heterozygous for a PD and a non-PD allele. The latter could be positively identified as an MLD allele in 11 of the 16 cases. In the remaining cases, identification of the non-PD allele was not possible. Both ASA activity in fibroblast homogenates and sulfatide degradation in cultured fibroblasts are intermediate between those in the PD/PD and those in MLD/MLD homozygotes. See table 1 for data on each proband and table 2 for a summary of the biochemical data. Sulfatide excretion in the urine was measured in nine of the probands and was either not detectable (3/9) or was found in traces (4/9). However, in two probands (cases 7 and 9) sulfatide excretion was about 10% of the mean in MLD patients.

The present clinical status is normal in 14 of 16 probands. One subject (proband 4) previously had symptoms that normalized during the observation period (see description of probands below). MRI was done in 12 cases, of which 10 were normal. The probands in whom either clinical status or MRI was evaluated as abnormal will be described briefly: proband 4 is a 5-year-old hyperactive boy with slightly clumsy fine-motor skills and weak verbal articulation. During 2 years of observation, he has completely normalized. His MRI shows confluent parietal periventricular increase in signal intensity (fig. 1). Sulfatide excretion in urine was determined twice; once it was found in trace amounts,

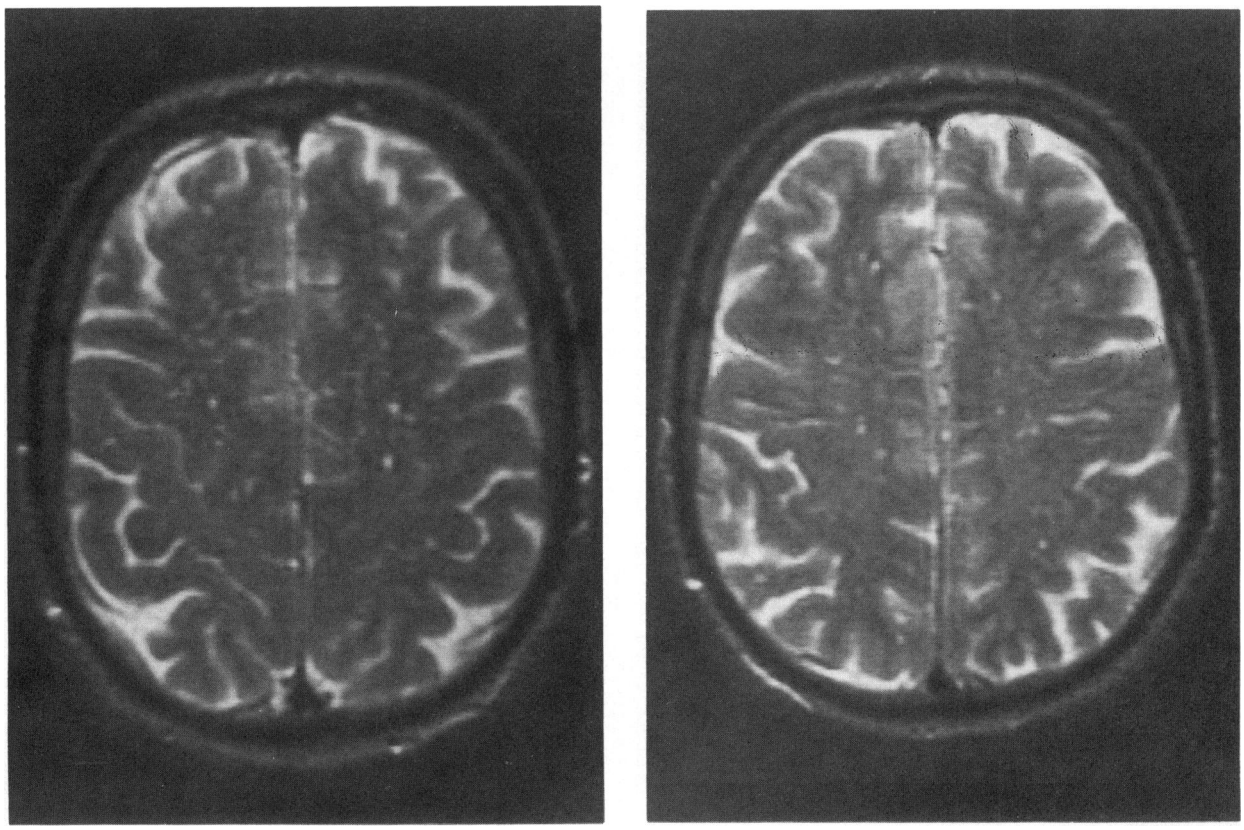

Figure 2 T2-weighted images (TR/TE = 2,500/100 ms) of a 56-year-old man (proband 7), which reveal multiple punctate lesions (*left*) and few linear white matter lesions in subcortical location (*right*).

and once it was not detectable (table 1). Additional neurophysiological studies (nerve conduction velocity and visual, acoustic, and somatosensory evoked potentials) were normal. Proband 7 is a healthy 56-year-old man. His MRI shows, in subcortical fronto-parietal location, multiple punctate lesions (fig. 2, *left*) and few linear lesions of high signal intensity (fig. 2, *right*) in T2-weighted images and shows correspondingly low signal intensity in T1-weighted images (fig. 3). Findings in a second examination 20 mo later were identical. In two independent determinations considerable sulfatiduria was found (table 1). Nerve conduction velocity was normal. Case 9 is a 13-year-old schoolboy; his neurological examination revealed both a slight apraxia of finger movements and dysdiadochokinesia with abundant associated movements. He has been followed for 9 years now, and there is no progression. He excretes considerable amounts of sulfatide in urine (table 1). MRI and neurophysiological studies (nerve conduction velocity and visual and acoustic evoked potentials) are normal. Case 10 is a 21-year-old woman who is mentally retarded and has had a clumsy gait and reduced control of fine finger movements since early childhood. She was intensively examined 11 years ago, at the time of her brother's diagnosis. Her status now does not show any progression. She excretes traces of sulfatide in urine. MRI is normal.

Discussion

In the present study 16 PD/MLD compound heterozygotes were examined biochemically and clinically. Determination of ASA activity in fibroblasts by using synthetic and natural substrates demonstrates that these individuals have residual enzyme activities intermediate between those in PD/PD and those in MLD/MLD homozygotes (see also Conzelmann and Sandhoff 1983/84). The in vivo sulfatide accumulation is probably best reflected by the amount of sulfatide excreted in the urine. In comparison with the sulfatide found in the urine of MLD patients, only trace amounts (in subjects 7 and 9, up to 10% of the mean) were found in PD/

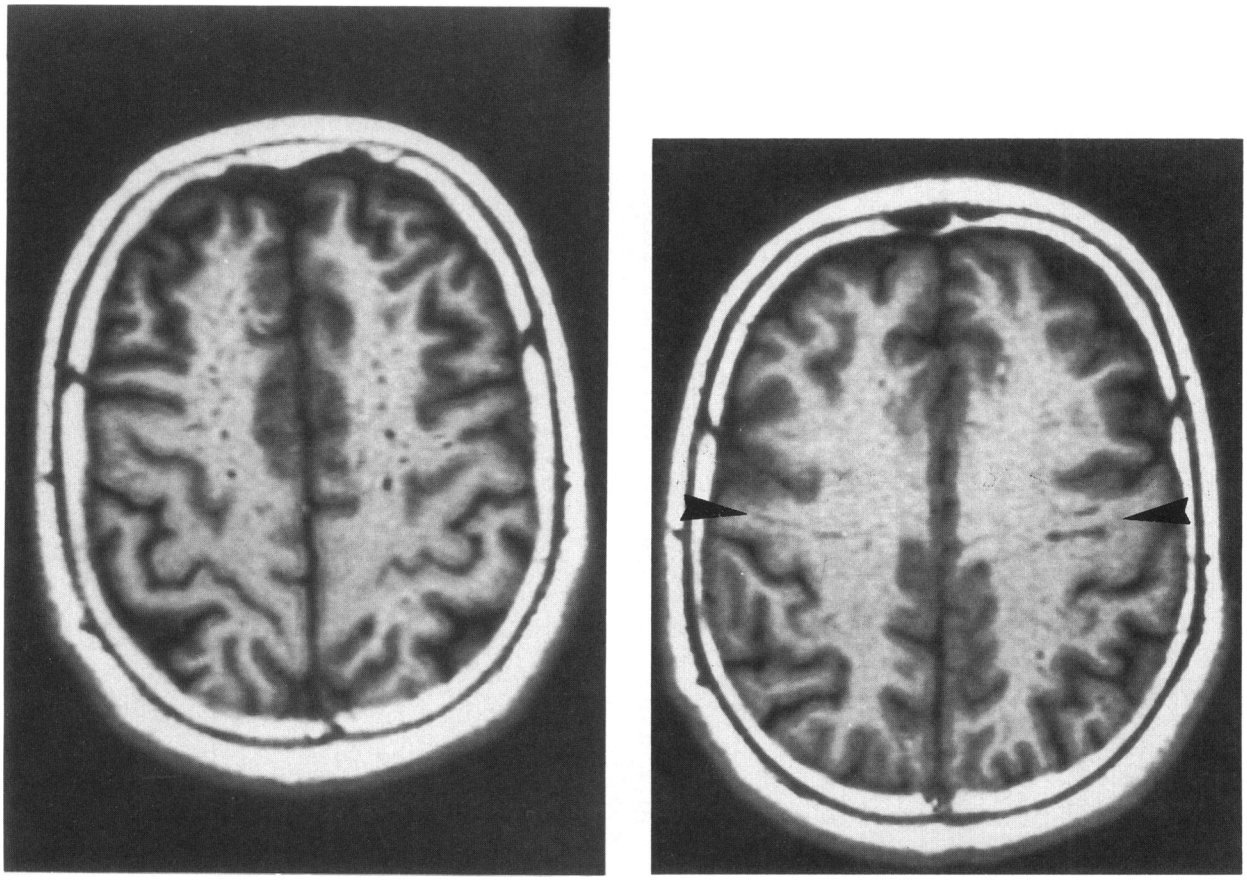

Figure 3 Inversion recovery images (TR/TE/TI = 1,500/20/610 ms) of proband 7 reveal the same lesions as shown on T2-weighted images (see fig. 2).

MLD heterozygotes. This finding suggests that their sulfatide metabolism is closer to normal than to that of MLD patients. On the other hand, sulfatide excretion in the two aforementioned probands is not a normal finding—it was observed neither in our normal controls nor in PD/PD subjects (for review, see Kolodny 1989). Thus, it might be an effect related to PD/MLD heterozygosity. At present, 14 of 16 probands are clinically normal. The two probands with symptoms had normal brain MRIs. Of the two individuals whose brain MRI was evaluated as abnormal, one was without neurological symptoms, whereas one individual had neurological soft signs at an earlier stage, which normalized during the 2-year observation time. At present he has no symptoms.

It can, however, be argued that the finding of 4 abnormal individuals among 16 is unexpectedly high. For several reasons, we think that it is likely that these symptoms are not related to the PD/MLD genotype. In the two cases in which neurological abnormalities were observed, they were distinct and did not resemble the symptoms of MLD (for review, see Kolodny 1989). The subjects were under clinical observation for up to 11 years, and no progression of symptoms was found. In addition, nerve conduction velocities, a reduction of which may be detectable in MLD patients before the disease becomes apparent (Clark et al. 1979), were normal in all probands who showed either clinical, biochemical, or neuroimaging abnormalities. Alterations in computed tomography scans of MLD patients had been found to begin periventricularly, and from there the demyelinization proceeds into the white matter of the hemisphere (Schipper and Seidel 1984). The alterations described in the two abnormal MRIs neither showed the typical MLD pattern nor resembled each other. During the period of observation, two MRIs were performed, and again no progression of abnormalities was found. If PD/MLD compound heterozygosity

causes clinical or neuroradiological symptoms, then one would expect that the appearance among different individuals is similar. The symptoms described here did not show any resemblance among the different individuals presented. Thus, in none of the individuals examined was there any evidence of findings similar to those of MLD, and we conclude that compound heterozygosity for PD/MLD alleles is not a sufficient condition for a progressive demyelinating disease.

However, two objections may be raised against this statement. First, symptoms due to compound heterozygosity may theoretically develop in senescence and may not be obvious in our probands. Second, it has recently been shown that the ASA genotype is one of the major determinants of the clinical course of the disease. However, among late-onset MLD patients with an identical genotype there was a remarkable variation of the age at onset of the disease (Polten et al. 1991). This suggests that other, unknown factors substantially influence the course of the disease. By analogy, it cannot be excluded that such a variation within the group of PD/MLD heterozygotes may allow the development of symptoms in some individuals. Our data suggest that, in the majority of compound heterozygotes, this will not be the case.

The question whether other factors in combination with PD/MLD compound heterozygosity may increase the risk for other demyelinating disorders was recently addressed by a study of 160 multiple sclerosis patients, in which an indication for a slight association of ASA PD was found. No PD/MLD compound heterozygotes were detected, however (Kappler et al. 1991*b*), which suggests that an association of reduced ASA activity with multiple sclerosis could only be very weak, if it exists at all.

We conclude that, in families with a member suffering from MLD, there is considerable probability for the existence of PD/MLD compound heterozygotes. These subjects are not at risk for demyelinating disease. For proper genetic counseling and prenatal diagnosis, it is of utmost importance to realize that low ASA activities may not be associated with a devastating metabolic disorder. A proper diagnosis can only be achieved by combinated determination of enzyme activities and sulfatide excretion, cerebroside sulfate loading assays, and molecular genetic techniques.

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