Late-onset metachromatic leukodystrophy

Genotype strongly influences phenotype

H. Rauschka, MD*; B. Colsch*; N. Baumann, MD, PhD; R. Wevers, PhD; M. Schmidbauer, MD; M. Krammer; J.-C. Turpin, MD; M. Lefevre; C. Olivier, PhD; S. Tardieu, BS; W. Krivit, MD; H. Moser, MD; A. Moser; V. Gieselmann, MD; B. Zalc, MD, PhD; T. Cox, MD; U. Reuner, MD; A. Tylki-Szymanska, MD; F. Aboul-Enein, MD; E. LeGuern, MD, PhD; H. Bernheimer, MD; and J. Berger, PhD

Abstract—*Background:* P426L and I179S are the two most frequent mutations in juvenile and adult metachromatic leukodystrophy (late-onset MLD), which, in contrast to infantile MLD, show marked phenotypic heterogeneity. *Objective:* To search for genotype–phenotype correlations in late-onset MLD. *Methods:* The authors reviewed the clinical course of 22 patients homozygous for mutation P426L vs 20 patients heterozygous for mutation I179S, in which the second arylsulfatase A (ASA) mutation had also been determined. *Results:* P426L homozygotes principally presented with progressive gait disturbance caused by spastic paraparesis or cerebellar ataxia; mental disturbance was absent or insignificant at the onset of disease but became more apparent as the disease evolved. In contrast, compound heterozygotes for I179S presented with schizophrenia-like behavioral abnormalities, social dysfunction, and mental decline, but motor deficits were scarce. Reduced peripheral nerve conduction velocities and less residual ASA activity were present in P426L homozygotes vs I179S heterozygotes. *Conclusion:* The characteristic clinical differences between homozygous P426L and compound heterozygous I179S patients establish a distinct genotype–phenotype correlation in late-onset metachromatic leukodystrophy.

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Metachromatic leukodystrophy (MLD) is an autosomal recessive sulfatide storage disease classically due to deficiency of the lysosomal enzyme arylsulfatase A (ASA). Normally, ASA initiates the degradation of sulfatides (3-*O*-sulfogalactosylceramides), which are an essential component of myelin. Decreased ASA activity induces sulfatide accumulation in the CNS and PNS, gallbladder, kidney, and other visceral organs. Neurologic symptoms result from progressive myelin degeneration and axonal loss in the CNS and PNS.[1]

Prior to the molecular era, four subtypes of MLD were described, according to onset, severity, and progression of the disease: late infantile (onset before age 4), early juvenile (age 4 to 6), late juvenile (age 6 to 16), and adult MLD (age >16).[1] A genotype–phenotype correlation has been established based on the two functionally different types of mutations: mutations encoding inactive ASA (0-alleles) and mutations encoding ASA with residual enzymatic activity (R-alleles). Genotypes comprising two 0-alleles will cause the severe late infantile type of MLD. Coincidence of a 0-allele and an R-allele induces predominantly the intermediate juvenile type, whereas two R-alleles usually cause the milder adult type of MLD, although juvenile and adult forms of MLD rather seem to form a continuum based on specific mutations as well as other, still unknown, genetic, epigenetic, and environmental factors.[1,2]

Patients with late infantile MLD show little phe-

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*These authors contributed equally to this work.

From the Center for Brain Research (H.R., M.K., F.A.-E., J.B.), Medical University of Vienna, Department of Neurology (H.R., M.S.), Hospital Lainz, and Clinical Institute of Neurology (H.B.), Medical University of Vienna, Vienna General Hospital, Austria; INSERM U711 (B.C., N.B., M.L., C.O., B.Z.), Universite Pierre et Marie Curie, Faculte de Medecine, and Association pour la Recherche en Neurochimie (J.-C.T.), Hopital de la Salpetriere, Neurogenetics Laboratory and Department of Medical Genetics (S.T., E.L.G.) and INSERM Unit 679 (E.L.G.), Pitié-Salpêtrière Hôpital, Paris, France; Institute Neurology (R.W.), University Medical Centre Nijmegen, Nijmegen, the Netherlands; Department of Pediatrics (W.K.), University of Minnesota, Minneapolis; Kennedy Krieger Institute and Department of Neurology (H.M., A.M.), Johns Hopkins University, Baltimore, MD; Institut für Physiologische Chemie (V.G.), Rheinische Friedrich-Wilhelms-Universität, Bonn, Germany; Department of Medicine (T.C.), University of Cambridge, Addenbrooke's Hospital, Cambridge, UK; Klinik und Poliklinik für Neurologie (U.R.), Universitätsklinikum Carl Gustav Carus-Technische Universität, Dresden, Germany; Zdrowia Dziecka Pomnik-Szpital (A.T.-S.), Children's Memorial Health Institute, Warszawa-Miedzylesie, Poland.

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Address correspondence and reprint requests to Dr. J. Berger, Center for Brain Research, Medical University of Vienna, Spitalgasse 4, A-1090 Vienna, Austria; e-mail: johannes.berger@meduniwien.ac.at

notype variation, regardless of the kind of 0 mutation. In contrast, marked clinical heterogeneity is found in late-onset (late juvenile and adult) MLD associated with the two late-onset genotypes (0/R and R/R). Among these, two different clinical presentations have been observed: patients with progressive motor or sensory deficits and, on the other hand, cases with mental disturbance.[3-12] It has been suggested that these differences may originate from different specific mutations.[1,7,12] To explore a possible genotype–phenotype correlation in late-onset MLD on the basis of specific mutations, we studied the molecular and clinical data of 22 patients homozygous for mutation P426L and of 20 patients heterozygous for mutation I179S. These two mutations are the most frequent R-type mutations in late-onset MLD.

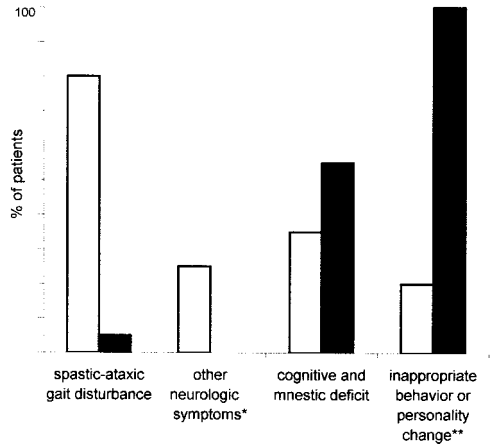

*Figure 1. Symptoms at onset of disease in P426L homozygous and I179S heterozygous patients with metachromatic leukodystrophy. White bars = P426L homozygotes; black bars = I179S compound heterozygotes; *declining visual acuity due to optic nerve atrophy, paravertebral pain, epilepsy; **lack of social inhibition, loss of interest, apathy ("frontal lobe type").*

Methods. *Patients.* We studied MLD patients with either mutation P426L in homozygosity or mutation I179S in heterozygosity when adequate biochemical and clinical data were available. In the I179S patients, the second mutation was also characterized. Twenty-one unpublished and 21 reported patients[3-22] fulfilled these criteria and were included (22 P426L homozygotes and 20 I179S compound heterozygotes) (see tables E-1 and E-2 on the *Neurology* Web site; go to www.neurology.org). In all patients, diagnosis of MLD was verified biochemically by determination of ASA activity in leukocytes or fibroblasts, sulfatide excretion in urine, or neuropathologic examination of nerve biopsy. As, owing to the low incidence of late-onset MLD and the paucity of cases recognized in the early clinical phase, a prospective approach is almost impossible, acquisition of clinical data were done retrospectively. All available clinical records of the hitherto unpublished patients were examined by experienced neurologists who could not be blinded to the genotype. The reviewer abstracted each symptom and sign given in the records to obtain an individual chronology for each patient. Similarly, the clinical courses of the reported cases were abstracted by one reviewer; in 10 cases, unpublished clinical data were provided by the respective authors.

Biochemical analysis. ASA activity was determined in leukocytes[23,24] and in urine.[25] ASA activities are reported as percentage of the mean ASA reference values.

Genetic analysis. In the 21 new patients and in the previously published cases, genomic DNA was extracted by standard procedures either from leukocytes or from fibroblasts.[26] Detection of the mutations P426L and I179S was performed by PCR restriction assays as previously described.[13,27] In 12 cases, mutation I179S was confirmed by sequencing.

To identify the complete genotype, both strands of the PCR fragment containing each of the eight ASA exons were sequenced in nine cases. The primers and laboratory methods are given in table E-3 and appendix E-1.

Statistical analysis. Nonparametric tests (e.g., Mann–Whitney) were used to compare clinical data between genotypes. Data were expressed as means ± SEM; unless stated otherwise, p values of <0.05 were considered significant.

Results. The complete molecular and clinical data of the 42 patients investigated are available in tables E-1 and E-2.

Genetic analysis. Our own PCR-based investigations on the common ASA mutations P426L and I179S revealed 13 cases homozygous for mutation P426L and 8 cases heterozygous for the I179S ASA allele. In the other cases, mutations were given as reported in the literature. To characterize the second ASA mutation in nine heterozygotes for I179S, the entire coding ASA sequence was analyzed. In six cases, the common splice site mutation 459 + 1 G→A known to be a 0-type ASA allele was identified. In two patients, a G-to-C transversion was identified at position 1,100, leading to the D255H substitution, and a C-to-G transversion at nucleotide 1,067 was identified in one patient, leading to the missense mutation R244C. Both mutations can be considered as 0-type mutation as both have been described in patients with late infantile MLD previously.[28,29]

Among the 12 additional I179S heterozygotes already reported in the literature, 8 have a second mutation, which belongs to the ASA 0-type: 5 with 459 + 1G→A, 1 with 1,204 + 1G→A, 1 with A212V, and 1 with L135P. In one patient, I179S was associated with D281Y, which has never been observed at the homozygous state or in any other combination than with I179S and thus cannot be categorized as a 0- or R-type mutation.[10] In three patients, the I179S allele was associated with P426L, a well-known R-type allele.

Eight patients, all carrying I179S/459 + 1G→A, shared three polymorphisms: T391S (2,161 C→G) in exon 7, 2,213 C→G in intron 7, and W193C (842 G→T) in exon 3. The 2,213 C→G and T391S polymorphisms have also been found in two patients carrying as second mutation D255H. In addition, polymorphism 2,213 C→G has been detected in one patient carrying R244C and polymorphism T391S in one patient carrying 459 + 1G→A as second mutation (tables E-1 and E-2).

Genotype–phenotype correlations. Clinical syndromes at onset and in advanced disease are summarized in figures 1 and 2. The phenotype and genotype of each patient are available in tables E-1 and E-2, and two characteristic cases are presented in appendix E-2.

Patients homozygous for p426l. Two cases were still asymptomatic at ages 14 and 32, both with symptomatic siblings. Age at onset was variable in the 20 patients (11 males, nine females) and ranged from 10 to 29 years (mean 19.9 ± 1.3 years). This classifies 14 cases as adult and 6 cases as late juvenile MLD.[1] Documented disease course ranged from 1 to 20 years (mean 6.7 ± 1.2 years).

Sixteen of the 20 patients presented with progressive gait disturbance due to spastic paraparesis or cerebellar ataxia. In seven cases, gait disturbance was accompanied

by cognitive disturbance, personality changes (apathy, loss of spontaneity), or disorganized behavior, in two by visual loss due to optic atrophy, and in one by epilepsy. In an advanced course of the disease, cognitive decline was observed in 18 patients and marked frontal lobe–type personality changes in 3 of the 20 patients. Symptoms of brainstem dysfunction (noncerebellar dysarthria, dysphagia, gaze palsy) developed in three patients, extrapyramidal symptoms (bradykinesia, athetosic movements) in two, and seizures in three patients.

Only four patients did not have gait disturbance at onset. Two of them presented with paravertebral pain: one with progressive optic atrophy and one with apathy and cognitive deficits initially diagnosed as major depression. In the further course, progressive spastic paraparesis and cerebellar ataxia as well as cognitive disturbance developed in three of these patients.

Two patients had sensory impairment: one with hemihypesthesia and one with a symmetric sensory deficit in the distal lower limbs developing 20 years after onset, very probably due to excessive alcohol consumption.

Otherwise, symptoms or clinical signs of PNS damage were generally absent.

Patients heterozygous for I179s. Age at onset in the 20 patients (11 males, 9 females) ranged from 8 to 60 years (mean 26 ± 3.0 years). This is statistically not different from the mean age at onset in the P426L homozygotes. Sixteen I179S heterozygotes were classified as adult and four as late juvenile MLD.[1] The mean age at onset in the 3 patients with an R-type mutation on the second allele (47 ± 6.3 years) was higher ($p < 0.01$) than in the 16 patients with a 0-type mutation (22.4 ± 2.6 years). Documented disease course ranged from 1 to 23 years (mean 7.8 ± 5.3 years).

Nineteen of the 20 patients presented with isolated neuropsychiatric symptoms and 1 patient with a combination of neuropsychiatric symptoms and gait disturbance. The initial neuropsychiatric symptoms were prominent behavioral and perceptual disturbances that are described for schizophrenia in the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders, namely, delusions and hallucinations in 3 patients, disorganized behavior in 12 patients, and social dysfunction in all. In addition, nine patients had signs of a frontal lobe syndrome: lack of social inhibition, emotional lability, or apathy. In the further course there was cognitive decline in 19 patients, leading to dementia in 7 cases, 4 to 14 years (median 7 years) after onset. Five patients were hospitalized in psychiatric institutions mainly with the diagnosis of schizophrenia until neurologic signs appeared or because the atypical course of the disease led to the performance of cerebral MRI. The majority of patients with isolated mental symptoms at onset developed neurologic disturbances 2 to 14 years (median 7 years) after onset: Seven patients had spastic or ataxic motor impairment, two patients epileptic seizures, and three patients had localized cortical disturbances such as aphasia, agraphia, acalculia, apraxia, or agnosia. Extrapyramidal symptoms, brainstem disturbances, optic atrophy, action and postural tremor, or unspecified gait disturbance developed in one patient. Eight cases did not develop any neurologic signs or symptoms, despite a disease duration of up to 23 years. Symptoms or clinical signs of PNS damage were generally absent.

Neuroimaging. In P426L homozygotes, results of cerebral CT were available in 16 cases and of cerebral MRI in 11 cases. In I179S heterozygotes, results of cerebral CT and MRI were available in 7 and 11 cases. Both groups, symptomatic as well as asymptomatic cases, showed the classic signs of leukodystrophy with symmetric periventricular white matter abnormalities and relative sparing of U-fibers, accompanied by cortical and subcortical atrophy. Two P426L homozygous patients showed signs of long fiber tract degeneration in brainstem MRI images. Brainstem changes were generally absent in the I179S patients, but in seven of them, white matter changes were frontally accentuated.

Electrophysiology. Nerve conduction studies were performed in 19 of 20 symptomatic P426L homozygotes, and a prolonged or severely prolonged nerve conduction velocity was generally found. Numerical data were available in 13 patients. Nerve conduction studies were done in 14 of 20 I179S heterozygotes. Numerical data were available in eight patients. In a further six patients, slightly reduced nerve conduction velocities were found. Mean motor nerve conduction velocity was slower in the upper limbs in P426L homozygotes (32 ± 1.9 m/s) than in I179S heterozygotes (42 ± 3.1 m/s; $p < 0.02$). In the lower limbs, differences were more pronounced (23 ± 0.9 m/s in P426L homozygotes vs 37 ± 3.1 m/s in I179S heterozygotes; $p < 0.0005$) (figure E-1). The time between disease onset and nerve conduction studies was not statistically different between the two groups. There was no correlation of nerve conduction velocity with disease duration at the time of examination.

EEG was available in seven P426L homozygotes and in four I179S heterozygotes. In both groups, diffuse slowing was most common, but focal slowing as well as focal epileptiform elements were additional findings.

CSF. Results of CSF analysis were available in 12 P426L homozygotes and 8 I179S heterozygotes. Cell count was generally normal, but protein was mildly elevated in about half of patients in both groups (range 0.46 to 0.73 g/L).

ASA activity. Mean ASA activity control values were available for 14 P426L homozygotes and 12 I179S heterozygotes, all with a 0-mutation on the second allele. In the remaining cases, ASA activity was below the range of control values (mean not known).

Although there is considerable overlap, the mean leukocyte ASA activity was lower in P426L homozygotes (5.8 ± 1.0%) than in I179S compound heterozygotes (10.6 ± 1.7%) ($p < 0.03$) (figure E-2).

Discussion. In this study, we present late-onset MLD patients with fully analyzed genotypes and characterize two distinct phenotypes, correlating with the ASA mutations P426L and I179S.

P426L homozygotes typically started with gait disturbance caused by spastic paraparesis or cerebellar ataxia, whereas all patients heterozygous for mutation I179S presented with inappropriate or disorganized behavior and social dysfunction. Because of the isolated psychiatric presentation and the relatively young age at onset, the patients are at risk to be misdiagnosed as having schizophrenia or affective disorder.

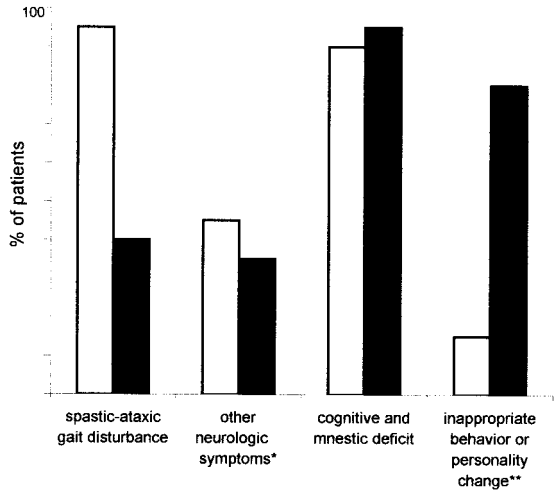

*Figure 2. Symptoms in advanced disease in P426L homozygous and I179S heterozygous patients with metachromatic leukodystrophy. White bars = P426L homozygotes; black bars = I179S compound heterozygotes; *declining visual acuity due to optic nerve atrophy, paravertebral pain, epilepsy, localized cortical disturbances as aphasia, agraphia, acalculia, apraxia, or agnosia, brainstem dysfunction, noncerebellar tremor; **lack of social inhibition, loss of interest, apathy ("frontal lobe type").*

In early disease, the clinical syndrome of patients heterozygous for I179S is distinct and different from that of patients homozygous for P426L (figure 1). However, in advanced disease, the two groups become clinically less distinct, as a combination of neurologic and neuropsychiatric disturbances is then often found in patients of both genotypes (figure 2).

Although nerve conduction studies show more severe changes in P426L homozygotes than in I179S heterozygotes, considerable overlap limits the clinical usefulness of these findings.

Neuroimaging also did not differentiate the two groups, although it is of interest that frontal dominance of the leukoencephalopathy was found in a proportion of I179S heterozygotes, but not in P426L homozygotes. It has been suggested that in patients with psychocognitive MLD, the prefrontal white matter is primarily involved, implying that psychosis in MLD is related in particular to a functional impairment of prefrontal temporolimbic connections.[30] Alternatively, the psychiatric presentation might be caused by cortical dysfunction, because when compared with changes in late infantile MLD, a relatively increased sulfatide accumulation in the cortical gray matter has been found in a patient with adult MLD with predominant mental disturbance.[31]

Considering the presence of two apparently identical mutant ASA alleles in P426L homozygotes, there is still remarkable heterogeneity of the clinical expression. This emphasizes the influence of the genetic background or unknown environmental factors. In contrast, I179S heterozygotes, harboring diverse heteroallelic ASA variants, show a remarkably consistent phenotype. The I179S allele was mainly associated with 0-type ASA alleles, which, in the homozygous state, causes late infantile MLD. If the I179S mutation was associated with an R-allele, this led to a significantly older age at onset than the association with a 0-allele, but otherwise the phenotype was essentially the same. Therefore, and as no patients with MLD homozygous for I179S have been detected up to now, a high residual ASA activity of this enzyme variant has to be expected. Mean leukocyte ASA activity in our I179S heterozygotes was significantly higher than in the P426L homozygotes, but the range was large with nearly complete overlap. As the correlation of leukocyte ASA activity to the relevant ASA activity in neurons and glia is unknown, leukocyte ASA activities do not permit conclusions regarding the clinical course of the individual patient.[32]

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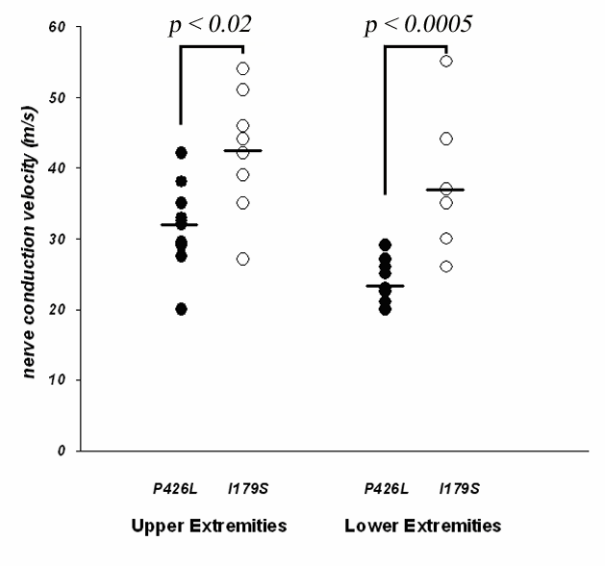
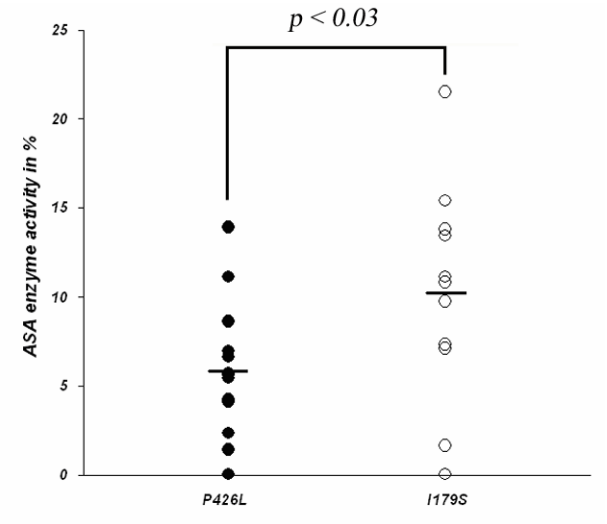
Figure (E) F-1:



Measurement of motor nerve conduction velocity of upper and lower extremities in patients with MLD. For each patient, the lowest conduction velocity of the respective extremity is given; *Black bars* indicate the means of each group; *black circles*: P426L patients; *white circles*: I179S patients.

Figure (E) F-2:



Leukozyte ASA enzyme activity in patients with MLD with P426L and I179S mutation. *Black bars* indicate the means of each group. *black circles*: P426L patients; *white circles*: I179S patients.

Table (E) T-1: Clinical and molecular data of patients homozygous for P426L

case no*	sex	age at onset	presentation	further disease course		documented course	mutation, (methodes)	origin, references
				neurological disturbance	*mental disturbance*			
1	m	13y	progressive Lhermitte´s sign like paravertebral pain	unsteady gait, spastic- tetraparesis, ataxia, diminished visual acuity	progressive cognitive and mnestic disturbance	20y	P426L / P426L' (a)	Austria, 13
2	m	14y	gait disturbance	spastic-tetraparesis, ataxia, dysarthria	progressive cognitive and mnestic disturbance	15y	P426L / P426L (a)	Austria, 13
3 (brother of 4)	m	23y	gait disturbance, epileptic fits	spastic tetraparesis	progressive cognitive and mnestic disturbance	3y	P426L / P426L (a)	France, 12
4 (brother of 3)	m	22y	bilateral optic atrophy leading to blindness within 1 year	spastic-tetraparesis, ataxia, ophtalmoplegia, dysphagia, epilepsy	progressive cognitive and mnestic disturbance	10y	P426L / P426L (a)	France, 12
5	m	19y	gait disturbance, diminishing visual acuity	spastic-tetraparesis, ataxia	progressive cognitive and mnestic disturbance	10y	P426L / P426L (a)	France, unpublished
6 (sister of 7)	f	(>14y)	(concentration difficulties, headache)	none	none	not known	P426L / P426L..(b), (c)	Germany, unpublished
7 (sister of 6)	f	10y	gait disturbance, deteriorating school performance	spastic-paraparesis, ataxia, intention-tremor	increasing learning and concentration difficulties	2y	P426L / P426L (b, (c)	Germany, unpublished
8 (sister of 9)	f	15y	gait disturbance, headache, learning difficulties	spastic-tetraparesis, ataxia, gaze-palsy, bulbar disturbance, athetosic movements , hemihypoesthesia	progressive cognitive and mnestic disturbance, apathy, inaedequat behavior and affect, finally dementia	7y	P426L / P426L (b), (c)	Germany, unpublished
9 (sister of 8)	f	16y	gait disturbance, learning difficulties	spastic-tetraparesis, ataxia, vertical gaze-palsy, dysarthria	progressive cognitive and mnestic disturbance, personality change with inaedequat affect , finally dementia	7y	P426L / P426L (b), (c)	Germany, 5
10	m	28y	gait disturbance due to weakness of the left leg	slowly progressive gait disturbance with frequent falls	reduced short term memory, reduced concentration	3y	P426L / P426L (c)	Germany, unpublished
11	m	22y	gait disturbance	spastic-paraparesis, ataxia	none	2y	P426L / P426L (c)	Germany, 3
12	f	22y	gait disturbance, memory disturbances	ataxia of upper and lower limbs	progressive cognitive and mnestic disturbance, finally dementia	3y	P426L / P426L (c)	Germany, 5
13 (sister of 14)	f	22y	gait disturbance, loss of spontaneity	bradykinesia, hypophonia, spastic tetraparesis	progressive mnestic and cognitive disturbance, finally dementia	1y	P426L / P426L (b)	Japan, 6
14 (brother of 13)	m	12y	gait disturbance	spastic-tetraparesis, ataxia, epilepsy	progressive mnestic and cognitive disturbance, finallydementia	11y	P426L / P426L (b)	Japan, 6
15	f	29y	gait disturbance, diminished visual acuity	spastic paraparesis, bilateral optic atrophy	progressive mnestic and cognitive disturbance, finally dementia	3y (death)	P426L / P426L (a), (b), (d)	Netherlands, unpublished
16	m	28y	depressed mood, cognitive disturbance	spastic-paraparesis, ataxia	progressive mnestic and cognitive disturbance	6y (death)	P426L / P426L. (a),.(b), (d)	Netherlands, unpublished
17	f	17y	gait disturbance, personality change, cognitive disturbance	spastic-tetraparesis, ataxia	progressive mnestic and cognitive disturbance, personality changes	5y (death)	P426L / P426L (a),.(b), (d)	Netherlands, unpublished
18	m	17y	gait disturbance, declining school performance, strange and inappropriate behavior and affect	spastic-paraparesis, epilepsy	progressive mnestic and cognitive disturbance, finally dementia	13y (death)	P426L / P426L (a), (b), (d)	Netherlands, unpublished
19	f	18y	gait disturbance	spastic-paraparesis,ataxia	progressive mnestic and cognitive disturbance, finally dementia	12y	P426L / P426L. (a), (b), (d)	Netherlands, unpublished
20 (sister of 21)	f	- (>32y)	(frequent headaches)	none	none	3y	P426L / P426L (a), (b), (d)	Netherlands, unpublished
21 (brother of 20)	m	25y	paravertebral pain, pain in the legs	remission of pain	none	2y	P426L / P426L (a), (b), (d)	Netherlands, unpublished
22	f	25y	gait disturbance	spastic-paraparesis, ataxia	Mnestic and cognitive disturbance	2y	P426L / P426L (a), (b), (d)	Netherlands, unpublished

* order of cases is according to time of inclusion into our study; mutations determined by: (a) mutated primer modulated PCR restriction fragment length polymorphism, (b) sequenzing analysis, (c) allele specific hybridisation, (d) single stranded conformation polymorphism

Table (E) T-2: Clinical and molecular data of patients heterozygous for I179S

case no*	sex	age at onset	presentation	further disease course		documented course	mutations, (types), (methode)	origin, references
				neurological disturbance	*mental disturbance*			
23 (brother of 24)	m	41y	memory disturbance, slowed speed of cognition, inadequate behavior	slowly progressive spastic-paraparesis,ataxia, explosive speech, extrapyramidal symptoms	slowly progressive cognitive and behavioral disturbance of frontal lobe type	8y	I179S / P426L, (R/R), (a)	Germany, 13-17
24 (sister of 23)	f	41y	apathy, depressive mood, emotional lability	gait disturbance, spastic- tetraparesis, ataxia, dysarthria, left facial palsy, bilateral optic nerve atrophy	rapid cognitive decline, finally dementia	6y (death)	I179S / P426L, (R/R), (a)	Germany, 13-17
25	m	24y	apathy, cognitive disturbance	spastic tetraparesis	bizarre behavior, cognitive decline, finally dementia	14y	I179S / 459+1G>A, (R/0), (a)	Austria, 13, 18
26	m	9y	moody, memory disturbance, inadequate behavior	ataxia	cognitive decline, severe personality changes	4y	I179S / 1204+1G>A, (R/0), (b)	Germany, 4
27	m	40y	inappropriate behavior, confusion	mild postural and action tremor	antisocial impulsive behavior, moderate cognitive decline	6y	I179S / A212V, (R/0), (b), (c)	Canada, 19, 20
28	m	11y	concentration difficulties, cognitive disturbance, strange behavior, gait disturbance	slowly progressive gait disturbance	strange behavior, outbursts of inappropriate laughter, confusion, cognitive impairment, finally dementia	5y	I179S / 459+1G>A , (R/0), (a), (b)	Poland, 7
29	m	60y	personality change, chaotic behaviour, memory problems	none	progressive personality change of frontal lobe type	2y	I179S / P426L, (R/R), (nk)	Netherlands, 11, 21
30 (sister of 31)	f	15y	inappropriate behavior and affect, cognitive disturbance	no spastic paraparesis except for Babinski sign, aphasia, apraxia, agnosia	bizarre behavior, blunted affect, disturbed thinking, confusion, cognitive decline, finally dementia	5y	I179S / 459+1G>A , (R/0), (a), (b)	France, 8, 12, 22
31 (sister of 30)	f	16y	inappropriate behavior, cognitive disturbance	no spastic paraparesia except for Babinski sign, apraxia, acalculia, agraphia, epilepsy	apathy, memory problems, cognitive decline, finally dementia	14y	I179S / 459+1G>A, (R/0), (a), (b)	France, 8, 12, 22
32	m	8y	concentration difficulties, inappropriate behavior	mild spastic paraparesis and severe apraxia	mild memory disturbance, mild cognitive decline	9y	I179S / 459+1G>A, (R/0), (a), (b)	Italy, 9
33	f	39y	moody, easily distracted	none	frontal lobe syndrome, memory disturbance, cognitive decline	1y	I179S / L135P, (R/0), (a), (b)	Italy, 9
34	f	16y	disorganized behavior with forgetfulness	none	frontal lobe syndrome, memory disturbances, cognitive decline	4y	I179S / D281Y, (R/0), (b)	UK, 10
35 (sister of 36)	f	17y	delusions, hallucinations, disorganized behavior	none	Inappropriate behavior, cognitive impairment, disorientation, inappropriate laughter	23y	I179S / 459+1G>A, (R/0), (a), (b)	France, unpublished
36 (brother of 35)	m	34y	disorganized behavior, maniaco-depressive state	none	Inappropriate behavior, cognitive impairment, disorientation	10y	I179S / 459+1G>A, (R/0), (a), (b)	France, unpublished
37	f	29y	delusions, hallucinations, disorganized behavior	cerebellar syndrome	frontal lobe syndrome, dementia	13y	I179S / 459+1G>A, (R/0), (a), (b)	France, unpublished
38	f	28y	manic states	ataxia, epilepsy	disorganized behavior, hallucinations, cognitive decline,dementia	6y	I179S / 459+1G>A, (R/0), (a), (b)	France, unpublished
39 (sister of 40)	f	21y	impulsiveness and poor judgement	gait difficulties	manic-depressive illness, cognitive- and memory deficits	8y	I179S / D255H, (R/0), (a), (b)	USA, unpublished
40 (brother of 39)	m	33y	mood swings, memory loss	none	worsening cognitive- and mnestic deficits	4y	I179S / D255H, (R/0), (a), (b)	USA, unpublished
41	m	25y	agressive behavior	none	worsening cognitive deficits	3y	I179S / 459+1G>A, (R/0), (a), (b)	USA, unpublished
42	m	21y	confusion, hallucinations, apathy, memory deficits	none	worsening cognitive deficits, frontal lobe syndrome	9y	I179S / R244C, (R/0), (a), (b)	USA, unpublished

* order of cases is according to time of inclusion into our study; mutations determined by: (a) mutated primer modulated PCR restriction fragment length polymorphism, (b) sequenzing analysis, (c) single stranded conformation polymorphism, (nk) not known

E-Table 3: Primers used for sequencing				
Exons	Position*		Sequence (5'→3')	Annealing temp (°C)
Exon1	76 → 57	Forward	TCT GCG GTA TCG GAA AGA GC	57
	289 → 308	Reverse	GAG ACA GAC GTT TTT CCC GC	
Exon2	333 → 352	F	TGT CTG TCT CAG GGA CTC TG	57
	649 → 668	R	ATC AAG GGC TGG GGG ACT TT	
Exon3	649 → 668	F	AAA GTC CCC CAG CCC TTG AT	57
	984 → 1003	R	AGA CTG GAG TTA GCA CTG GG	
Exon4	879 → 908	F	ATG ACC TCA TGG CCG ACG CCC AGC GCC AGG	65
	1198 → 1214	R	GCC GCA GCA CCC AGC TG	
Exon5	1471 → 1490	F	GCT CAT GAG CGC CTC CTG TG	58
	1623 → 1652	R	AGG GTT CCA AGG AGA GGG CCT GCG GAC TGA	
Exon6	1680 → 1699	F	AAC TGA GTG ACT GAC CAG CC	57
	1916 → 1935	R	CAC TGA GGC ACA GAC TCT CA	
Exon7	2034 → 2053	F	CCC AGG TAT GTG CAG TGC TT	57
	2279 → 2298	R	CCA ATT CTG TGC ACA GGG CA	
Exon8	2305 → 2324	F	ACC CAG GCT CTG CCC ACA GT	58
	2663 → 2682	R	GCC ATC ACA TGC CCA GGC CA	

* Refers to the gene nucleotide numbering, with the A of ATG as nucleotide number 1

E-Appendix 1

To identify the complete genotype both strands of the PCR fragment containing each of the eight ASA exons were sequenced in nine cases (28 and 35 – 42). Briefly, a total of 25 cycles was performed with the following steps: 10s at 96°C, 10s at 50°C and 4min at 60°C in a thermocycler (Hybaid), using the primers given in Table 3. PCR products were purified (Millipore filtration system) and controlled on a 1% agarose gel. Sequencing was performed in 20µl of a mix containing 1µl Big Die Terminator, 3.4µl 5x sequencing buffer containing 10pmol of each primer (forward and reverse), 11.4µl distilled water and 2µl purified PCR products. Sequence products were purified with G50 superfine columns (Amersham) and diluted (10µl) in distilled water (30µl). The 96 wells-plate was settled in a 3100 sequencer (Applied Biosystems). Sequence electrophoretograms were analysed by SepScape or Autoassembler (Applied Biosystems) software.

E-Appendix 2

Characteristic cases

Case 1: P426L homozygote with gait disturbance and late cognitive decline

In 1976, a boy of 13 years, who was previously healthy, developed paravertebral thoracic pain on ventral flexion of the cervical spine. X-ray examination of the spine was unremarkable. Orthopaedic treatments failed, and the pain spread to the whole spine. Nevertheless, he was able to serve his apprenticeship with a locksmith. In 1983, he became aware of an increasing gait disturbance. Neurological investigation revealed a spastic tetraparesis with cerebellar ataxia. Sensitivity was unremarkable, as was psychiatric investigation. Brain CT showed central atrophy and a left parietal hypodensity, the EEG revealed slowing over the left hemisphere, and CSF immunoglobulines were elevated. A cervical myelography was unremarkable, as was needle EMG, but nerve conduction studies were not done. Progressive multiple sclerosis was diagnosed and ACTH treatment was started. After temporary improvement, the spastic tetraparesis with ataxia worsened, but the patient was still able to walk. In 1989, concentration and memory deficits were noticed and a general IQ of 87 was measured. Brain MRI showed diffuse bilateral leukoencephalopathy, central atrophy and symmetric pyramidal tract degeneration. Elevated sulfatides in urine and severely reduced ASA-activity in leukocytes established the diagnosis of MLD 13 years after onset. In the following years, there was profound mental deterioration and bilateral reduction of visual acuity due to optic atrophy. Since 1996 the patient was wheelchair bound, but still able to manage living alone in his apartment.

Case 35: I179S / 459+1G>A compound heterozygote with psychocognitive disturbance

The patient, born in 1963, was a healthy girl and had a normal school performance until 17 years of age. At this time, she presented with behavioral problems, delusions, hallucinations and a disorganized speech. These symptoms were treated by neuroleptics which improved her temporarily. When she was 24 she was diagnosed as having classical schizophrenia according to the criteria of DSM 4. She persisted with delusions and hallucinations, had a disorganized speech, unmotivated laughing, stereotypes and personality changes with abnormal social behavior. She was never in a depressed or manic state. Her brother, case 36, presented cognitive troubles and disorientation, which led to hospitalization in a neurological department where MRI showed leukodystrophy. Therefore, she was re-examined when 39 years old, and the diagnosis of MLD was suspected. At that age she was pleasant, able to communicate, but had cognitive deficiencies. She ran away several times from home and was unable to find her way by herself, unable to dress herself, but was able to eat by her own. Neurological examination was entirely normal as were nerve conduction studies. MRI performed at age 40 showed fronto-parieto-occipital demyelination and a cortico-subcortical atrophy. She presented decreased level of ASA (Nine units; range of controls 55-80 units) and sulfatiduria. Her abnormal behavioral manifestations and cognitive impairment made her unable to live by herself. Up to now, although she has major mental deficiencies, she is still able to communicate and still has no neurological signs.