MOLECULAR BASIS OF DIFFERENT FORMS OF METACHROMATIC LEUKODYSTROPHY

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Abstract Background. Metachromatic leukodystrophy is an autosomal recessive inherited lysosomal storage disorder caused by a deficiency of arylsulfatase A. Three forms of the disease can be distinguished according to severity and the age at onset: late infantile (1 to 2 years), juvenile (3 to 16), and adult (>16).

Methods and Results. To understand the molecular basis of the different forms of the disease, we analyzed arylsulfatase A alleles associated with metachromatic leukodystrophy. Two alleles (termed I and A) were identified and accounted for about half of all arylsulfatase A alleles among 68 patients with metachromatic leukodystrophy whom we examined. Sufficient information was available for 66 of the patients to allow classification of their disease. Of the six instances of homozygosity for allele I, all were associated with the late-infantile form of the disease; of the eight instances of homozygosity for allele A, five were associated with the adult form and three with the juvenile form. When both alleles were present, the juvenile form resulted (seven of seven instances). Heterozygosity for allele I (with the other allele unknown) is usually associated with late-infantile disease, and heterozygosity for allele A with a later onset of the disease. The clinical variability can be explained by the different levels of residual arylsulfatase A activity associated with these genotypes.

Conclusions. Like many lysosomal storage disorders, metachromatic leukodystrophy shows clinical heterogeneity that seems to reflect genetic heterogeneity. One of the known alleles (allele I) is associated with earlier and more severe disease than the other (allele A). (N Engl J Med 1991; 324:18-22.)

ARYLSULFATASE A is a lysosomal enzyme involved in the degradation of cerebroside sulfate, a polar glycolipid that is found mainly as a component of the myelin sheaths of the nervous system. Deficiency of arylsulfatase A results in metachromatic leukodystrophy, a lysosomal storage disease characterized by the accumulation of cerebroside sulfate. The storage of sulfatide mainly affects the nervous system, where it is associated with progressive demyelination and loss of white matter. Clinically, there may be neurologic symptoms, such as weakness, ataxia, progressive spastic tetraparesis, optic atrophy, and dementia. In older patients psychiatric symptoms may precede the neurologic manifestations. The age of onset ranges from less than two years to the third or fourth decade of life. Three forms of metachromatic leukodystrophy can be distinguished according to the age at onset: late infantile (1 to 2 years), juvenile (3 to 16), and adult (>16). The late-infantile form is fatal within a few years, whereas the course of the juvenile and adult forms is more protracted. The incidence of metachromatic leukodystrophy among whites is estimated to be 1 in 40,000, suggesting a frequency of 0.5 percent for metachromatic leukodystrophy alleles. Approximately 0.5 percent to 2 percent of the population has very low levels of residual arylsulfatase A activity but is asymptomatic. This condition has been called arylsulfatase A pseudodeficiency and is in most cases caused by homozygosity for the arylsulfatase A pseudodeficiency allele. Estimates of the frequency of this allele range from 7.3 percent to 15 percent. We recently analyzed the mutations in this allele and found that the low residual activity is due to reduced synthesis of arylsulfatase A, which can be explained by the loss of a polyadenylation signal. However, at least in those who are homozygous for the arylsulfatase A pseudodeficiency allele, enough arylsulfatase A is synthesized to prevent clinically apparent disease.

In this report we describe four genotypes that are combinations of two arylsulfatase A alleles that cause metachromatic leukodystrophy and the pseudodeficiency allele. These genotypes are associated with different levels of residual arylsulfatase A activity — from 0 to approximately 10 percent — and represent the clinical spectrum from the most severely affected children to apparently healthy adults.

Methods Amplification of Fragments from Genomic DNA

Procedures used for the amplification of DNA fragments have been described elsewhere. For the amplification of fragment D, the elongation time was increased to five minutes. The sequences of oligonucleotides used to amplify the fragments shown in Figure 1 were as follows: for fragment A, oligonucleotide 1 sequence 5’ TCAATTCTTCGCTGAGCGCAAGTAGGCCTT and oligonucleotide 3 sequence 5’ GCAAGACTGGAGTACAGC’; for fragment B, oligonucleotide 4 sequence 5’ CCGAATTCTTCTGATGCGGAACTGATGACG’ and oligonucleotide 5 sequence 5’ GCCAGAGTCTCCTATGTTACCAGG’; and for fragment D, the sequence of oligonucleotide 1 was the same as that in fragment A and oligonucleotide 2 sequence 5’ GAGGAATCCAGTGAGGAAGGCACTGAGG’; the fragments were subcloned into M13mp18 and M13mp19 and sequenced according to standard techniques.

Hybridization of Allele-Specific Oligonucleotides

The genotype of the patients was determined by hybridization of allele-specific oligonucleotides. Fragments A and C (Fig. 1) were amplified, blotted onto nylon filters, and hybridized to oligonucleotides end-labeled with [32P]ATP and T4 kinase. The methods used have been described elsewhere. The sequence of oligonucleotides used to detect the splice-site mutation of allele I was 5’ TGTTCTCCTACTTCTGTTG’ (57°C) for the normal allele and 5’ TGTTCTCCTACTTCTGTTG’ (57°C) for the mutant allele. The sequence of oligonucleotides used to detect the exchange of leucine for proline at position 426 in allele A was 5’ CATAGAGCAAGCAGAAGCGATCGG’.
Figure 1. Mutations in the Arylsulfatase A Gene.

Boxes indicate exons; solid parts represent translated and hatched parts untranslated regions. Lines depict introns, and triangles potential sites of N-glycosylation. ATG and TGA are the initiation codon and termination codon, respectively. A, C, and D are the fragments that were amplified: A and C for allele-specific oligonucleotide hybridization, and C and D for subcloning and sequencing. ON 1 through 5 indicate the binding sites of the oligonucleotides (ON) used for the amplification of DNA (see Methods). Arrows show the location of the mutations. The mutations and the codons in which they occur are shown. Adjacent sequences are shown for the splice-site mutation.

GGGGGCT3’ (59°C) for the normal allele and 5’CATAGAGCGAG-GAGGGGCT3’ (59°C) for the mutant allele. (Values in parentheses indicate the temperatures at which the filters were washed to give an allele-specific signal.)

Transfection and Western Blot Analysis

Transfection of the arylsulfatase A complementary DNA (cDNA) into baby-hamster kidney cells was performed as described elsewhere. Western blot analysis and treatment of cultured human fibroblasts with carbobenzoxy Phe-Ala diazomethylethlykete were carried out as described elsewhere.

RESULTS

Identification of Mutations in the Arylsulfatase A Gene

The arylsulfatase A gene has been characterized recently. It can be amplified in two overlapping fragments (Fig. 1). To identify the molecular defects in metachromatic leukodystrophy, we amplified the arylsulfatase A gene of a patient with juvenile onset of the disease. The amplified fragments were subcloned into M13mp18 and M13mp19 and sequenced in both directions.

Two different metachromatic leukodystrophy alleles were identified in this patient. One allele — designated allele I — differed in three positions from the published gene sequence for arylsulfatase A: a G → T transversion changing tryptophan at position 193 to cysteine, a C → G transversion changing threonine at position 391 to serine, and a G → A transition destroying the splice donor site of exon 2 by changing the classic exon—intron boundary consensus sequence AG gt . . . to AG at . . . . The exchange of serine for threonine at position 391 is a polymorphism that was found in the DNA of four of eight healthy controls (unpublished data).

The exchange of cysteine for tryptophan at position 193 was introduced into the arylsulfatase A cDNA by site-directed mutagenesis, and a mutated cDNA was transiently expressed in baby-hamster kidney cells after transfection. The arylsulfatase A activity measured in the transfected cells was comparable to that in cells transfected with wild-type cDNA, indicating that the exchange of cysteine for tryptophan at position 193 is functionally silent. Of the three changes found in allele I, only the loss of the splice donor site was considered to be of relevance for metachromatic leukodystrophy.

The second metachromatic leukodystrophy allele — designated allele A — differed from the published arylsulfatase A sequence in one position: a C → T transition causing the change of proline at position 426 to leucine. The introduction of this mutation into the arylsulfatase A cDNA and its expression in baby-hamster kidney cells led to a small increase in the activity of arylsulfatase A in the transfected cells. Three independent transfection experiments showed that this increase was only 3 percent (range, 2 to 5) of that observed in cells transfected with the normal arylsulfatase A cDNA. Metachromatic leukodystrophy allele A thus codes for low residual arylsulfatase A activity.

Frequencies of Alleles I and A among Patients with Metachromatic Leukodystrophy

Fragments A and C (Fig. 1) were amplified from the DNA of 68 patients affected with different clinical forms of the disease, and the frequencies of alleles I and A were determined by allele-specific oligonucleotide hybridization. Of the 68 patients, 50 carried at least one of the two metachromatic leukodystrophy alleles (I or A). Twenty-three patients were homozygous for either allele I or allele A or heterozygous for both alleles. In 18 patients neither allele I nor allele A was found. In total, 37 I alleles and 36 A alleles were found. Thus, the two alleles accounted for about half of all arylsulfatase A alleles in this fairly typical selection of patients with metachromatic leukodystrophy.

Distribution of Alleles I and A among Patients with Different Clinical Forms of Metachromatic Leukodystrophy

Sufficient information was available for 66 of the patients with metachromatic leukodystrophy to allow classification of their disease as late infantile, juvenile, or adult. Table 1 shows the correlation between the genotype and the clinical form of the disease. All six patients with metachromatic leukodystrophy who were homozygous for allele I (loss of the splice donor site of exon 2) had the late-infantile form, whereas five
Table 1. Genotypes and Clinical Form of Metachromatic Leukodystrophy in 68 Patients.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Patients</th>
<th>Form of Metachromatic Leukodystrophy*</th>
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<td>??</td>
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*Denotes an allele with the loss of the splice donor site of exon 2; A an allele with the substitution of leucine for proline at position 426; and ? an unidentified abnormal allele.

For two patients, there was insufficient information for classification.

of the patients who were homozygous for allele A (substitution of leucine for proline at position 426) had the adult form and three had the juvenile form. Among the patients with juvenile-onset metachromatic leukodystrophy, seven had both allele I and allele A. The I allele was found in combination with an unidentified allele in 14 patients with the late-infantile form, 2 with the juvenile form, and 1 with the adult form of the disease. The A allele was found in combination with an unidentified metachromatic leukodystrophy allele in 10 patients with either the adult or the juvenile form of the disease.

These results suggest that homozygosity for allele I predisposes patients to the late-infantile form of metachromatic leukodystrophy, homozygosity for allele A to the adult form, and compound heterozygosity of both alleles to the juvenile form. This is supported by the correlation of the age at onset with the genotype (Fig. 2). The age at onset of the disease was known for 19 patients with metachromatic leukodystrophy who had a fully identifiable genotype. In patients who were homozygous for allele I, the disease started around the age of 2 years; in patients who were homozygous for allele A, the onset was between 8 and 22 years (mean, 17.3); and in patients carrying both alleles, the onset was between 4 and 7 years (mean, 5.8).

More complete clinical information was available for five of the six patients with the late-infantile form who were homozygous for allele I. In all cases the initial symptoms were gait disturbances due to muscular hypotonia. In the older patients the first symptoms were either neurologic or psychiatric. A summary of the clinical data for most of the patients with the late-onset form shown in Figure 2 has been presented elsewhere.

Biochemical Characterization of Patients Homozygous for Allele I or Allele A

Patients homozygous for allele I who had late-infantile metachromatic leukodystrophy were characterized biochemically by the absence of arylsulfatase A polypeptides after metabolic labeling of fibroblasts with [35S]methionine (data not shown). Fibroblasts...
contain three forms of arylsulfatase A RNA; the smallest, a 2.1-kilobase (kb) species, is efficiently polyadenylated and appears to be the predominant form of messenger RNA used for translation, whereas the two larger forms (3.7 kb and 4.8 kb) are poorly polyadenylated.2 We have previously reported that the 2.1-kb species was not detectable and levels of the two larger forms were severely diminished in measurements of total RNA in a patient with metachromatic leukodystrophy who was homozygous for allele I.6 We conclude that the deficiency of the splice donor site of exon 2 renders the three forms of arylsulfatase A RNA unstable and that the presence of allele I does not lead to the synthesis of detectable amounts of polypeptides that cross-react with arylsulfatase A.

The biochemical phenotype of two patients with the adult form of metachromatic leukodystrophy who proved to be homozygous for allele A in this study was reported earlier.9 Incubation of fibroblasts from these patients with inhibitors of cysteine proteinases partially restored the arylsulfatase A activity. The deficiency was thought to result from an increased susceptibility of the mutant arylsulfatase A polypeptides to lysosomal cysteine proteinases.9,13 The accumulation of arylsulfatase A polypeptides in fibroblasts from a patient homozygous for allele A in response to incubation with a cysteine proteinase inhibitor is shown in Figure 3. We conclude from these results that the exchange of leucine for proline at position 426 increases the susceptibility of arylsulfatase A to lysosomal cysteine proteinases and results in a severe reduction in the half-life of the mutant polypeptides.

Identification of Compound Heterozygotes for the Arylsulfatase A Pseudodeficiency Allele and Allele I

We previously identified the mutations in the arylsulfatase A pseudodeficiency allele.7 The gene frequency of this allele is estimated to range from 7.3 to 13 percent, and persons who are homozygous for the pseudodeficiency allele are clinically healthy.9,14 We have identified two persons with compound heterozygosity for the pseudodeficiency allele and the metachromatic leukodystrophy allele I. The combination of these alleles is expected to reduce arylsulfatase A activity to about 10 percent3 of normal. Both persons are in their third decade of life and so far have no symptoms characteristic of metachromatic leukodystrophy.

Discussion

It is apparent from this study that a clear correlation exists between the arylsulfatase A genotype and the clinical phenotype. The most severe type of metachromatic leukodystrophy, the late-infantile form, is associated with homozygosity for allele I, which does not encode for functional arylsulfatase A polypeptides. One copy of allele A, which encodes for an unstable but active arylsulfatase A, is sufficient to mitigate the clinical course and produce the juvenile form, whereas two copies of this allele allow for the mildest course of the disease, the adult form of metachromatic leukodystrophy. One copy of the arylsulfatase A pseudodeficiency allele is sufficient to sustain a normal phenotype. Although one cannot be certain that symptoms will not develop in these persons very late in life, it seems that the critical threshold is approximately 10 percent residual arylsulfatase A activity. Small variations in residual arylsulfatase A activity may greatly influence the accumulation of its substrates and its clinical manifestation.

Bone marrow transplantation has been proposed as a treatment for metachromatic leukodystrophy. Improvement of the clinical symptoms was recently reported in a girl who had received a bone marrow transplant several years earlier.15 Our data support the view that the low levels of enzyme delivered to the brain by the microglial cells derived from the donor’s bone marrow may be sufficient to alter the course of the disease. Patients with the juvenile or adult form of metachromatic leukodystrophy should be better candidates for bone marrow transplantation than those with the late-infantile form, because their level of residual arylsulfatase A activity is higher and therefore less enzyme needs to be replaced.

Although the average age at onset is clearly different in the three forms of metachromatic leukodystrophy, there is a remarkable variability among the patients who are homozygous for allele A. This genotype can be found both among patients with the juvenile form and among those with the adult form of the disease. Such variability can occur within a single family16 and suggests the existence of additional loci that can influence the clinical phenotype of metachromatic leukodystrophy. For example, the pattern of expression and activity of lysosomal proteinases may differ from person to person, and this in turn may influence the half-life of the mutant arylsulfatase A protein and explain the variability of the clinical manifestations in adult patients with metachromatic leukodystrophy.

References

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THE EFFECT OF EPILEPSY OR DIABETES MELLITUS ON THE RISK OF AUTOMOBILE ACCIDENTS

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Abstract. Background. Previous studies of possible associations between chronic medical conditions and traffic safety have been inconsistent and subject to bias because of the incomplete identification of affected persons. Recent advances in the diagnosis and management of epilepsy and diabetes mellitus have improved the control of these disorders and suggest a need to reexamine the risk of traffic mishaps among patients with these conditions.

Methods. We conducted a population-based retrospective cohort study of 30,420 subjects 16 to 50 years of age, with and without epilepsy or diabetes mellitus. Subjects included all the licensed drivers in seven contiguous ZIP Code areas in which the Marshfield Clinic and St. Joseph’s Hospital, Marshfield, Wisconsin, are the primary sources of medical care. Standardized rates of moving violations and accidents over a four-year period (1985 through 1988) were compared in affected and unaffected cohorts.

Results. Standardized mishap ratios for subjects with diabetes were 1.14 for all moving violations (P = 0.23) and 1.32 for accidents (P = 0.01); for subjects with epilepsy the ratios were 1.13 for moving violations (P = 0.25) and 1.33 for accidents (P = 0.04).

Conclusions. We conclude that drivers with epilepsy or diabetes mellitus have slightly increased risks of traffic accidents as compared with unaffected persons. The increases in risk observed in our study were generally smaller than those in previous studies, and we believe they are not great enough to warrant further restrictions on driving privileges. (N Engl J Med 1991; 324:22-6.)

EVER since automobiles were introduced to the public in the late 1900s, some medical conditions have been recognized as posing risks of driving accidents. Conditions such as epilepsy and diabetes mellitus that can impair consciousness or cause loss of body control have been of special interest to those concerned with traffic safety. By 1940, medical advances such as the discoveries of electroencephalography and of new antiepileptic drugs such as phenytoin had demonstrated that epilepsy could often be controlled. In the case of diabetes, insulin and other medications used to control the disease may actually increase the risk of traffic accidents, because the frequency and severity of hypoglycemia are increased among patients with insulin-dependent diabetes treated intensively.1 Laws governing the issuance of driver’s licenses to patients with an epileptic-seizure disorder or diabetes mellitus differ from state to state. Driving is important to Americans for social, educational, economic, recreational, and other reasons, but motor vehicle accidents are an important cause of injury, death, and disability. Physicians are frequently asked whether impaired persons should continue to have full driving privileges, and they weigh the loss of the patient’s mobility against the risk of a traffic accident to the patient and the community. These decisions have been based on studies that were sometimes poorly controlled and that often gave conflicting results.

A previous study has suggested that drivers with chronic medical conditions, especially alcoholism, have higher accident rates than the rest of the driving public.2 In contrast, Swedish investigators have con-