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Genetics

Arylsulfatase A pseudodeficiency: Loss of a polyadenylylation signal and N-glycosylation site

(lysosomal storage disorders/metachromatic leukodystrophy)

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ABSTRACT **Metachromatic leukodystrophy is a metabolic disorder caused by the deficiency of arylsulfatase A. Deficiency of this enzyme is also found in apparently healthy individuals, a condition for which the term pseudodeficiency was introduced. The arylsulfatase A (cerebroside-3-sulfate 3-sulfohydrolase; EC 3.1.6.8) (ASA) encoding gene was isolated from an individual homozygous for the ASA pseudodeficiency allele. Sequence analysis revealed two A → G transitions. One changes Arg-350 to serine, which leads to the loss of a utilized N-glycosylation site. This loss explains the smaller size of ASA in ASA pseudodeficiency fibroblasts. The introduction of Ser-350 into normal ASA cDNA does not affect the rate of synthesis, the stability, or the catalytic properties of ASA in stably transfected baby hamster kidney cells. Therefore, the loss of the N-linked oligosaccharide does not contribute to the reduction of ASA activity in ASA pseudodeficiency. The other A → G transition changes the first polyadenylylation signal downstream of the stop codon from AATAAC to AGTAAC. The latter causes a severe deficiency of a 2.1-kilobase (kb) mRNA species. The deficiency of the 2.1-kb RNA species provides an explanation for the diminished synthesis of ASA seen in pseudodeficiency fibroblasts. Amplification of genomic DNA and hybridization with allele-specific oligonucleotides detected both mutations in four unrelated individuals with ASA pseudodeficiency.**

Metachromatic leukodystrophy is a lysosomal storage disorder caused by the deficiency of arylsulfatase A (cerebroside-3-sulfate 3-sulfohydrolase; EC 3.1.6.8) (ASA), which leads to the intralysosomal accumulation of cerebroside sulfate (1). The incidence of this recessively inherited disease is estimated to be 1:40,000. The sulfatide storage affects mainly the central nervous system, causing a progressive demyelination that eventually leads to the death of the patient. Based on the age of onset three clinical variants of metachromatic leukodystrophy are differentiated: late infantile, juvenile, and adult (1). The molecular basis for this heterogeneity is not known, and the determination of the residual ASA activity does not permit differentiation between the various forms.

Occasionally, the deficiency of ASA is found in apparently healthy individuals, a condition known as ASA pseudodeficiency (2). The ASA deficiency in metachromatic leukodystrophy and pseudodeficiency are caused by allelic mutations of the same gene (3). It has been shown that the ASA synthesized in fibroblasts from individuals with ASA pseudodeficiency is reduced in quantity and smaller in size when compared to normal (4). While the size difference has been attributed to altered glycosylation, the reasons for the attenuated ASA activity remained unclear.

The high frequency of the ASA pseudodeficiency allele of 7–15% (5, 6) and the inability to distinguish reliably homo- and heterozygotes for nonfunctional and pseudodeficiency ASA alleles by ASA activity determinations with artificial or natural substrates pose serious problems in the genetic counseling and prenatal diagnosis of metachromatic leukodystrophy (7). Thus, the availability of allele-specific tests would facilitate genetic counseling and pre- and postnatal diagnosis in families at risk for metachromatic leukodystrophy.

We show here that in the ASA pseudodeficiency allele one of two utilized N-glycosylation sites is missing. The attenuated ASA activity, however, is due to a second mutation in a polyadenylylation signal, used to generate the major poly $(A)^+$ ASA mRNA species in normal fibroblasts. Deficiency of this mRNA species in ASA pseudodeficiency explains the severely diminished synthesis of ASA polypeptides, which are smaller in size but equivalent in stability and catalytic activity to normal ASA. Based on the two mutations allele-specific tests were developed that allow identification of the ASA pseudodeficiency allele.

MATERIALS AND METHODS

Restriction enzymes and DNA modifying enzymes were from Boehringer Mannheim, New England Biolabs, or BRL. *Thermus aquaticus* (*Taq*) polymerase was purchased from Cetus. [^{35}S]methionine (specific activity > 600 Ci/mmol; 1 Ci = 37 GBq), [α-^{32}P]CTP (specific activity > 3000 Ci/mmol), and [α-^{32}P]ATP (specific activity > 3000 Ci/mmol) were from Amersham. MLD cell lines used were from the cell deposit of the Hôpital Debrousse Lyon (439, 2301), from K. Sandhoff (Bonn) (Kn), or from our own diagnostic samples (77k).

Cell Culture and Metabolic Labeling. Human fibroblasts and baby hamster kidney (BHK)-21 cells were maintained in culture as described (8). Metabolic labeling of cells, immunoprecipitation of ASA, and endoglucosaminidase H digestion of the immunoprecipitates were done according to refs. 8 and 9.

RNA Isolation and Northern (RNA) Blot Analysis. Total and poly $(A)^+$ RNA were isolated, separated on a gel, blotted, and hybridized as described (10).

Oligonucleotides and DNA Probes. Oligonucleotides were synthesized and labeled as described (10). DNA probes were labeled according to ref. 11.

Isolation of Genomic ASA Clones. The normal ASA allele was cloned from a human genomic library in EMBL3 (Clontech) by standard techniques (12). A 6-kilobase (kb) *Eco*RI fragment within the 14-kb insert of the isolated clone containing the entire ASA gene (J.K., unpublished work) was subcloned into Bluescript (Stratagene). The pseudodeficiency allele was cloned from a library made from size-selected genomic DNA of an individual with ASA pseudodeficiency. One hundred eighty micrograms of DNA isolated

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Abbreviations: ASA, arylsulfatase A; BHK, baby hamster kidney; nt, nucleotides.

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from peripheral blood cells was digested with *Eco*RI and four enzymes, which do not cut within the genomic 6-kb ASA fragment (*Xba* I, *Sst* I, *Eco*RV, and *Bcl* I). Digested DNA was separated on 0.6% agarose gel, and the region between 5 and 7 kb was cut into seven slices (13), which were electroeluted in 0.2 TBE (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA, pH 8.3) overnight. The electroeluted material was concentrated to ≈300 μl in a Speedvac vacuum, extracted with phenol and chloroform, and after vacuum addition of 3 μg of carrier tRNA precipitated overnight with ethanol at −20°C. The pellets were redissolved, and the fraction with the highest frequency of ASA DNA was determined by Southern blot hybridization. An aliquot of this fraction was ligated to λgt10 arms (Promega), packaged (Stratagene packaging extracts), and plated. Of 16,000 independent plaques, 14 hybridized with the ASA cDNA probe. One of these plaques was purified, and its 6-kb *Eco*RI fragment was subcloned into Bluescript. Restriction fragments of the normal and the pseudodeficiency DNA were subcloned into M13mp18 and mp19. Regions known to code for the potential glycosylation sites and the polyadenylylation signal were sequenced by using ASA-specific oligonucleotides as primers.

Amplification of Genomic DNA and Hybridization with Allele-Specific Oligonucleotides. One microgram of genomic DNA was amplified in 100 μl of 0.2 mM dNTP/10% dimethyl sulfoxide/50 mM KCl/10 mM Tris·HCl, pH 8.3/1.5 mM $MgCl_2$/0.01% gelatine using the oligonucleotides ON 16 and ON 3.9 (ON 16, 5′-TTGATGGCGAACTGAGTGAC-3′; ON 3.9, 5′-TTCCTCATTCGTACCACAGG-3′) as primers. After initial denaturation for 7 min at 95°C, 2.5 units of *Taq*-polymerase (Cetus) was added. Conditions for each of the 40 cycles were 1-min denaturation at 95°C, 1-min hybridization at 50°C, and 4-min elongation at 72°C. Final extension was 7 min. About 100 ng of amplified fragments was loaded on a 1% agarose gel, denatured, and blotted onto Hybond-N filters (Amersham). Filters were prehybridized for 2 hr at 55°C in 4 ml of 6× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7)/0.1% SDS/0.05% sodium pyrophosphate/salmon sperm DNA at 100 μg/ml. Oligonucleotides A–D (A, 5′-AAGGTGACATTGGGCAGTGG-3′; B, 5′-AAGGTGACACTGGGCAGTGG-3′; C, 5′-CTGGTGTTATTACGTTATC-3′; and D, 5′-CTGGTGTTACTACGTTATC-3′) were phosphorylated by using T4 polynucleotide kinase and [α-^{32}P]ATP (specific activity > 3000 Ci/mmol). Approximately 5×10^5 cpm/ml were added and allowed to hybridize for 4 hr. Hybridization temperature for oligonucleotides A + B was 55°C, for oligonucleotides C + D it was 48°C. Filters were washed at room temperature twice for 15 min and then for 10 min in 6× SSC/0.1% SDS/0.05% sodium pyrophosphate at 62°C (oligonucleotide A), 60°C (oligonucleotide B), or 52°C (oligonucleotides C and D).

Site-Directed Mutagenesis and DNA Transfections. The normal ASA cDNA was cloned into M13mp18, and single-stranded DNA was isolated. Site-directed mutagenesis was done as described (14). After mutagenesis double-stranded DNA was prepared, and the *Eco*RI cDNA fragment was isolated and cloned into the expression vector pBEH (15). Transfections of BHK cells were done as described (10). Expression vectors were cotransfected with pSV2pac (16). Two days after transfection the medium was supplemented with puromycin at 5 μg/ml for the selection of stably transfected cells.

RESULTS

Synthesis of ASA in Normal and ASA Pseudodeficiency Fibroblasts. Fibroblasts were labeled with [^{35}S]methionine in the presence of 10 mM NH_4Cl/2 mM deoxymannojirimycin. NH_4Cl was added to induce secretion of the newly synthesized ASA into the medium (17). The amount of ^{35}S-labeled ASA in the medium is not affected by degradation in lysosomes and reflects, therefore, the rate of ASA synthesis more faithfully than the ^{35}S-labeled ASA isolated from cells labeled without NH_4Cl. Deoxymannojirimycin was added to prevent the processing of N-linked oligosaccharides to complex type structures (18), thereby facilitating the complete deglycosylation of ASA by endoglucosaminidase H.

Normal fibroblasts secrete a 62-kDa ASA polypeptide (Fig. 1), whereas the ASA secreted by ASA pseudodeficiency fibroblasts is ≈2.5 kDa smaller. The amount of ^{35}S-labeled ASA secreted by ASA pseudodeficient cells is only 10% compared to normal. Both media contained comparable amounts (±10%) of ^{35}S-labeled polypeptides and of ^{35}S-labeled cathepsin D, a lysosomal enzyme not affected in ASA pseudodeficiency (data not shown). Thus, the apparent rate of ASA synthesis is severely reduced in ASA pseudodeficiency. Digestion with endoglucosaminidase H reduced the size of the normal ASA by 5 kDa and that of the pseudodeficiency ASA by 2.5 kDa and produced deglycosylated polypeptides of the same size (Fig. 1). This result indicates that normal and pseudodeficiency ASA have polypeptide backbones of the same size and differ in the number of N-linked oligosaccharides.

Loss of the Third Potential N-Glycosylation Site in the ASA Pseudodeficiency Allele. A 6-kb ASA genomic clone containing the entire ASA gene was isolated from a library made from size-selected genomic DNA of an individual homozygous for ASA pseudodeficiency allele (5). Knowledge of the ASA cDNA sequence and of the intron–exon organization of the ASA gene (J.K., unpublished work) allowed the sequencing of the exons that contain the potential N-glycosylation sites. The N-glycosylation sites corresponding to cDNA nucleotides 472–480 and 550–558 (10) were present in the ASA pseudodeficiency allele. In the third N-glycosylation site at position 1048–1056 an A → G transition was found at nucleotide (nt) 1049 (Fig. 2*A*), which changes Asn-350 to a serine residue and causes the loss of this N-glycosylation site.

Expression of Normal ASA and ASA Lacking the Third Potential N-Glycosylation Site in BHK Cells. The A → G transition changing Asn-350 to a serine residue was introduced into wild-type ASA cDNA by site-directed mutagenesis. Both the wild-type and the mutated cDNA were cloned into the expression vector pBEH and cotransfected with a plasmid containing a puromycin resistance gene in BHK cells. Stably transfected cells were selected with puromycin.

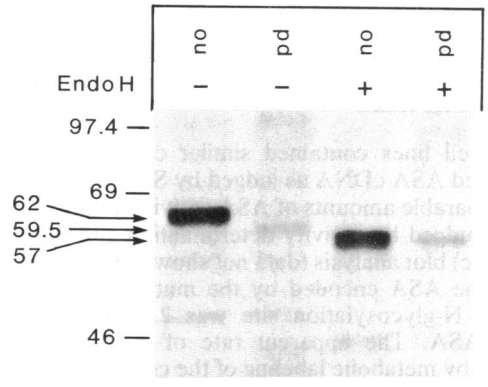

FIG. 1. ASA synthesized in normal (no) and ASA pseudodeficiency (pd) fibroblasts. Fibroblasts (25 cm^2 flasks) were labeled for 16 hr with 50 μCi of [^{35}S]methionine in 10 mM NH_4Cl/2 mM deoxymannojirimycin. ASA was immunoprecipitated from the medium. Immunoprecipitates are shown before (left) and after (right) digestion with endoglucosaminidase H (Endo H). Sizes of the glycosylated (62- and 59.5-kDa) and deglycosylated (57-kDa) ASA polypeptides and the position of M_r standards (46, 69, and 97.4 kDa) are given at the left.

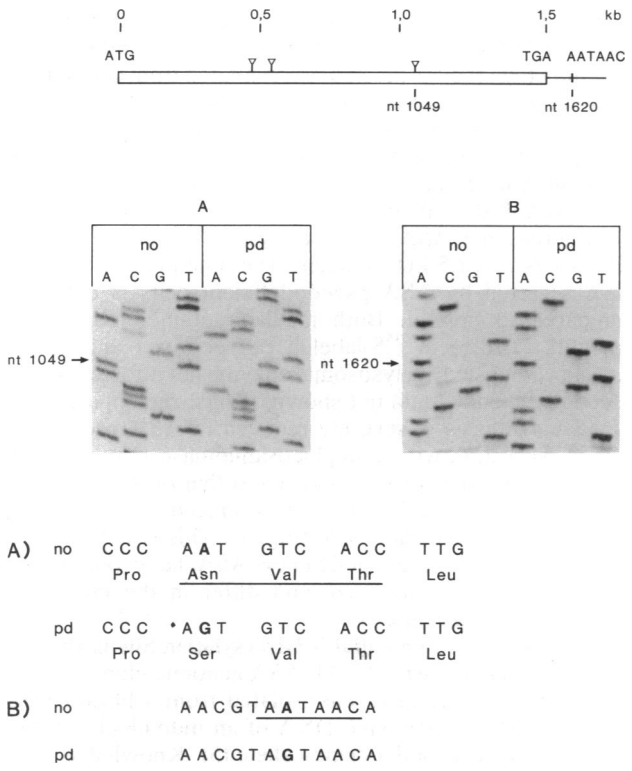

FIG. 2. Mutations in the pseudodeficiency allele. (*Top*) Map of the ASA cDNA (10). ATG indicates the start codon and TGA the stop codon; the coding region is boxed. ▽ shows potential glycosylation sites; AATAAC is the first polyadenylylation signal downstream of the termination codon. Positions of nucleotides exchanged in the pseudodeficiency (pd) allele are indicated. (*Middle*) Sequences surrounding the third N-glycosylation site (*A*) and the (first) polyadenylylation signal downstream of the termination codon (*B*) in the normal (no) and pseudodeficiency (pd) allele. A, C, G, and T indicate the nucleotides to be read. Sequences of the normal allele are at the left of and the pseudodeficiency allele are at the right of each panel. (*Bottom*) The nucleotide sequences and deduced amino acid sequences around the third N-glycosylation site (*A*) and in the polyadenylylation signal (*B*) are shown. A → G transitions are shown in boldface letters, and the potential N-glycosylation site and the polyadenylylation signal in the normal allele are underlined. Sequencing was done with ASA-specific oligonucleotides as primers. In *A* the coding strand was sequenced; in *B* the noncoding strand was sequenced. To allow direct reading of the sequences the sequencing gel shown in *B* is reproduced after upside–down and frontside–backside inversion. Both mutations were sequenced on both strands. In addition to the sequences shown here ≈90% of the coding regions of the ASA pseudodeficiency gene were sequenced, and no other mutations were found.

Both cell lines contained similar copy numbers of the transfected ASA cDNA as judged by Southern blot analysis and comparable amounts of ASA activity and ASA polypeptides as judged by activity determination and Western (immunologic) blot analysis (data not shown). Only the apparent size of the ASA encoded by the mutant lacking the third potential N-glycosylation site was 2.5 kDa smaller than normal ASA. The apparent rate of ASA synthesis was analyzed by metabolic labeling of the cells in 10 mM NH_4Cl. The medium contained similar amounts of labeled ASA polypeptides and ASA activity (Fig. 3), showing that the third N-glycosylation site is glycosylated in wild-type ASA. Deficiency of this oligosaccharide side chain in the pseudodeficiency ASA does not affect the specific catalytic activity of the ASA polypeptides. Because an apparently normal rate of synthesis results in normal enzyme levels, stability of the ASA polypeptides is not affected by loss of the N-linked oligosaccharide. The A → G transition causing the altered

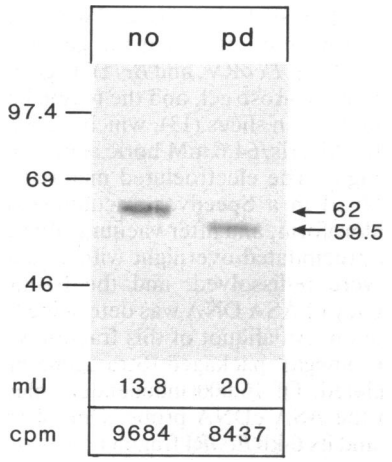

FIG. 3. Immunoprecipitation of ASA from transfected BHK cells. BHK cells expressing normal ASA or ASA deficient in the third N-glycosylation site were labeled for 3 hr with 30 μCi of [^{35}S]methionine in 10 mM NH_4Cl. ASA activity was determined in the medium, and ASA polypeptides were immunoprecipitated from the medium. The ^{35}S-labeled ASA polypeptides were excised from the gel and measured. ASA activity [nmol/min, milliunits (mU)] in the medium and the radioactivity in the ASA polypeptides (cpm) are shown at bottom. The ASA activity and ^{35}S-labeled ASA polypeptides in nontransfected BHK cells are below the limit of detectability (data not shown). Position of molecular mass standard (at left) and the molecular masses of ASA polypeptides (at right) are indicated in kDa.

glycosylation of ASA explains, therefore, the smaller size of ASA, but not the severely diminished polypeptide and activity levels of ASA in ASA pseudodeficiency fibroblasts.

Mutation in a Polyadenylylation Signal Is Associated with Loss of the Major Poly(A)$^+$ ASA mRNA Species in ASA Pseudodeficiency. ASA cDNA hybridizes to three different RNA species of 2.1, 3.7, and 4.8 kb in poly(A)$^+$ RNA from normal human fibroblasts (10). The 2.1-kb mRNA species that represents 90% of poly(A)$^+$ ASA mRNA was severely diminished in poly(A)$^+$ RNA from individuals with ASA pseudodeficiency. Trace amounts of 2.1-kb mRNA were detectable upon prolonged exposure of the blots (Fig. 4). Because the three RNA species arise from the use of different polyadenylylation signals (J.K., unpublished work), the deficiency of the 2.1-kb mRNA species may be caused by loss of the polyadenylylation signal used for its generation. Sequencing revealed an A → G transition at a position corresponding to nt 1620 of the ASA cDNA (10). This substitution changes the first polyadenylylation signal downstream of the termination codon from AATAAC (19) to AGTAAC (Fig. 2*B*). Loss of the polyadenylylation signal associated with deficiency of the 2.1-kb mRNA species provides an explanation for the decreased synthesis of ASA in pseudodeficiency.

Deficient termination of transcription at the first polyadenylylation signal in ASA pseudodeficiency should result in increased amounts of the larger 3.7- and 4.8-kb ASA RNA species (20); this was not seen in the poly(A)$^+$ (Fig. 4). When total RNA from normal fibroblasts was assayed, the 3.7- and 4.8-kb ASA RNA species were the major RNAs hybridizing to ASA cDNA. A similar pattern was seen in RNA from four patients with metachromatic leukodystrophy, where the level of ASA RNA from one patient was severely reduced, if present at all (Fig. 5). In total RNA the 2.1-kb ASA mRNA accounted for 30–40% of the ASA RNA. The total RNA from an individual homozygous for ASA pseudodeficiency was deficient in the 2.1-kb ASA mRNA, whereas the amount of the 3.7- and 4.8-kb ASA RNA was comparable to that in the other RNA samples. The variation of the ASA RNA signal

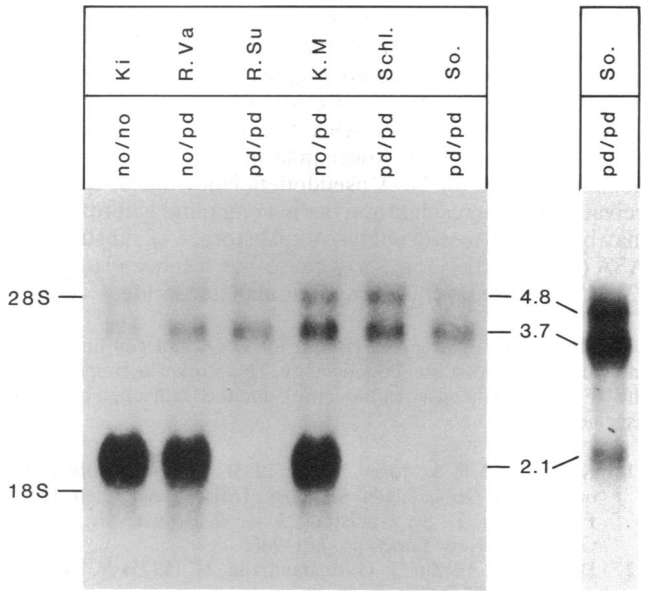

FIG. 4. Northern blot analysis of poly(A)$^+$ RNA from cultured human fibroblasts. Poly(A)$^+$ RNA was isolated from 40 μg of total RNA. Approximately 1 μg of poly(A)$^+$ RNA was loaded on the gel. The gel was blotted and hybridized with the ASA cDNA probe (10). The fibroblast lines are identified by the initials of their donors. Genotype of the donors is shown below; no, normal ASA allele; pd, ASA pseudodeficiency allele. 28S and 18S ribosomal RNA are shown as size markers at left; the sizes of ASA mRNAs are shown at right. The right lane shows an eight times longer exposure of lane So.

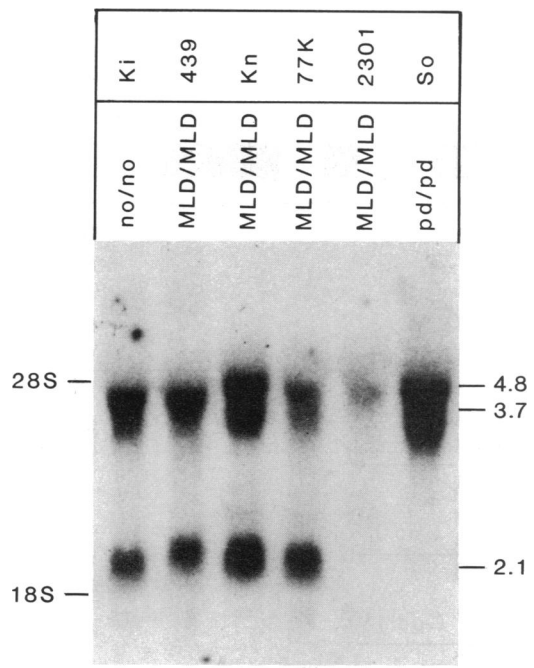

FIG. 5. Northern (RNA) blot analysis of total RNA from cultured human fibroblasts. Total RNA was isolated from cultured human fibroblasts. Five micrograms of RNA was loaded on the gel. The gel was blotted and hybridized with the ASA cDNA probe. Fibroblast lines are identified by numbers or initials of their donors; genotype of the donors is as follows: no, normal allele; pd, ASA pseudodeficiency allele; MLD, ASA metachromatic leukodystrophy allele. 28S and 18S ribosomal RNA are shown as size markers on left; sizes of the ASA RNAs are shown at right. Due to compression effects caused by the 28S ribosomal RNA in some lanes the 3.7- and 4.8-kb RNA species could not be separated. However, in poly(A)$^+$ RNA these species can clearly be distinguished (lanes Ki in Figs. 4 and 5).

(the ratio of the ASA RNA to actin RNA varied between 11% and 100%) did not allow a decision about whether amounts of the 3.7- and 4.8-kb ASA RNA increase in pseudodeficiency. However, these results clearly indicate that transcription of the ASA gene is not significantly altered in ASA pseudodeficiency.

Detection of the Pseudodeficiency Allele with Allele-Specific Oligonucleotides. The presence of both mutations was analyzed in four unrelated individuals with ASA pseudodeficiency. A 1.1-kb fragment encompassing the third N-glycosylation site and the polyadenylylation signal was amplified from genomic DNA. Four allele-specific oligonucleotides were hybridized to the amplified DNA. Washing conditions were chosen such that hybridization signals were seen only when all bases match (Fig. 6). In each of the four unrelated individuals with ASA pseudodeficiency both mutations were detectable. In two obligate heterozygotes the normal and pseudodeficiency alleles were found.

DISCUSSION

The present study shows that the phenotype of ASA pseudodeficiency is caused by two separate mutations. One changes an asparagine within an N-glycosylation site into a serine residue, and the other converts a canonical polyadenylylation signal into a nonfunctional sequence. The missing N-glycosylation site is one of two sites that are N-glycosylated in wild-type ASA. It is conceivable that an underglycosylated ASA is less stable and/or has a lower catalytic activity. We could demonstrate, however, that introducing the Asn → Ser exchange into wild-type cDNA results in synthesis of an underglycosylated ASA, which has normal stability and activity. This excludes that the underglycosylation contributes to the attenuated ASA activity in ASA pseudodeficiency.

The AATAAC polyadenylylation signal that is converted into a nonfunctional AGTAAC sequence in ASA pseudodeficiency is used for the generation of a 2.1-kb ASA mRNA species, which accounts for ≈90% of the ASA RNA in poly(A)$^+$ RNA of human fibroblasts. In fibroblasts from three individuals with ASA pseudodeficiency the 2.1-kb ASA mRNA was markedly deficient. The severe deficiency of the 2.1-kb ASA mRNA correlates well with diminished synthesis of ASA polypeptides and ASA activity attenuation in ASA pseudodeficiency fibroblasts.

The diminished termination at the first polyadenylylation signal codon is expected to yield increased amounts of the larger 3.7- and 4.8-kb ASA RNA species (20, 21). Because these species represent 60–70% of the ASA RNA in total RNA and the amount of ASA RNA was variable in total RNA of the cell lines analyzed, it was not possible to evaluate whether amounts of the 3.7- and 4.8-kb ASA RNA increased by 30–40% in total RNA from ASA pseudodeficiency fibroblasts. We conclude from normal amounts of ASA RNA in total RNA that transcription of the ASA gene is unaffected in ASA pseudodeficiency. The low recovery of the 3.7- and 4.8-kb ASA RNA species in the poly(A)$^+$ RNA suggests that these species are poorly polyadenylylated. The attenuated synthesis of ASA polypeptides in the ASA pseudodeficiency indicates that these nonpolyadenylated species might be translationally inactive. However, whether the low amounts of the 3.7- and 4.8-kb poly(A)$^+$ ASA RNAs can be translated or whether trace amounts of the 2.1-kb ASA RNA species found in the pseudodeficiency account for the residual ASA synthesis is presently unclear.

The four individuals with ASA pseudodeficiency that have been examined so far carried both mutations. Because the mutation affecting the N-glycosylation site is unrelated to the attenuated ASA activity in ASA pseudodeficiency, individ-

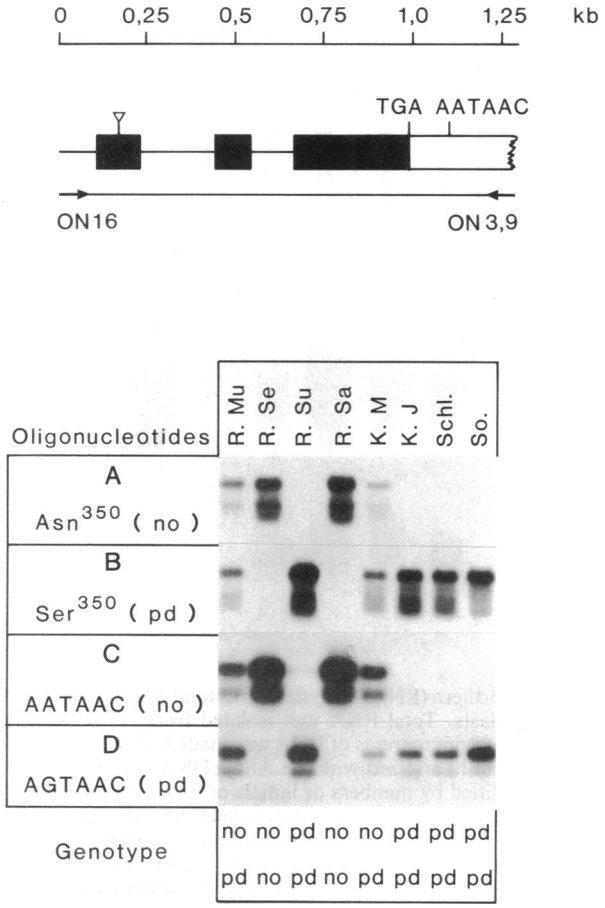

FIG. 6. Hybridization of allele-specific oligonucleotides to amplified genomic DNA. (*Top*) Scheme of the last three exons of the ASA gene. Exons are boxed, coding regions are black, and 3'-untranslated sequences are white. TGA, the stop codon; AATAAC, polyadenylylation signal; ▽, the potential glycosylation site; arrows, location of oligonucleotides used for DNA amplification. (*Bottom*) Southern blots of amplified DNA hybridized with different oligonucleotides. Top lane identifies the individuals by their initials; four were homozygous (R. Su., K. J., Schl., So.), and two were obligate heterozygous (R. Mu., K. M.) for the ASA pseudodeficiency allele. The oligonucleotides (A, B, C, and D) used and the mutation detected by the oligonucleotides are given at left. Deduced genotypes are shown at bottom.

uals with ASA pseudodeficiency possibly could be found that carry only the mutation in the polyadenylylation signal and express a normally glycosylated ASA due to a segregation of both mutations. Furthermore, ASA carrying only one oligosaccharide may be found in individuals with normal ASA activity.

For diagnostic purposes we have so far analyzed 31 individuals, of which 6 were homozygous and 16 were heterozygous for the pseudodeficiency allele. In none of these individuals has a segregation of the mutations occurred.

The allele-specific tests described here permit precise differentiation between ASA pseudodeficiency and ASA deficiency leading to metachromatic leukodystrophy; this distinction is particularly needed for the genetic counseling of metachromatic leukodystrophy in families in which nonfunctional and pseudodeficiency alleles for ASA occur. Furthermore, these tests allow the identification of compound heterozygotes for the pseudodeficiency and a nonfunctional ASA allele. Given the gene frequency for nonfunctional ASA alleles causing metachromatic leukodystrophy (0.5%) (22) and ASA pseudodeficiency alleles (7–15%) (5, 6) the frequency of compound heterozygotes is ≈1:1000, which is higher than the prevalence of cystic fibrosis (1:2000) (23) in Caucasians. The residual ASA activity in tissues of compound heterozygotes is lower than in tissues of individuals homozygous for the ASA pseudodeficiency allele. It is conceivable that the residual activity in compound heterozygotes may be too low to prevent lysosomal storage of substrates of ASA (24) and, therefore, may cause—as has been suggested (24, 25)—neuropsychiatric symptoms later in life.

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