# Arylsulfatase A pseudodeficiency incidence in Turkey

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**SUMMARY: Emre S, Topçu M, Terzioğlu M, Renda Y. Arylsulfatase A pseudodeficiency incidence in Turkey. Turk J Pediatr 2000; 42: 115-117.**

Pseudodeficiency (Pd) in arylsulfatase A (ASA) is a relatively frequent condition in healthy individuals. It produces a reduction in enzyme activity similar to that found in metachromatic leukodystrophy (MLD). A variable incidence of the Pd allele was found in different populations; it was 10-20 times higher than that of metachromatic leukodystrophy. Twelve of the 52 unrelated, healthy individuals were found to be heterozygous for the ASA Pd allele. In Turkey we estimated the incidence of the Pd allele as 11.5 percent. Out of 18 cases with MLD, one patient was found homozygous for the Pd allele and the other patient was found heterozygous. Turk J Pediatr 2000; 42: 115-116.

*Key words: metachromatic leukodystrophy, arylsulfatase A, pseudodeficiency.*

Metachromatic leukodystrophy (MLD, McKusick 250100) is a severe lysosomal storage disorder caused by deficiency of the lysosomal enzyme commonly known as arylsulfatase A (ASA) (E.C.3.1.6.1)[8]. Occasionally, low levels of urinary and/or leukocyte ASA activity, similar to those described for MLD, are found in apparently healthy individuals[5,10]. This condition is known as arylsulfatase A pseudodeficiency (ASA Pd). In most Pd cases, two A → G transitions, of which only the one destroying the polyadenylation signal is the cause of the lowered enzyme activity, are the common mutations[7]. Discrimination of MLD and pseudodeficiency based on ASA activity is diffucult and unreliable. This is a serious problem in carrier detection and prenatal diagnosis in MLD families who also carry a Pd allele. Detection of the Pd mutation by polymerase chain reaction (PCR) and Mae III cleavage is very rapid and reliable method[16].

The Pd allele is relatively frequent, with a variable incidence in different populations ranging from 6 to 23%, 6% in Denmark[16], 10% in Australia[13], and Israel[18], 12% in France[3], Spain[2], 13% in the United Kingdom[1], 10.1% in Italy[15], and 23% among the Jewish population from Yemen[18] and 6% in Poland[4].

The aim of the present study was to estimate the prevalence of the Pd allele in a sample of the normal Turkish population. A group of 18 patients are further examined for one mutation previously reported in exon 2[6,14].

## Material and Methods

*Detection of the Pd-allele*: Fifty-two unrelated, healthy volunteers were used as DNA donors. DNA was isolated from peripheral leukocytes by the ammonium acetate salting out procedure[12].

Polymerase chain reaction was carried out using a modification of Salamon's procedure in which one set of primers is used alternatively to amplify the 301 bp fragment of the ASA gene[16]. PCR product was cleaved by IU Mae III in three hours at 55 °C. The bands were separated on a three percent agarose.

Exon 2 of the ASA was amplified by PCR with exon specific primers. The amplified fragment of 350 bp was digested with MvaI according to the manufacturer's recommendations, and the products were analysed on a two percent agarose gel[6,14].

*ASA activity measurements*: ASA was assayed with q-nitrocatechol sulphate in sonicated leukocyte pellets as described by Lee-Vaupel and Conzelmann[9]. Protein was determined by the method of Lowry et al[11].

## Results

Because measurement of ASA activity is one of the most frequently performed lysosomal enzyme tests, the presence of pseudodeficiency mutations in a significant proportion of the population is important in the diagnosis of MLD.

There was a large variation in frequencies of the ASA pseudodeficiency alleles between the populations. The polyadenylation signal site mutation was found only among the Caucasian groups. Interestingly, this allele was found at a very low frequency in the Finnish population as compared to Noth American Caucasians and other European, Middle Eastern, and Australian populations.

In this study, the presence of the ASA Pd allele was analyzed in 52 unrelated healthy individuals. The polyadenylation site mutation was found in 12 out of 104 chromosomes (Table I).

Table I. Frequencies of the ASA Pseudodeficiency Genotypes and Alleles

| Genotypes | | | |
|---|---|---|---|
| no/no | no/pd | pd/pd | Total |
| 40 (76.9%) | 12 (23.1%) | – | 52 |
| **Alleles** | | | |
| | no | pd | Total |
| | 92 (88.5%) | 12 (11.5%) | 104 |

ASA: arylsulfatase A. no: normal allele. pd: pseudodeficiency allele.

Twelve of the 52 unrelated, healthy individuals were found to be heterozygous for the ASA Pd allele. We found the Pd allele with a frequency of 11.5 percent among healthy, unrelated individuals. We asked the donors about their ethnic background, all of them Turkish citizens. Among MLD diagnosed patients (in total 18 patients), Pd allele frequency was 8.3 percent (Table II). Our data suggest that the frequency of the Pd allele is in agreement with previously published results from Australia and western Europe.

Table II. Frequencies of the ASA Pseudodeficiency Genotypes and Alleles

| Genotypes | | | |
|---|---|---|---|
| no/no | no/pd | pd/pd | Total |
| 16 (88.9%) | 1 (5.5%) | 1 (5.5%) | 18 |
| **Alleles** | | | |
| | no | pd | Total |
| | 33 (91.7%) | 12 (8.3%) | 45 |

ASA: arylsulfatase A. MLD: metachromatic leukodystrophy. no: normal allele. pd: pseudodeficiency allele.

## Discussion

MLD occurs in forms ranging from late-infantile to adult-onset, with age of onset largely determined by the amount of residual ASA enzymatic activity. While reduced levels of ASA enzymic activity in pseudodeficiency do not cause disease, ASA activity in persons with adult-onset metachromatic leukodystrophy and persons homozygous for the pseudodeficiency mutations can be very similar. Identification of patients and carriers of MLD becomes critical when values of ASA activity are below 15% of normal. In such situations, the correct diagnosis can easily be achieved by a PCR test that detects the ASA Pd allele. The technique described here amplifies DNA of the ASA genomic region from a single pair of primers and does not require a specific set for each genetic variant. In addition to DNA analyses, the metabolism of radioactively-labeled sulfatide in cultured fibroblast cells from ASA pseudodeficiency. This is important because the presence of pseudodeficiency mutations does not negate the possibility of an MLD mutation on the same ASA gene[18].

Out of the 18 cases with MLD examined of so far, one patient was found homozygous for the Pd allele and had undetectable enzyme activity. The other patient was found heterozygous for the Pd allele and had undetectable enzyme activity. The remainder of the patients with low ASA activity did not have the Pd mutation. Low ASA activity may be due to other mutations.

The exon 2 donor splice site mutation has been reported to be the most common mutation causing MLD. We did not identify a mutation in exon 2 of the ASA gene in Turkish patients with MLD. Further studies will concentrate on finding population specific mutations for the ASA gene.

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