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# Arylsulfatase A Pseudodeficiency – Incidence in Poland

## Key Words

Arylsulfatase A  
Pseudodeficiency

## Abstract

Arylsulfatase A (ASA) pseudodeficiency (Pd) was defined as the in vitro measurement of low enzyme activity in a healthy person. A variable incidence of the Pd allele was found in different populations; it was 10–20 times higher than that of metachromatic leukodystrophy (MLD). In Poland we estimated the incidence of the Pd allele at 6% and that of isolated 1788 mutation (loss of glycosylation site) at 3%. Out of 8 cases with neurological symptoms and low ASA activity, 2 were found to be homozygous for the Pd allele; 2 MLD patients had healthy siblings homozygous for the Pd allele and another patient's allele bore two mutations, Pd and that causing MLD.

## Introduction

Low arylsulfatase A (ASA) activity is observed not only in metachromatic leukodystrophy (MLD), a very severe inborn storage disease, but also in some healthy individuals. The latter phenomenon has been named ASA pseudodeficiency (Pd). A number of mutations causing MLD have been described to date [1]. In most Pd cases, two A → G transitions in the ASA gene have been found, namely in positions 1788 and 2725 [2]. The first leads to a loss of a glycosylation site without any change in enzyme activity, the second causes a 90% reduction in enzyme activity due to a nonfunctional polyadenylation signal. Cases of isolated mutations 1788 (No\*) [3, 4] or 2725 Pd\* [4, 5] have also been described, as well as cases with both mutations – MLD and Pd – in the same allele [6].

The presence of the Pd allele can be shown using the polymerase chain reaction (PCR) [7]. Pd presents considerable diagnostic problems, for example, if the MLD patient's parents and/or sibling have the MLD/Pd genotype. Individuals with Pd/Pd or Pd/MLD genotypes pre-

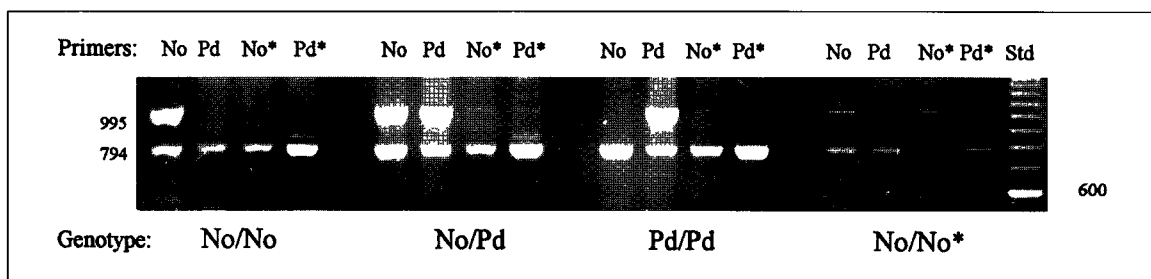
senting neurological signs that imitate MLD may be misdiagnosed. The Pd allele is relatively frequent, with a variable incidence in different populations ranging from 6 to 23% (6% in Denmark [8], 10% in Australia [9], and Israel [3], 12% in France [10] and Spain [11], 13% in the UK [12] and 23% among the Jewish population from Yemen [3]). Thus its frequency is 10–20 times higher than that of the MLD allele.

The aim of the present study was to estimate the prevalence of the Pd allele in the population of Poland.

## Materials and Methods

Fifty unrelated, healthy volunteers (staff members, students, etc.) were used as DNA donors. Eight cases suspected of MLD as well as their family members were referred to our Institute for biochemical diagnosis.

DNA was isolated from peripheral leukocytes by phenol extraction. PCR was carried out using a modification of Gieselmann's [7] procedure in which four pairs of primers are used alternatively to amplify the 995-bp fragment of the ASA gene between nucleotides



**Fig. 1.** Electrophoresis of amplified 995 bp and 794 bp fragments of ASA gene from: No/No (lanes 1–4), No/Pd (lanes 5–8), Pd/Pd (lanes 9–12) and No/No\* (lanes 13–16) persons; allele specific pair of primers used in PCR are indicated (see table 1 for details).

1788 and 2725 (i.e. between points of mutations). Their 3' terminal bases must match the adenines or guanines in the normal ASA (No) or mutated allele, respectively. The 995 bp fragment is amplified only if the terminal bases of the primers match the DNA template. The additional pair of primers that allows allele-independent amplification of the 774-bp ASA gene fragment served as an internal control of the PCR reaction (table 1, fig. 1).

ASA activity was determined with *p*-nitrocatechol sulfate as substrate at 37 or 0°C [13].

## Results and Discussion

Six of the 50 unrelated, healthy individuals were found to be heterozygous for the ASA Pd allele (fig. 1). This would suggest a frequency of about 6%. We are not able to say whether some of these Pd alleles do not bear a second mutation, i.e. MLD.

Additionally, 3 individuals were heterozygous for an isolated 1788 mutation (No\*), i.e. the loss of a glycosylation site, which suggests that the frequency of this mutation is 3%. We did not find any case of isolated 2725 mutation in our material (fig. 1).

Our data suggest that the Pd allele may be less frequent in Poland than in Western Europe. We did not ask the donors about their ethnic background, but all of them were Polish citizens. By chance we know that the No/No\* person is of Ashkenazi Jewish ancestry and another (No/Pd) of Byelorussian ancestry. Despite this, our data seem to represent the real frequency of Pd alleles in the contemporary Polish society and enable an estimation of the prevalence of both studied mutations at 6% for the double Pd mutation, at 3% for the isolated 1788 mutation and at <<1% for the isolated 2725 mutation.

Out of 8 cases with neurological symptoms and low ASA activity (i.e. below 30% of mean control value)

**Table 1.** Primers used for amplification of ASA gene fragment

Primers	Amplified gene fragment from allele
Asn/A <sup>+</sup> , No	995-bp fragment from normal allele
Ser/A <sup>-</sup> , Pd	995-bp fragment from the pseudodeficiency allele, i.e. the double mutation 1788 and 2725
Ser/A <sup>+</sup> , No*	995-bp fragment from the isolated mutation 1788 (loss of a glycosylation site)
Asn/A <sup>-</sup> , Pd*	995-bp fragment from the isolated mutation 2725 (non-functional polyadenylation signal)
26/27	Mutation independent 774 bp ASA gene fragment (control of PCR reaction)

examined so far, 2 were found to be homozygous for the Pd allele; as they did not excrete sulfatides in the urine, the underlying cause of their clinical picture was not MLD; moreover the healthy mother of one of the patients was also homozygous for the Pd allele [14]. In two families a healthy sibling of the MLD patient was homozygous for the Pd allele. Another case of MLD, confirmed by determination of urinary sulfatides, was heterozygous for the Pd allele of paternal origin; this allele most probably bore both, Pd and MLD mutations.

These examples illustrate the importance of DNA analysis in cases suspected of MLD.

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