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# Clinical heterogeneity of alpha 1-antitrypsin deficiency

**B. Maliseva-Lace[1], V. Baumanis[2], A. Krumina[1]**

*[1] Department of Medical Biology, Rīga Stradiņš University, Dzirciema 16, LV-1007 Riga, Latvia*
*[2] Biomedical Research and Study Center, Latvia.*
*E-mail: baibam@latnet.lv*

Alpha 1-antitrypsin (AAT) deficiency is a disease caused by the genetically determined AAT deficiency. The most common deficiency allele in North Europe is PI Z, and the majority of individuals with severe AAT deficiency are PI type ZZ. Of Caucasians, 0.2–0.4% are carriers for PI Z mutation. We performed DNA analysis for 251 healthy Latvians and acquired the frequency of PI Z allele in Latvia to be 1:303, *i.e*. the highest population frequency in the world. The estimated number of persons homozygous for PI ZZ is 1:833. Homozygous persons show an approximate number of persons who are under the risk of developing AAT deficiency.

On the basis of clinical symptoms of AAT deficiency we analyzed 22 patients referred from clinicians in the past three years. The PI ZZ mutation was found only in 4 of them. The clinical symptoms and AAT levels in blood (in spite of the same mutation for all of them) differed significantly.

We would like to explain the difference from the estimated number of patients compared to a substantial and variable clinical severity of AAT deficiency with the high clinical heterogeneity of AAT deficiency. Probably AAT deficiency varies between no clinical symptoms to severe invalidity.

**Key words:** AAT deficiency, DNA diagnostics, PI Z mutation

## INTRODUCTION

Alpha 1-antitrypsin (AAT) deficiency is a genetic disorder characterized by low serum levels of AAT and a high risk of pulmonary emphysema and a liver disease at a young age [1]. Alpha 1-antitrypsin deficiency is one of the most common inherited diseases in Caucasians. Allelic frequency for the most common protease inhibitor (PI) Z mutation is 1–2% in Caucasians of Northern Europe descent [2, 3].

Z mutation is caused by a single base substitution in exon V of the normal M1 allele leading to glycine to lysine amino acid change at position 342 in a molecule. This amino acid change causes a loss of the normal internal salt bridge between two amino acids, Gly342 and Lys290, of the AAT molecule. The homozygous Z mutation leads to AAT protein forming two-dimensional Z form, which folds at a slow rate, allowing AAT molecules to aggregate. Spontaneous conformation of AAT promotes insertion of the reactive center loop of one molecule into the A β-plated sheet of another to form chains of polymers [1, 4].

AAT is the main protease inhibitor in human serum. Lack of the AAT enzyme leaves proteases such as neutrophil elastase, trypsin, chymotrypsin and catepsin G uncleaved and consequently disturbs the balance of proteolytic activity. Production of AAT mainly occurs in hepatocytes, smaller amounts are synthesized in macrophages. Conformational changes of a mutated AAT molecule lead to development of several pathological pathways. Since ZZ homozygotes secrete only 15% of AAT in plasma, this decreased amount of AAT leads to an increased proteolytic activity in tissues. The remaining 85% accumulate in the endoplasmic reticulum of hepatocytes. While most of AAT is degraded, the remainder aggregates to form insoluble intracellular inclusions. As one of the co-factors in early response of inflammation, AAT deficiency can initiate a systemic disease [1, 5, 6].

Clinical symptoms are dependent on the age of a patient, environmental factors and lifestyle. Most likely there are other, thus far unknown, modifying factors that influence development of the disease.

Accumulation of AAT Z polymers in hepatocytes causes liver disease mostly in childhood. There is only one report of liver disease due to AAT deficiency in a late adulthood. Liver disease could vary from neonatal jaundice to severe liver cirrhosis. Approximately 50% of newborns homozygous for PI ZZ mutation have only slightly elevated liver enzymes as a clinical manifestation of AAT deficiency. Elevated liver enzymes, hyperbilirubine-

mia and jaundice are typical of cholestatic hepatitis and are observed in 10% of AAT deficiency cases. AAT deficiency could also be a reason for failure to thrive in early childhood. More than 3% patients with AAT deficiency develop liver cirrhosis. One of the modifying factors for such an atypical clinical presentation is considered a polymorphism of protein – ubiquitin. On the background of a specific form of ubiquitin, MM, homozygosity for the PI Z allele leads to more pronounced clinical manifestations of the disease [7]. Atypical clinical presentation of AAT deficiency often misguides physicians, therefore World Health Organization (WHO) recommends measuring AAT level in blood for all cases of cholestatic hepatitis with unknown etiology [8–10].

The main impact of AAT deficiency in human serum leads to the development of Chronic Obstructive Pulmonary Disease (COPD) and emphysema later in life. The most typical clinical manifestations are: dyspnoea, chronic bronchitis, COPD and bronchial asthma [8, 11]. Morphologically emphysema of the lower lobes of the lungs is observed on a chest computer tomography.

The British Thoracic Society studied 166 patients with AAT deficiency due to ZZ genotype. They found that the mean time of dyspnoea onset is 40 years and grade 2, 3 or 4 dyspnoea was observed in 86% of patients. Chronic bronchitis usually presents at age 40 as well in 50% of cases [12]. Certain environmental factors (dust, smoke) and smoking will induce clinical symptoms much earlier and they will be more severe. Approximately 80% of cases had some radiological evidence of lower zones emphysema. In the study by Piitulainen, 124 PI Z subjects were examined and nearly 20% of them had wheezing and bronchial asthma [6].

During the early stage of inflammation in lungs, when neutrophils release proteases, AAT makes a complex 1:1 with target protease and cleaves it. Due to lack of AAT, concentration of proteases increases. Increased amount of protease PR3 causes significant production of antinuclear antibodies (ANCA). There are some controversial data showing that AAT deficiency may be associated with Wegener's granulamatosis, Henoch–Schonlein purpura and systemic vasculitis in about 10% of cases. AAT deficiency alone is not sufficient to cause symptoms of diseases, but can be a contributing factor [13].

Liver cirrhosis and portal hypertension may result in a splenic artery aneurism. It has been shown that AAT deficiency leaves walls of arteries unprotected to proteolytic activity. The exact relationship between AAT deficiency and arterial aneurism formation is not clear yet and now is under investigation [14].

## MATERIALS AND METHODS

DNA was extracted [15] from venous blood of 251 inhabitants who had lived in selected regions of Latvia for at least three generations and 22 patients referred by physicians with symptoms suggestive of AAT deficiency. Physicians completed a questionnaire that provided genealogical information, demographical data and clinical status of the patients.

PI gene was first amplified by polymerase chain reaction (PCR). Total reaction volume of 20 µl included 1 µl genomic DNA, 5 pmol of each primer, 1.25 U of Taq DNA polymerase, 200 µmol of dNTPs, 10× buffer, $MgCl_2$ and BSA (Fermentas). Each sample was subjected to amplification on a Perkin–Elmer 2400 thermocycler under the following conditions: Initial denaturation was performed at 94 °C for 140 s, 55 °C for 140 s, 72 °C for 50 s, followed 38 cycles: each 45 s at 94 °C, 45 s at 55 °C, 60 s at 72 °C and final extension 94 °C for 140 s, 55 °C for 140 s, 72 °C for 300 s [13].

The PCR products were digested with Taq1 restriction enzyme. One µl of Taq1 added to 10 µl PCR product and incubated for 180 min at 65 °C.

The results were visualized in 8% polyacrylamide gel electrophoresis. The length standard we used was Puc19DNA/MspI (Fermentas) (Fig. 1). Z mutation causes the DNA to be cut differently resulting to an additional third band that is 210 base pairs.


Fig. 1. PI Z analysis in 8% PAGE
W – wild type, Z – mutation Z, NC – negative control.

## RESULTS AND CONCLUSIONS

Based on the analysis of 251 individuals enrolled in the study, the frequency of PI Z allele in ethnic

Latvians was calculated to be 33:1000. This represents the highest population frequency of Z allele in the world reported so far (Fig. 2) [2, 16].

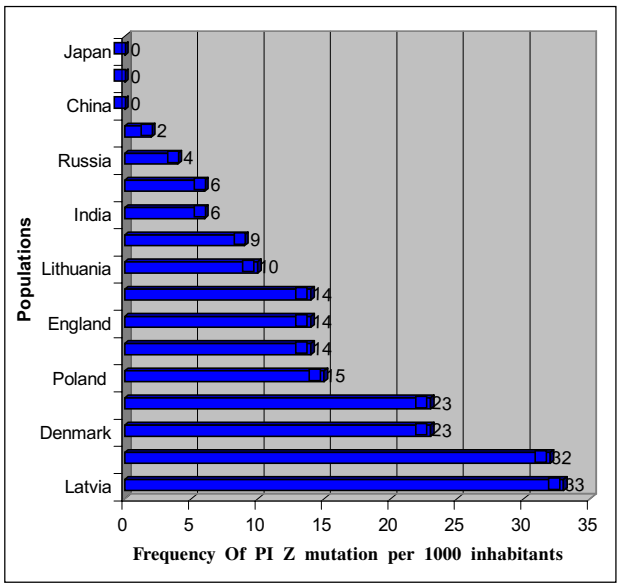


Fig. 2. Allele PI Z frequency per 1000 inhabitants in different populations

Using laws of population genetics, the estimated number of persons homozygous for PI ZZ is 1:833. Homozygous individuals are at a risk of developing AAT deficiency.

We analyzed the PI gene in 22 patients with clinical symptoms of AAT deficiency who were referred by clinicians in the past three years. PI Z mutation was found only in 4 of 22 patients. Two of them were homozygous and two heterozygous for Z allele. Presumably both heterozygous are compound heterozygotes (we did not perform full sequencing of PI gene). All of the patients with mutation Z have a decreased blood concentration of AAT. The AAT level in blood differed more than 10 times in the two homozygous individuals. However, it did not appear to affect the severity of disease.

We speculate that the AAT level can vary during lifetime and is dependent upon the presence or absence of an inflammation process in the patient and the functional status of the liver. Therefore we recommend PI genotyping in all patients with a decreased or low normal AAT level in blood.

The AAT level measured several times in a PI Z heterozygous person showed only moderately decreased levels, in spite of clinical symptoms. The patient had COPD and grade 3 dyspnoea. In contrast, a homozygous PI ZZ individual in our study had a much lower concentration of AAT, while having significantly less clinical symptoms. Thus, the clinical variability of AAT deficiency is dependent upon other, yet unknown, co-existing modifying factors.

Most of the patients referred from clinicians had bronchial asthma as the initial diagnosis; therefore AAT deficiency should be considered in patients with bronchial asthma. Bronchial asthma is a common diagnosis and tends to be overdiagnosed instead of considering the possibility of chronic obstructive pulmonary disease. The latter diagnosis would more likely suggest AAT deficiency as a possible underlying etiology.

Ten percent of newborns with cholestatic hepatitis of unknown etiology, 5 to 10% of young COPD and bronchial asthma patients and approximately 10% of patients with systemic vasculites have AAT deficiency. Therefore, it is important to measure AAT levels in the above listed categories of patients, which is a recommendation of WHO [7].

Additionally, the following groups of patients should have AAT blood level tested at least once: all patients with bronchial asthma, advanced age patients with liver disease of unknown etiology and patients with Wegener's granulamatosis, Henoch–Schonlein purpura and systemic vasculitis.

Correct and timely diagnosis, cessation of smoking, favorable working environment and appropriate genetic consultation will protect the health of AAT deficiency patients and prolong their lifespan.

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**B. Mališeva-Lacė, V. Baumanis, A. Krumina**

**ALFA 1-ANTITRIPSINO NEPAKANKAMUMO KLINIKINIS HETEROGENIŠKUMAS**

S a n t r a u k a

Alfa 1-antitripsino (AAT) nepakankamumas yra paveldima liga. Šiaurės Europoje labiausiai paplitęs *PI Z* alelis, ir dauguma asmenų serga ūmiu *PI ZZ* tipo AAT nepakankamumu. Kaukaziečių *PI Z* mutacijos dažnis yra 0,2–0,4%. Darbe ištirtas 251 sveikas latvis ir nustatytas 1 : 303 *PI Z* alelio dažnis Latvijoje, t. y. didesnis negu pasaulio žmonių populiacijoje. Vidutinis dažnis yra 1 : 833. Iš 22 tirtų ligonių *PI ZZ* mutacija nustatyta tik keturiems. Be to, klinikiniai jų simptomai ir AAT kiekis kraujyje labai skyrėsi. Daroma prielaida apie didelį AAT heterogeniškumą populiacijoje – nuo klinikinių požymių nebuvimo iki visiško invalidumo.