

## Alpha-1-Antitrypsin Deficiency: A Case of a Two-year Old Boy with Inherited Disease

Aleksandar Petlichkovski<sup>1</sup>, Sonja Peova<sup>2</sup>, Dejan Trajkov<sup>1</sup>, Todor Arsov<sup>1</sup>, Ana Strezova<sup>1</sup>, Slavica Hristomanova<sup>1</sup>, Eli Djulejic<sup>1</sup>, Jordan Petrov<sup>1</sup>, Mirko Spiroski<sup>1</sup>

<sup>1</sup>Institute of Immunobiology and Human Genetics, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia; <sup>2</sup>Clinic for Pediatrics, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia

### Abstract

#### Key words:

AAT deficiency; PIZ; PIM; PIS; hybridization assay; AAT genotyping.

#### Correspondence:

Dr. Aleksandar Petlichkovski  
Institute of Immunobiology and Human Genetics, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje. Ul. "50 Divizija" No. 6, P.O. Box 60, 1109 SKOPJE, Republic of Macedonia  
Phone: +389 2 110 556;  
Fax: +389 2 110 558  
E-mail: petlichkovski@yahoo.com

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Alpha-1-antitrypsin (AAT) is a protease inhibitor which plays an important role of protector of the lung tissues against the proteolytic effect of elastase secreted from neutrophils. Its deficiency is associated with liver disease in children and emphysema in adults. So far, more than 75 variants of this protein are defined, but most of the cases of AAT deficiency are caused by homozygosis for the deficient allele PIZ or by heterozygous combination of the 2 most common deficient alleles, PIS and PIZ. A diagnosis in the case of a suspicion of AAT deficiency is carried out by measuring the alpha-1 antitrypsin level in blood and by genotyping the alpha-1 antitrypsin alleles. The importance of early diagnosis resides in the possibility of undergoing a lifestyle modification (such as vigorous smoking cessation, for example) and treatment of pulmonary disease thus significantly decreasing the morbidity. The family studying is important to identify individuals at high risk, and initiation of AAT replacement therapy in individuals. Having in mind the underdiagnosis of this disease, we hope to contribute with this case report to the medical community in Republic of Macedonia to raise the awareness of this disease, and also of the possibilities of exact diagnosis.

### Introduction

Alpha-1-antitrypsin (AAT) is a protease inhibitor, deficiency of which is associated with emphysema and liver disease. It was first discovered by Laurell and Erikson in 1963 (1) who analyzed the plasma concentration of alpha-1 globulins in patients with an increased prevalence of emphysema. Their results represented the first direct evidence of a genetic risk factor for the development of chronic obstructive pulmonary disease (COPD). Several other diseases (rheumatoid arthritis, connective tissue disorders, bronchiectasis, liver diseases and cancer) are later reported to occur at a higher frequency in people with alpha 1AT deficiency (2-4).

Alpha 1-antitrypsin is 52 kDa serpine, one of the most important protease inhibitors in the serum. Sometimes, it is referred to as protease inhibitor 1 (OMIM +107400, PI1) (5). It is mainly synthesized in the liver, but is also produced by alveolar macrophages and peripheral blood monocytes (6). AAT is one of the few enzymes that can inhibit neutrophil elastase, an important enzyme in the pathogenesis and development of COPD, for example.

The protein is encoded by a gene (PI) located on the distal long arm of chromosome 14 (14q32.1). A salient polymorphism is noted within this protein, with more than 75 variants identified today (7, 8). Around 30 of known polymorphic variants have been connected to

some pathologic consequences. The protein level in serum and the enzyme activity depend on the genotype and are distributed in a broad range from 0 to 100% (9), but from a clinical point of view, AAT variants are classified into 4 categories: normal variants, deficient variants, null variants and dysfunctional variants, characterized with normal amount of AAT, which does not function properly (e.g. PI Pittsburg). (Figure 1):

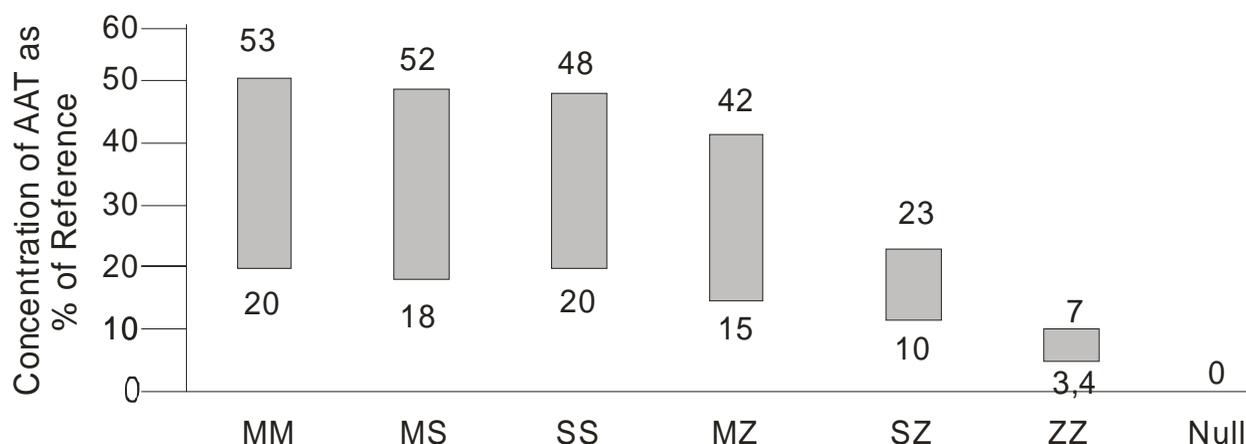


Figure 1: Serum concentration of AAT according to genotype. On the X axis, the mean PI genotypes are given, and on the Y axis the corresponding plasma concentration of AAT are shown as a proportion of the normal range. The risk for pulmonary disease increases with the ratio dropping below 35%.

The AAT variants are distributed at variable frequencies in different populations (10).

The most cases of AAT deficiency are caused by homozygosity for the deficient allele PIZ or by heterozygous combination of the 2 most common deficient alleles, PIS and PIZ. The Z variant of AAT (Glu342Lys) is found in approximately 1% of the general population. It encodes for a protein with deficient antiproteolytic function, yet the principal defect is in the incorrect processing of the protein and its retention in the rough endoplasmic reticulum of the hepatocytes (about 85% of the produced protein). This leads to formation of aggregates in the liver, what subsequently causes a liver disease and also decreases the AAT plasma concentration (11). Intriguingly, only 10-15% of PiZZ individuals develop clinical symptoms of liver disease in childhood (12).

The allele frequency of the PIS variant (Glu264Val), ranges between 0,2% to around 10% in general populations from different countries, with a tendency to increase towards Southern European populations (13). When in homozygosity, the S allele leads to decrease of the concentrations of AAT to about 60% of normal values, but patients are usually not affected with clinical disease. However, population-

based studies have proved an increased risk of developing COPD in those individuals if they smoke cigarettes, with levels of obstruction similar to homozygous PIZ individuals. In contrast, this risk is not present in non-smokers (14-16).

A diagnosis in the case of a suspicion of AAT deficiency is carried out by measuring the alpha-1 antitrypsin level in blood. A strongly reduced or non-

measurable concentration of the protein points to a homozygous defect. The AAT concentration in heterozygous patients is usually in the lower normal region. As alpha-1 antitrypsin is an acute phase protein, a slightly raised concentration can be measured in heterozygous patients during an infection or treatment with oestrogen or steroids. The measurement of the alpha-1 antitrypsin level in blood is not therefore appropriate for identifying heterozygous carriers. A certain diagnosis can only be achieved by typing the alpha-1 antitrypsin allele.

## Case report

A baby boy, forty days old, from first pregnancy of the mother with controlled normal follow-up, regular peri- and postnatal period admitted to the Clinic of Pediatrics at the Faculty of Medicine in Skopje because of a prolonged jaundice, which started on the fourth day after birth. At the day of reception he was conscious, afebrile, with intensive icteric coloring of the skin and mucous membranes. He had enlarged hepato as determined by palpation and later confirmed by ultrasonography, and stable vital parameters. With possible diagnosis of infective hepatitis, the patient

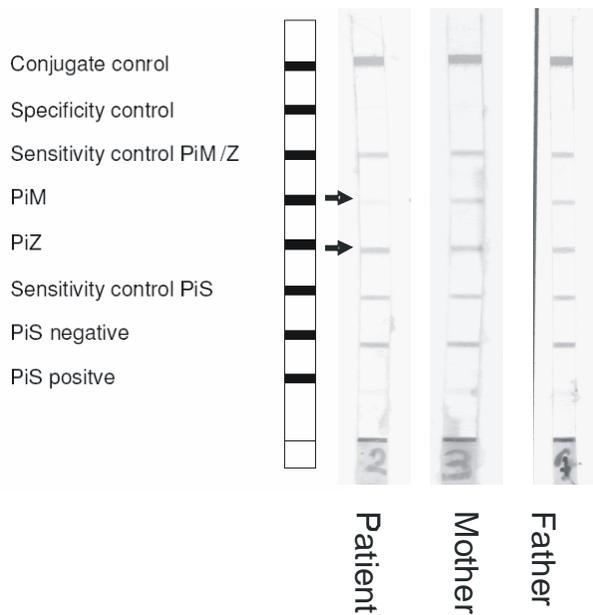


Figure 2: Scan of AAT hybridization tests of the patient and his parents. Two arrows show the absence of "normal" PiM allele in the patient (upper arrow) and presence of PiZ allele (lower arrow). Both parents have both, PiM and PiZ alleles.

was treated with hepatoprotectants and vitamino-therapy until resolution of jaundice and was sent home with advice for free nursing.

Eight months later, the baby readmits to the Clinic febrile, with diarrhea, coughing, dyspnoic, with notable loss of appetite as noted by his mother. He had nasal obstruction while on auscultation a prolonged expirium together with bilateral basal crepitations were noted. The liver was further more enlarged both on physical and on ultrasonographic examination. After isolation of *Pseudomonas aeruginosa* from the upper respiratory tract and inclusion of antibiotic infusion, the condition improved fast.

From the different laboratory analysis, a significant decrease of the concentration of alpha 1 antitrypsin was prominent (0,49 g/L and 0,5 g/L for the repeated analysis) after which genetic test for AAT genotyping was requested. DNA was isolated from the peripheral blood leukocytes from the patient and his first-degree relatives after signing a written consent, using phenol-chlorophorm extraction method (17) and the samples were subsequently stored in the Macedonian Human DNA Bank (18). The detection of the alpha-1 antitrypsin deficiency alleles PiZ and PiS was performed using the commercial Alpha-1 antitrypsin kit (AID GmbH, Straßberg, Germany), based on re-

verse hybridization technique (19, 20). Briefly, a multiplex PCR is first carried out allowing amplification of two fragments of the alpha 1 antitrypsin gene using specific, biotin-labeled primers. The band pattern is analyzed using the template supplied (Figure 2).

Homozygous presence of PiZ allele in the patient was confirmed by a genetic test performed at the Institute for Immunobiology and Human Genetics at the same Faculty of Medicine, while both parents were found to be heterozygous for the same allele. Diagnosis of inherited alpha 1 antitrypsin deficit was assigned. The parents were advised for home dietetic regiment of the patient and routine controls at the Clinic were scheduled on a monthly basis.

## Discussion

Increased intracellular degradation of PiZ in the liver results in severe plasma AAT deficiency. The deficiency of AAT protein may predispose an individual to several illnesses. Less commonly, and characteristically in children, AAT deficiency may cause progressive liver damage (cirrhosis) or liver cancer (less than 3%) requiring a liver transplant in some patients. The most common illness in adult individuals with AAT deficiency is lung disease. Most commonly it is associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema.

Alpha-1 antitrypsin deficiency is the most common genetic cause of liver disease in children and is the most common genetic disease for which liver transplantation is undertaken in children. Some children show signs of liver failure at birth including jaundice, swelling of the abdomen, and poor feeding. In some children, the signs of AAT deficiency do not become apparent until early childhood or adolescence when they may develop hepatitis, enlarged spleen, ascites, pruritus and other signs of liver injury. In some families one child may not show any signs of liver disease whereas a brother or sister may be seriously affected. It is possible to have AAT deficiency without having developed symptoms yet or that has been misdiagnosed. The reasons for the wide variability, from normal life span and normal activity through life, to severe liver or lung disease are largely unknown (21).

In 1997, the World Health Organization recommended determination of serum concentrations of AAT in all patients with COPD. They further recommend determination of the phenotype in patients with anomalous results obtained in this screen (22). In another

more recent consensus document, issued by joint activity of the American Thoracic Society and European Respiratory Society concerning the diagnosis and treatment of AAT deficiency a diagnostic testing is recommended for patients with early-onset emphysema or in the absence of recognized risk factors for emphysema (smoking and occupational dust exposure), emphysema with prominent basilar hyperlucency, unexplained liver disease, necrotizing panniculitis, antiproteinase 3-positive vasculitis (C-ANCA), or family history of the above, and bronchiectasis without unexplained etiology. In the same report, type A recommendation (genetic predispositional testing) is recommended for individuals with unexplained liver disease, including neonates, children and adults, siblings of a patient with ATT deficiency, relatives of those with homozygous or heterozygous ATT deficiency, and those with a family history of unexplained chronic obstructive pulmonary disease (COPD) or liver disease (23-26).

Based on these recommendations, some countries (e.g. Italy, Germany, USA, Spain) have started screening programs (27-29). This could be an important issue, since these population-based studies conclude that AAT deficiency is an underdiagnosed disease and that diagnosis is often delayed. The average delay in diagnosis, measured as the time between diagnosis of COPD and diagnosis of AAT deficiency, was found to be 10 years (30-32).

The importance of early diagnosis or diagnosis early in life resides in the possibility of undergoing a lifestyle modification and thus significantly decrease the morbidity associated with this chronic disease. The latest refers to ways for helping prevent tissue damage in the lung such as: receiving immunizations for flu and pneumonia; receiving early treatment for lung infections by seeing a doctor at the first sign of a cold or other lung problem; avoiding tobacco smoke, noxious fumes, dust, and pollution; staying fit by doing regular exercise. Further, there is a possibility for family studies in order to identify individuals at high risk for developing the disease or patients at earlier stages of development of the disease. The possibility of initiating AAT replacement therapy, and also genetic counseling should be considered in individuals who meet the established criteria.

In Republic of Macedonia, to our knowledge, the AAT deficiency has not been studied nor do recommendations for eventual screening exist. There is no data regarding the public health aspect and frequency of the genetic defect. We hope to contribute with this case report to the medical community in Republic of

Macedonia to raise the awareness of this disease, and also of the possibilities of exact diagnosis.

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