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The incidence of alpha-1-antitrypsin (A1AT) deficiency alleles in population of Central Poland — preliminary results from newborn screening

Ocena częstości występowania głównych alleli deficytowych genu alfa-1 antytrypsyny w populacji województwa mazowieckiego — wstępne wyniki badania przesiewowego noworodków

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Abstract

Inherited alpha-1 antitrypsin deficiency (A1ATD) is listed among the three most common genetic disorders in Caucasians. It considerably increases the risk of progressive obstructive lung diseases, mostly chronic obstructive pulmonary disease. Data on the A1ATD prevalence in Poland are scarce, no studies with large enough groups representative for whole Polish population have been performed. Here, we present the preliminary data on the incidence of A1AT main deficiency alleles from the newborn screening in Mazovia (Central Poland) region. Real-time PCR genotyping and A1AT blood concentration measurement by nephelometry were performed from the dry blood spots (DBS) samples of 658 newborns. Deficiency alleles PI*Z i PI*S were present in 28 children, respectively in 2.8% and 1.5%. Their existence corresponded with significantly lower A1AT blood concentration. Estimated incidence of deficiency alleles was 13,7/1000 (95% CI 5.8–21.5) for PI*Z and 7.6/1000 (95% CI 1.7–13.5) for PI*S. The calculated prevalence for the main deficiency genotype ZZ was 1/5345. The study is on-going.

Key words: alpha-1 antitrypsin deficiency; S allele, Z allele; genotyping, blood concentration, DBS, prevalence, newborns, Poland

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Deficiency of alpha-1 antitrypsin (A1AT), a serum inhibitor of serine proteases, is one of the most common inborn disorders in Caucasians. It contributes to development of various diseases of the respiratory tract, particularly in subjects smoking cigarettes or with professional/environmental exposition to noxious substances [1]. Clinical manifestations of inherited A1AT deficiency are variegated, including early development of emphysema in young adults, leading to respiratory handicapping as well as formation of bronchiectases and progression to chronic obturatory pulmonary disease (COPD) [2].

Alpha-1 antitrypsin is coded by the *SERPINA1* gene located in the long arm of chromosome 14, in which numerous polymorphisms have been identified. Until now, more than 130 genetic variations of the A1AT protein were detected, resulting from

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mutations of the gene. Normal gene codes for a functional protein (M variant). When blood level of the protein is adequate, A1AT contributes to a normal balance state between proteases and antiproteases in the body, and especially in the respiratory tract. Two most commonly occurring deficiency alleles Z (PI*Z) and S (PI*S) result from *SERPINA1* gene mutation at Glu342Lys and Glu264Val positions, respectively. These lead to synthesis of A1AT protein which cannot achieve a normal spatial conformation, and thus is accumulated inside hepatocytes (Z protein) or immediately degraded (S protein) in the cells. In result, blood level of A1AT is critically decreased [3].

Available epidemiological data suggest that in Europe severe homozygous A1AT deficiency (PIZZ) is more common in Scandinavia (2.3%) as compared to southern regions of the continent (1.0%), and occurs on average in 1 per 4.727 Caucasian neonates [4]. Poland remains one of the few European countries with no comprehensive data on prevalence of A1AT deficiency.

The presented data includes preliminary results of a study aimed to investigate prevalence of deficiency alleles Z and S in inhabitants of Masovia region.

Material and methods

Study group

The study included all children born alive in the Duchess Anna Mazowiecka Public Teaching Hospital in Warsaw between September 1st and December 31st 2011; this population included the total of 658 neonates. Parents were provided extensive written information on the aims of the study, and agreed to participation of their children in it, leaving a written consent. The study protocol was approved by the institutional bioethical committee.

Analysis was performed on cord or venous blood sampled onto dry blood spots (DBS). All investigations were performed on biological material isolated from three pieces of filter paper of 13 mm in diameter each.

Methods

Measurement of A1AT concentration

Concentration of alpha-1 antitrypsin was measured in DBS material by nephelometry, using an IMMAGE 800 analyser (Beckman Coulter, USA) and goat antibodies against human A1AT (Beckman-Coulter, USA). Concentrations were read from standard curve ranging between 20–250 mg/dl, as described by Giorrini et al. [5].

A1AT genotyping

Genotyping analysis of A1AT was performed in DBS eluates prepared using Extract-N-Amp Blood PCR Kits (Sigma-Aldrich), as previously described [6]. Two most commonly occurring mutations in *A1AT* gene (Z and S) were identified by real-time polymerase chain reaction (RT-PCR) using two hydrolysing, fluorescently marked probes (VIC, FAM), which detect a native allele (without S or Z mutation) or mutated allele (PI*S or PI*Z) in a single PCR reaction. Primer and probe sequences as well as PCR reaction conditions were previously described by Struniawski et al. [6].

Statistical significance was verified using nonparametric Kruskal-Wallis test. Prevalence of deficiency alleles Z and S and of A1AT genotypes was calculated using Hardy-Weinberg equation.

Results

Six hundred and thirty among all 658 tested children (95.7%) did not have a deficiency allele PI*Z or PI*S. Mutated alleles were detected in 28 children, including PI*Z allele in 18 (2.8%) and PI*S allele in 10 neonates (1.5%). Blood A1AT concentration was significantly lower in both these groups (tab. 1).

Based on these results, the estimated prevalence of deficiency alleles Z and S was 13.7 per 1.000 persons for PI*Z (95% confidence interval [CI] 5.8–21.5), and 7.6 per 1.000 persons for PI*S (95% CI 1.7–13.5). Estimates for both alleles are presented in table 2.

Discussion

Prevalence of inborn A1AT deficiency alleles was previously randomly studied in Poland, and analyses were performed mostly in regions of Małopolska or Wielkopolska [7–10]. Interpretation of these results is difficult due to low numbers of analysed subjects (630–1262 healthy persons), especially when concerning an overall low incidence of the deficiency in the country [8–12]. Kowalska et al. used electrofocusing to analyse phenotypes and prevalence of respective A1AT alleles in adult inhabitants of Poznań voivodship. Prevalence of PI*Z allele was 15 per 1.000 and PI*S 14.2 per 1.000 in the studied population in their study [10]. Kaczor et al. studied 859 adult inhabitants of city of Cracow using real-time PCR, and found the respective prevalence rates of PI*Z and PI*S to be of 10.5 and 17.5 per 1.000 persons [8]. Results of the analysis performed in a small population of inhabitants of Gdańsk Pomeranian region (228

Table 1. Peripheral blood A1AT concentration in respective newborns groups with PI*Z or PI*S alleles. Statistical significance towards non-S non-Z group was calculated by the non-parametric Kruskal-Wallis one-way analysis on variance

Allele type	Mean A1AT concentration in the blood	SD	95% Confidence interval	P
Non-S Non-Z	220.09	48.39	185.17–247.44	
PI*Z	138.43	16.65	127.68–150.69	$p < 0.000$*
PI*S	178.14	32.90	158.23–199.92	$p < 0.000$*

Table 2. Estimated frequency for A1AT deficiency genotypes with PI*Z or PI*S alleles

Estimated genotype frequency (1/Hardy-Weinberg)					
Non-S Non-Z	Z Non-S	ZZ	S Non-Z	SS	SZ
1/1,04	1/37	1/5345	1/67	1/17319	1/4810

persons) were different, showing the prevalence of PI*Z to be 15.3, and that of PI*S 21.9 per 1.000 persons, respectively [11]. Statistical analysis in the above mentioned studies was performed by Kaczor et al., and the average calculated prevalence of PI*Z allele was 14.5 per 1.000 and that of PI*S allele 10.9 per 1.000 persons in a combined population of 2.653 subjects analysed using various laboratory techniques [8].

Currently presented preliminary results suggest a similar prevalence of the deficiency allele PI*Z (13.7 per 1.000), and slightly lower prevalence of PI*S (7.6 per 1.000 persons). However, the estimated prevalence of the main deficiency phenotype ZZ is significantly higher in the presented study as compared to estimates made by Kaczor et al. (1 per 5.345 vs. 1 per 9.110) [8].

It should be emphasised that ultimate results will be obtained when a sufficiently big group of children is analysed, and the intended patient population is of approximately 4.000 neonates. This patient number is sufficient for a valid analysis, given the estimated prevalence of PI*Z allele.

The choice of a sensitive and reliable diagnostic tool is also of utmost importance. The only known Polish study which concerned a relatively big group of healthy volunteers (3.560 persons) was performed in the beginning of 1970s. Authors performed electrophoresis in starch gel for A1AT phenotyping. This method was later abandoned due to its unsatisfactory sensitivity [7], as reflected by the study results demonstrating prevalence of PI*Z to be 1.4 and that of PI*S to amount 15.6 per 1.000, respectively.

Authors of the presented study performed analyses using a technique applied in modern genetic testing (real-time PCR), the applicability of which was already verified by Kaczor et al. [8]. Furthermore, biological material in this study was collected using methodology that was not previously applied. This permits a low-invasive and convenient sampling and transferring neonate blood to filter paper as part of routine screening for genetically determined diseases. Introduction of a test which permits measuring A1AT concentration in a few drops of blood collected on filter paper is an unique achievement of this study.

Conflict of interests

None to declare.

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