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Joanna Chorostowska-Wynimko[1], Dariusz Gawryluk[2], Radosław Struniawski[1], Beata Popławska[1], Justyna Fijołek[2]

[1]Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, Warsaw
Head: Prof. J. Chorostowska-Wynimko MD PhD
[2]III Department of Lung Diseases, National Institute of Tuberculosis and Lung Diseases, Warsaw
Head: Prof. K. Roszkowski-Śliż MD PhD

Incidence of alpha-1 antitrypsin Z and S alleles in patients with granulomatosis with polyangiitis — pilot study

Występowanie alleli Z i S kodujących niedobór alfa-1 antytrypsyny u chorych na ziarniniakowatość z zapaleniem naczyń — wyniki wstępne

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Abstract

Introduction: Inherited alpha-1 antitrypsin (AAT) deficiency is one of the three most common genetic disorders in Caucasians. It considerably increases the risk of progressive obstructive lung diseases, mostly chronic obstructive pulmonary disease. It has also been suggested that AAT deficiency might be instrumental vasculitis associated with the anti-neutrophil cytoplasm antibodies (cANCA) and subsequent lung tissue injury.
Material and methods: We present the results from a pilot study involving 51 patients with granulomatosis with polyangiitis, formerly known as Wegener's granulomatosis (GPA), 43 of whom were cANCA positive. The control group consisted of 658 individuals. AAT blood concentration assessment by nephelometry, phenotyping by isoelectrofocusing and real-time PCR genotyping were performed.
Results: Deficiency alleles PI*Z and PI*S were detected in 3 (5.88%) and in 2 patients (3.92%) with GPA, respectively. All of them were cANCA positive. In the controls, PI*Z was observed in 2.8% while PI*S in 1.5% of cases. Accordingly, the increased incidence of main deficiency alleles was demonstrated in GPA, and particularly in cANCA$^+$GPA patients, when compared to the controls. The estimated frequency for PI*Z in GPA, cANCA$^+$GPA patients and controls was, respectively, 29.4/1000, 34.9/1000 and 13.7/1000, whereas for PI*S it was 19.2/1000, 23.2/10,00 and 7.6/1000. However, the observed differences did not reach statistical significance due to the considerable size disproportion between groups.
Consclusions: We believe that our preliminary data confirm the clinical importance of AAT deficiency in GPA patients and the need to screen for AAT deficiency alleles. The study is on-going.

Key words: inherited alpha-1 antitrypsin deficiency, S allele, Z allele, granulomatosis with polyangiitis, ANCA

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Streszczenie

Wstęp: Wrodzony niedobór alfa-1 antytrypsyny jest jedną z trzech najczęstszych chorób genetycznych rasy kaukaskiej i wiąże się z istotnie wyższym ryzykiem rozwoju postępujących obturacyjnych chorób płuc, zwłaszcza przewlekłej obturacyjnej choroby płuc. Niedostateczna aktywność hamująca alfa-1 antytrypsyny wobec proteinaz, a szczególnie proteinazy 3, skutkująca ich nadmierną aktywnością przypuszczalnie odgrywa również znaczącą rolę w patomechanizmie ziarniniakowatości z zapaleniem naczyń, dawnej ziarniniakowatości Wegenera, zwłaszcza postaci z przeciwciałami skierowanymi przeciwko proteinazie 3 (cANCA). cANCA-dodatnie zapalenie naczyń jest uznanym wskazaniem do diagnostyki w kierunku wrodzonego niedoboru alfa-1 antytrypsyny.
Materiał i metody: Pilotowym badaniem objęto grupę 51 chorych z potwierdzoną ziarniniakowatością z zapaleniem naczyń (GPA) i zajęciem układu oddechowego, u 43 z nich stwierdzono przeciwciała cANCA (cANCA$^+$GPA). Grupę kontrolną stano-

Address for correspondence: Prof. Joanna Chorostowska-Wynimko MD PhD, Laboratory of Molecular Diagnostics and Immunology, National Institute of Tuberculosis and Lung Diseases, ul. Płocka 26, 01–138 Warszawa, tel.: +48 22 431 21 58. fax: +48 22 431 23 58, e-mail: immuno@igichp.edu.pl

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wiło 658 noworodków przebadanych w ramach populacyjnego badania przesiewowego realizowanego na Mazowszu. W próbkach krwi obwodowej wykonano pomiar stężenia alfa-1antytrypsyny metodą nefelometryczną, fenotypowanie metodą ogniskowania izoelektycznego oraz genotypowanie metodą *real-time* PCR.
Wyniki: Allel deficytowy PI*Z stwierdzono u 3 (5,88%), a PI*S u 2 chorych (3,92%) z GPA, u wszystkich choroba przebiegała z obecnością przeciwciał cANCA. W grupie kontrolnej było to odpowiednio u 2,8% oraz 1,5% badanych. Zaobserwowano zwiększoną częstość występowania głównych alleli deficytowych u chorych na GPA, 29,4/1000 dla PI*Z, w tym 34,9/1000 w podgrupie cANCA$^+$GPA w porównaniu z kontrolą 13,7/1000, natomiast dla PI*S odpowiednio 19,2/1000, 23,2/1000 dla cANCA$^+$GPA oraz 7,6/1000 w grupie kontrolnej. Obserwowane różnice nie były istotne statystycznie w odniesieniu do kontroli, prawdopodobnie ze względu na różnice w liczebności porównywnych grup.
Wnioski: Wstępne wyniki badania potwierdzają klinicznie istotną rolę niedoboru AAT oraz potrzebę prowadzenia diagnostyki w tym kierunku u chorych na GPA. Badanie jest kontynuowane.

Słowa kluczowe: wrodzony niedobór alfa-1 antytrypsyny, allel S, allel Z, ziarniniakowatość z zapaleniem naczyń, ANCA
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Introduction

Alpha-1 antitrypsin (AAT) deficiency, one of the most common inherited disorders in Caucasian population, is a proven risk factor of lung diseases, mainly early-onset emphysema, COPD and bronchiectasis. The quantitative deficit of alpha-1 antitrypsin, encoded mainly by the PI*S and PI*Z deficiency alleles, results in impaired proteinase-antiproteinase balance in deficient individuals and promotes progressive lung damage [1]. AAT is the main protein inactivating neutrophil elastase as the preferred target. Still, it is also a cognate inhibitor of other neutrophil-derived proteases, such as proteinase 3 (PR3) and cathepsin G [2]. PR3 is known to play an important role in the mechanisms driving anti-neutrophil cytoplasm antibodies (cANCA) associated with vasculitis and ensuing lung tissue injury [3]. Hence, it has been suggested that impaired PR3 inhibition, due to inadequate AAT antiproteinase activity and subsequent PR3 hyperactivity, might assist in anti-PR3 autoantibody development and therefore the pathomechanism of vasculitis. Recently, Mota et al. demonstrated significantly diminished trypsin inhibitory capacity as well as specific AAT activity in the sera of 27 patients with GPA; meanwhile, PI*S or PI*Z allele was detected in only 5 of them [4]. This link has been further strengthened by the current guidelines of the ATS/ERS as well as of the Polish Respiratory Society enlisting cANCA-associated vasculitis as the recommendation for AATD screening, next to emphysema and COPD [5, 6]. Here, we present the preliminary results from the on-going screening program aimed at analysing the frequency of AAT main deficiency alleles in patients with granulomatosis with polyangiitis, formerly known as Wegener's granulomatosis.

Material and methods

Study group

The study group consisted of 51 patients, 28 female (55%) and 23 male (45%), mean age 46.2 (17-83) years with diagnosis of granulomatosis with polyangiitis in different stages of disease (active, on therapy or in remission) based on the clinical symptoms supported by either positive ANCA assay or a diagnostic biopsy and histopathological evaluation. ANCA were evaluated by ELISA and immunofluorescence methods. Forty-three subjects proved cANCA positive and pANCA negative (84.2%), 2 were cANCA negative and pANCA positive (3.9%), and 4 were cANCA and pANCA negative (9.5%). The ANCA status of 2 subjects was unknown. The study protocol was approved by the local bioethical committee.

The comparative data for the S and Z allele prevalence in the general Polish population came from the preliminary results of the on-going newborn screening study in Central Poland (Mazovia region). PI*Z and PI*S were assessed in the group of 658 consecutive neonates born alive at the Duchess Anna Mazowiecka University Hospital in Warsaw between September 1st and December 31st, 2011 [7].

Materials

Serum and dry blood spot (DBS) samples on filter paper were collected simultaneously from all GPA patients. Similarly, DBS sampling was performed in newborns, as previously described [7].

Quantitative determination of serum AAT concentration by immune nephelometry

The AAT measurement was performed in serum using a rate immune nephelometric method (Immage 800 Immunochemistry System, Beckman-Coulter, USA) with commercially available reagents containing goat anti-human AAT antibody

(Beckman-Coulter, USA). The normal range for AAT in serum samples was 88–174 mg/dL.

AAT phenotyping by isoelectrofocusing (IEF)

Phenotype analysis of serum AAT was performed by IEF on polyacrylamide gel with a pH of 4.2–4.9 using a Multiphor II Electrophoresis System (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), as described previously [8]. The AAT phenotype was determined by visual inspection and comparison to control M1M2, MS, MZ and ZZ samples.

PI*S and PI*Z AAT allele genotyping by real-time PCR

Genomic DNA was extracted using a commercially available kit: Extract-N-Amp Blood PCR Kits (Sigma-Aldrich). Genetic material present in the DBS eluate was directly used for AAT genotyping without the need for DNA purification from blood, as previously described [9]. The identification of the two most common mutations of the AAT gene (Z, S) was performed in a single reaction by real-time PCR method using hydrolysing probes coupled with fluorescent dyes (VIC or FAM) complementary to the mutant variants (PI*S or PI*Z). Primer and probe sequences as well as PCR reaction conditions were previously described by Struniawski et al. [9].

Prevalence of deficiency alleles Z and S and of AAT genotypes was calculated by Hardy-Weinberg equation.

Results

Mutated PI*Z or PI*S deficiency alleles were detected in 4 subjects out of 51 with GPA (7.84%), including PI*Z in 3 (5.88%) and PI*S allele in 2 patients (3.92%). All of them were cANCA positive; consequently, in the subgroup of cANCA$^+$ GPA patients PI*Z and PI*S subjects constituted, respectively, 6.98% and 4.65%. For comparison, in the controls, PI*Z was observed in 2.8% and PI*S in 1.5% of cases.

Phenotype distribution was as follows: MM in 47 subjects (92.16%), MZ 2 subjects (3.92 %), MS in 1 subject (1.96 %) and SZ in 1 subject (1.96%). Mean AAT serum concentration in the total GPA group was 201.8 mg/mL (66.1; 178-224; SD; 95% confidence interval), while in the cANCA$^+$ GPA group it was 186.3 mg/mL (67.2; 166–207), both within normal limits. Still, mean AAT serum level was 119.43 mg/mL (SD = 48.3) in MZ heterozygotes, 124 mg/mL in MS heterozygote and 58.8 mg/mL in SZ patient.

The estimated frequency of PI*Z and PI*S allele deficiency in the total GPA group was respectively, 29.4/1000 and 19.2/1000, while in the controls it was 13.7/100 and 7.6/1000, as recently reported for the Polish population (Tab. 1). Still, a certain trend towards higher incidence of AAT deficiency alleles in GPA subjects might be observed. The PI*Z and PI*S incidence in cANCA$^+$ group was even higher at 34.9/1000 and 23.2/1000, respectively. As before, the difference was not statistically significant.

The AAT genotypes distribution in total GPA group, cANCA$^+$ GPA subgroup and general population are demonstrated in Table 1.

Discussion

Antineutrophil cytoplasmic antibody (ANCA), associated granulomatosis with polyangiitis, is characterized by predominant lung involvement as well as by autoantibodies against neutrophil derived enzymes. In GPA, ANCA are predominantly directed against proteinase 3 (cANCA). Accordingly, in our material cANCA were present in 84.2% (n = 43) of patients, while autoantibodies against neutrophil myeloperoxidase (p-ANCA) were detected in 3.9% only (n = 2).

The high incidence of AAT deficiency alleles demonstrated by our pilot study in the examined GPA group, and particularly in cANCA$^+$ patients, seems to be meaningful, although the observed differences

Table 1. Estimated frequency for main AAT deficiency alleles and genotypes

	Estimated frequency of deficiency alleles		Estimated genotype frequency (1/Hardy-Weinberg)					
	PI*Z (95% CI)	PI*S (95% CI)	Non-S Non-Z	Non-SZ	ZZ	S Non-S	SS	SZ
GPA n = 51	29,4 (–3,3–62,2)	19,2 (–7,2–46,5)	1/1,10	1/18	1/1156	1/27	1/2601	1/867
cANCA$^+$ GPA n = 43	34,9 (–3,9–73,6)	23,2 (–8,6–55,1)	1/1,12	1/15	1/821	1/22	1/1849	1/616
Controls n = 658	13,7 (5,8–21,5)	7,6 (1,7–13,5)	1/1,04	1/37	1/5345	1/67	1/17319	1/4810

CI — confidence interval; GPA — Wegener's granulomatosis; cANCA$^+$ GPA — cANCA positive Wegener's granulomatosis

did not reach statistical significance. We believe this is attributable to the considerable size differences between all compared groups and subgroups.

Importantly, our preliminary results are in agreement with several, though not all, previously published reports. A genetic predisposition to GPA has been suggested in the early 90s due to several case reports suggesting familial clustering [10]. However, Abdou et al. presented discordant evidence, finding no familial case within 701 GPA patients group, including 12 pairs of twins [11].

The strongest evidence of genetic susceptibility to GPA came from the gene association studies aimed at identifying the candidate genes. In particular, those encoding the major histocompatibility complex (MHC) demonstrating a convincing link to ANCA vasculitides. Specifically, HLA-B8, -B50 or DR-1, DR-9 and DR2 haplotypes were pointed out by most, but not all, studies [12]. Recently, Lyons et al., in a cohorts of 1233 UK (discovery cohort) and 1454 Northern European patients (replication cohort) with ANCA-associated vasculitis, confirmed the significance of MHC in the genome-wide association study [13]. Out of four SNPs (single nucleotide polymorphisms) that exceeded the threshold of significance, three were located in the MHC region, with gene encoding HLA-DPB1 demonstrating the strongest link. Interestingly, the fourth was located within SERPINA 1 gene encoding alpha-1 antitrypsin.

The association between AATD (mainly PI*Z allele) and GPA has been previously indicated. In 1993 Esnault et al. postulated an association between the AAT deficiency phenotypes [14]. Soon afterwards Elzouki et al. implied the significantly increased frequency of PI*Z allele in GPA patients of Scandinavian origin, identifying it in as many as 17% of their cANCA$^+$ GPA group [15]. In 2001 Borgmann et al. analysed a population of 79 GPA German patients, confirming PI*Z in 7 of them (8.86%) and thus the higher prevalence of AAT deficient alleles in ANCA vasculitis [16]. Mahr et al. studied Z and S allele distribution in 443 Caucasians with GPA, detecting PI*Z in 7.4% and PI*S in 11.4% [17]. Quite a similar relationship is implied by our data with PI*Z present in 5.88% of the total GPA group and 6.98% of cANCA positive patients. Accordingly, the incidence of PI*Z and PI*S alleles in our GPA and cANCA$^+$ GPA groups is higher than in the Polish population, but not significantly. While our pilot study is on-going with the intention of extending the GPA group, the need for more insightful clinical analyses of Z or S carriers with GPA in particular cANCA$^+$ should be emphasized. The direct causative link between cANCA vasculitis and AAT deficiency alleles S and Z has not been proven beyond doubt although a considerable number of observations and indirect data seem to suggest its existence.

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Conflict of interest

The Authors declare no conflict of interest.

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