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The prevalence of alpha₁-antitrypsin deficiency in a representative population sample from Poland ☆

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Summary
Aim: Severe alpha₁-antitrypsin (AAT) deficiency is one of the most common genetic disorders in Caucasians. The aim of the present study was to assess an unbiased frequencies of PI*S and PI*Z alleles using genotyping of a representative sample from the general population of Poland.
Methods: A random sample of age- and gender-stratified residents, aged 20 years or older, was drawn from the municipal directory of Kraków, Poland. The two most common deficiency alleles: PI*S and PI*Z were genotyped with qualitative real-time PCR using degenerative dual-labeled allele-specific fluorescent probes.
Results: In the total population of 859 adult subjects (mean age: 49.5 years; range: 20–90), 28 heterozygotes MS, 18 heterozygotes MZ and one homozygote S were diagnosed. The frequency of PI*S allele was 17.5 (95% CI: 11.6–23.9) per 1000; and that of PI*Z was 10.5 (95% CI: 5.8–15.7) per 1000. Therefore, the estimated prevalence of inherited severe AAT deficiency (homozygotes Z) in Poland is 1/9110 (95% CI: 1/4057–1/29,727).
Conclusions: In the whole population of Poland comprising 38 millions, one may expect of about 4189 (95% CI: 1284–9406) subjects with severe AAT deficiency. These numbers are high enough to consider genetic testing being introduced into a common clinical practice.
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Introduction

In 1962, a Swedish biochemist Carl-Bertil Laurell and a clinician Sten Eriksson noticed that an absence of a protein band in the alpha₁ region of serum electrophoregram was associated with pulmonary emphysema.[1] Few years later, we have described the first case of alpha₁-antitrypsin (AAT) deficiency in Poland.[2] Severe AAT deficiency is one of the most common Mendelian disorders in Caucasians. AAT is

produced mainly in the liver. Its main function is to protect the lung against proteolytic damage from neutrophil elastase. The imbalance of protease–antiprotease ratio in the lung tissue leads to the early-onset (below 45 years of age) emphysema and chronic obstructive pulmonary disease (COPD). Abnormal protein can polymerize in hepatocytes causing liver disorders, such as prolonged neonatal jaundice, cirrhosis and cancer. AAT deficiency is also associated with panniculitis and Wegener's granulomatosis.[3]

Over 120 different variants of AAT gene (*SERPINA1*) were described, the most common causing clinically relevant deficiency of the protein being: PI*S (Glu^{264}→Val) and PI*Z (Glu^{342}→Lys).[4] Unfortunately, the condition is often undiagnosed or misdiagnosed.[5] Moreover, the current trends in diagnosis are not improving.[6] A reliable determination of the allelic frequencies in the particular population is crucial to estimate the numbers of subject at risk of complications related to severe AAT deficiency and to organize the national healthcare system for patients affected with this condition. The aim of this study was to assess the unbiased frequencies of PI*S and PI*Z using genetic tests and the representative sample of Polish population.

Material and methods

Random sample of population

Kraków with the community of approximately 741,000 inhabitants (578,000 adults) is the third largest city in Poland. Clearance from the local authorities was obtained and a random sample of 1500 age- and gender-stratified individuals, aged 20 years or older, was drawn from the Kraków municipal directory using computerized method. Taking into consideration a distance from the place of screening visit and representativeness to city population, the selection was restricted to the five (I, V, IX, XI and XIII) of 18 city districts. An invitation to participate included short information on study aims and was sent by post. Additionally, to improve the response rate, free of charge tests of blood cell counts and serum lipids were offered and the information in local media (TV, radio and newspapers) on conducting survey and a request, pointed to all persons who might receive the invitation letter to participate in, were carried out. A second reminder was sent to non-responders 1 year after the first letter.

In order to increase the precision of allelic frequency estimations, additional 309 samples of DNA were also genotyped. They were collected in a previous study by us, using random sampling, from inhabitants of Kazimierz, another district of Kraków.

Data and blood samples collection

A signed informed consent forms, including genetic testing, were obtained from each participant in the study. The survey was carried out in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the Jagiellonian University in Kraków. Blood samples were collected, and general information on height, weight, COPD and asthma symptoms, smoking expressed in "pack-years", medical and family history were collected in a standardized

manner by one investigator (MK). Laboratory tests were carried out on site using a standard hospital facility. The serum level of AAT was measured by a method of automated immunonephelometry, using rabbit's sera to highly purified human AAT (Dade Behering, Magdeburg, Germany). Plasma and serum samples were stored in −70 °C for further investigations.

DNA isolation and AAT genotyping

DNA was isolated from the whole blood drawn on EDTA using chaotropic lysis of white cells (DNAzol; Invitrogen, Carlsbad, USA).[7] DNA samples were then stored in −18 °C. Its concentrations were in a range of 50–300 µg/ml. The two most common AAT deficiency alleles PI*S and PI*Z, were genotyped using a qualitative real-time PCR, which employed two sets of oligonucleotides: a pair of primers and a pair of dual-labeled fluorescent probes. This technique, called 5′-nuclease assay, does not need post-amplification processing and is relatively inexpensive, considering costs of reagents and labor, and time-saving. The amplification and fluorescence detection was carried out with iCycler iQ (Bio-Rad, Hercules, USA) and genotypes were discriminated using iCycler iQ Optical System Software v.3 (Bio-Rad). PCR conditions and oligonucleotides' sequences were as published before.[8] This assay was designed in our laboratory and validated using reference test and external interlaboratory quality control.

Statistical analysis

Statistical analysis was performed using Statistical Analysis Software (v.9.1, SAS Institute). The allelic frequencies were determined by the prevalence of heterozygotes and calculated using bootstrap method with boot number of 100,000. The prevalence of the genotypes was calculated from the Hardy–Weinberg formula. The data on the number of individuals in Kraków and Poland were obtained from the Central Statistical Office (Warsaw, Poland). The 95% confidence interval (CI) was estimated for each value. Comparisons of the age of responders and non-responders were performed with ANOVA; differences between median AAT serum levels according to genotype, given with interquartile range (IQR), were tested with Kruskal–Wallis test. The χ^2 test was used for categorical variables. All results were regarded as significant for the alpha error of 0.05 or less. A statistical Precision Factor Score (PFS) was estimated according to method developed by Fernández-Bustillo.[9] In order to maintain a concordance to the previously published values of other surveys, calculations of PFS were performed with CI formulae using the original spreadsheet kindly provided by Dr. Ignatio Blanco.

Results

Response rate

In the first step of enrollment, 1500 letters with invitation were sent by post, 20 of them have been returned with information: "addressee unknown" or "addressee dead".

Five hundred and twenty three persons responded positively; the lowest response rate was noted in the youngest age group. In the second step, the reminders were sent to the subjects aged 20–39 years. Nevertheless, in this group the response rate was only 6.3% and no further recruitment was continued. Together, 550 randomly selected inhabitants (248 men and 302 women) were recruited from a general population of Kraków for this study (Table 1). The response rate was 36.7%. Significantly higher response was noted in the older age groups, than in the work-active strata. The mean age of responders was higher about 4.13 (95% CI: 2.23–6.02) years than in the group of non-responders ($p < 0.001$).

AAT genotyping

In the random sample of Kraków population ($n = 550$), 17 heterozygotes MS, 12 heterozygotes MZ and 1 homozygote S were diagnosed. Therefore, the frequency of PI*S allele was 17.3 (95% CI: 10.0–25.5) per 1000; and PI*Z–10.9 (95% CI: 5.5–17.3) per 1000. Combining with the earlier collected population of 309 randomly selected Kazimierz residents, the overall results were: 28 heterozygotes MS, 18 heterozygotes MZ and one homozygote S. These two population samples did not differ significantly and were in the Hardy–Weinberg equilibrium. In the total sample of 859

subjects, the estimated alleles' frequencies per 1000 were as follows: PI*M (non-S, non-Z)–972.1 (95% CI: 963.9–979.6); PI*S–17.5 (95% CI: 11.6–23.9); and PI*Z–10.5 (95% CI: 5.8–15.7). Therefore, basing on allelic frequencies, the prevalence of severe deficiency (homozygotes Z) was 1/9110 (95% CI: 1/4057–1/29,727), of the other deficiency combinations: SS–1/3279 (95% CI: 1/1751–1/7432); SZ–1/2733 (95% CI: 1/1333–1/7432) and of carriers: MZ–1/49 (95% CI: 1/33–1/89); MS–1/29 (95% CI: 21–45). The total PI*S and PI*Z prevalence was 1/18.

In the Polish population one may expect about 4189 (95% CI: 1284–9406) subjects with severe AAT deficiency. Details on the expected numbers of AAT genotypes in population of Kraków and Poland are given in Table 2.

Serum level of AAT

In the group of subjects with wild genotype (non-S and non-Z), the median level of AAT in serum (normal range: 0.9–2.0 g/l) was 1.23 g/l (IQR: 0.22); in MS heterozygotes—1.08 g/l (IQR: 0.22) and in MZ heterozygotes—0.77 g/l (IQR: 0.60). Differences in these levels between genotypes were statistically significant ($p = 0.0001$). The sensitivity of AAT measurement in serum, for prediction of partial deficiency, was 46.7% (95% CI: 28.3–65.7) and specificity—99.0% (95% CI: 97.8–99.7).

Table 1 The characteristic of the population of Kraków, randomly selected sample and participated group-strata according to age and sex.

Age (in years)	Kraków population*		Selected		Participated	
	Men (%)	Women (%)	Men (%)	Women (%)	Men (%)	Women (%)
20–39	109,942 (19.0)	113,783 (19.7)	286 (19.1)	295 (19.7)	74 (13.5)	94 (17.1)
40–59	99,381 (17.2)	116,073 (20.1)	258 (17.2)	301 (20.1)	102 (18.5)	122 (22.2)
60 and above	54,853 (9.5)	84,151 (14.6)	142 (9.5)	218 (14.5)	72 (13.1)	86 (15.6)
Combined	578,183		1500		550	

*Data of the Central Statistical Office on 31 December 2001.

Table 2 Expected numbers of AAT genotypes in Kraków and in Poland.

Genotype	Kraków ($n = 757,762$)*		Poland ($n = 38,161,313$)*		Prevalence 1/x	95% confidence interval 1/x
	Number	95% confidence interval	Number	95% confidence interval		
MM (non-S, non-Z)	716,011	704,039–723,158	36,058,689	35,455,798–36,418,624	–	–
MS	25,725	16,945–35,384	1,295,522	853,382–1,781,974	29	21–45
MZ	15,435	8473–23,244	777,313	426,691–1,170,585	49	33–89
SZ	227	102–569	13,964	5135–28,639	2733	1333–7432
SS	231	102–433	11,636	5135–21,798	3279	1751–7432
ZZ	83	25–187	4189	1284–9406	9110	4057–29,727

*Data of the Central Statistical Office on 30 June 2005.

Table 3 Published frequencies of AAT deficiency alleles in Polish population.

Study	No.	Method	PFS	Allele frequency (per 1000)		Genotype diagnosed				
				S (95% CI)	Z (95% CI)	MS	MZ	SZ	SS	ZZ
Current study	859	Genotyping (real-time PCR)	5.7	17.5 (11.6–23.9)	10.5 (5.8–15.7)	28	18	0	1	0
Walter et al.[12]	423	IEF	4.2	16.5 (8.3–26.0)	14.2 (7.1–22.5)	14	12	0	0	0
Kowalska et al.[13,14]	630	IEF	5.0	14.3 (7.9–21.4)	14.3 (7.9–21.4)	18	18	0	0	0
Kowalska et al.[15]	741	IEF	3.9	9.4 (4.7–14.8)	6.7 (2.7–11.5)	12	10	0	1	0
Combined (row 2–4)	1794		4.4*	12.8 (9.2–16.7)	11.1 (7.8–14.8)	44	40	0	1	0
Combined (all)	2653		4.8*	14.5 (11.3–17.9)	10.9 (8.3–13.8)	72	58	0	2	0

PFS—precision factor score; IEF—isoelectric focusing.
 *Weighted mean.

Discussion

In the random sample of general population comprising 859 subjects, we found the allelic frequency of PI*S—17.5 (95% CI: 11.6–23.9) per 1000, and that of PI*Z—10.5 (95% CI: 5.8–15.7) per 1000. Our sample size exceeds the proposed minimal value for such estimations.[10] This is the first study ascertaining the prevalence of AAT deficiency in Poland by genotyping and a random sampling method. The results do not differ significantly from the previously published surveys, except the study by Opolska[11] reporting seven-fold lower frequency of Pi*Z. This discrepancy could easily be explained by a low reliability of starch gel electrophoresis in diagnosis of Z band due to diminished level of the protein. Because of these limitations, the early data by Opolska were removed from further analysis of AAT deficiency prevalence in Poland. The detailed results of previous surveys,[12–15] using more reliable method of isoelectric focusing (IEF), are given in Table 3 and are illustrated by Figure 1 with references to their geographical origin and sampling methods.

Over the last few years, Blanco and de Serres published systematic reviews and meta-analyses of AAT deficiency phenotypes in Europe,[9,16–18] and developed a precision factor score for assessing the statistical quality of data, in terms of precision (or imprecision) of each published survey. Our study fulfills these inclusion criteria, with the precision factor score of 5.7, which is the highest among the Polish surveys (Table 3). Moreover, it seems to be the first study in Europe on the prevalence of AAT deficiency in general population using genotyping; and the second, after the work by Blanco et al.,[19] in which recruitment scheme warranted a representative population sample.

In the latest meta-analysis on the prevalence of AAT deficiency in Europe,[18] Blanco and de Serres presented the results for Poland: PI*S—15 (95% CI: 13–17) per 1000 and PI*Z—4 (95% CI: 3–5) per 1000. As some of original studies were available only in the Polish language, a bias in the extraction of primary data could have occurred; e.g. two
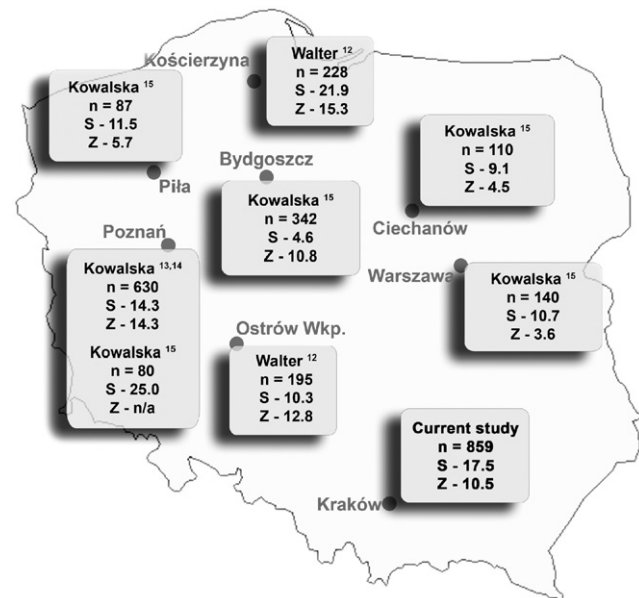

Figure 1 Geographic locations of surveys on frequencies (per 1000) of AAT deficiency alleles in Poland. Methods of sample recruitment: Walter et al.[12]—195 unrelated residents city of Ostrów Wielkopolski and 228 Kashubes from Koscierzyna; Kowalska et al.[13,14]—blood donors ($n = 181$) and unrelated inhabitants ($n = 450$) city of Poznań and environs; Kowalska et al.[15]—umbilical cord serum from 741 newborns collected in five cities of Central Poland: Bydgoszcz, Warsaw, Poznań, Piła, Ciechanów.

surveys included[20,21] referred to AAT phenotypes frequencies in general population based on the results by Opolska.[11]

The most important problem in epidemiological studies is representativeness of the results for the general population.[22–24] In our study, the response rate was moderate—36.7%, which limits the validity of conclusions. However, the allelic frequencies were determined mainly
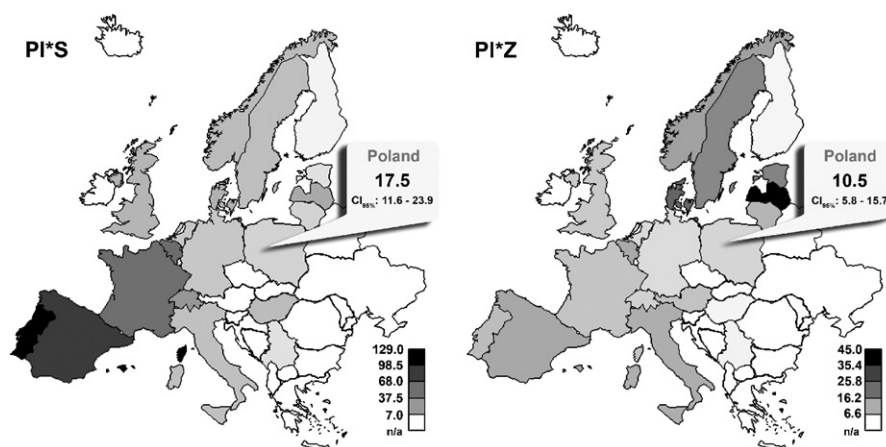
Figure 2 The frequencies (per 1000) of AAT deficiency alleles in Poland and other European countries. The data for the other countries are from the meta-analyses by Blanco et al.[18] A gradient distribution of AAT deficiency alleles declines across Europe from the North to the South for PI*Z, and from the West to the South for PI*S. n/a—data not available.

by the prevalence of heterozygotes MZ and MS. In such carriers for AAT deficiency, symptoms manifests only in case of additional exposure to other risk factors.[25–29] Therefore, we avoided any influence of over-mortality within ZZ homozygotes, potentially leading to underestimation of PI*Z allele in the older age strata. Similarly, an over-estimation of severe AAT deficiency due to higher response rate of affected subjects was avoided. Based on all of the Polish studies, a combined allelic frequency per 1000 is 14.5 (95% CI: 11.3–17.9) for PI*S and 10.9 (95% CI: 8.3–13.8) for PI*Z (Table 3), which seems well fitting to a cross-European gradient in frequency of this allele (Figure 2). Early diagnosis of this genetic predisposition, with the appropriate medical follow-up and change of life style can prevent or at least postpone an over AAT deficiency complications.

Conflict of interest statement

None of the authors have a conflict of interest to declare in relation to this work.

Acknowledgments

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