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Gene Mutations Of Alpha-1-Antitrypsin In The Kazakh Population.

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ABSTRACT

An article is considered the Alpha1-antitrypsin (AAT) deficiency, which is a rare, and it is under-diagnosed of the condition in patients with chronic obstructive pulmonary disease (COPD) and it mainly affects the Caucasian population. Research was aimed on identification of AAT deficiency cases in Kazakh of patients with COPD, in order to estimate the prevalence of this rare disease in Kazakhstan. There were recruited 111 Kazakh subjects with COPD. Blood samples were collected as dried blood spot. The AAT concentration was determined by nephelometry. Genotype and phenotype analysis were performed if AAT concentration was lower than 1.04 g/L. Demographic and clinical data were reported. Genotyping of 111samples were revealed 2 (1.8%) PI*MZ, and 1 (0.9%) PI*MS. Phenotyping was identified also two samples (1.8%) with phenotype PIMI. There were resulted Allelic frequencies of pathological mutations Z, S and 0.9%, 0.4%, 0.9%, respectively, in COPD Kazakh population. The results of the present study support the general concept of targeted screening for AAT deficiency in countries like Kazakhstan, with a large population of COPD patients and low awareness among care-givers about this genetic condition.

Keywords: alpha-1 antitrypsin deficiency, chronic obstructive pulmonary disease, genetics, genotyping, phenotyping.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a major and increasing cause of morbidity and mortality worldwide [1,p.1256–1276]. In 2010, COPD alone was estimated to have cost the global economy $400 billion [2,p.477-57]. COPD clusters within families, suggesting that heritable factors play a role in the pathogenesis of this disease [3, p. 1770 – 8. 15, p. 818–*855*]. The only genetic factor that is widely accepted to be associated with COPD is severe deficiency of α_1-antitrypsin [4, p. 496–504].

Alpha-1 antitrypsin (AAT) deficiency is a hereditary disorder first reported in the early 1960s when emphysema was described in patients with low plasma levels of AAT protein [5, p. 132–140]. The condition is associated with substantially increased risk for the development of pulmonary emphysema by the third or fourth decades of life and is also associated with risks for development of hepatic disease [6, p. 1316–1321], cutaneous panniculitis [7, p. 296–299.], bronchiectasis [8,p. 137–141], vasculitis [9, p. 489–494], Wegener's granulomatosis [10, p. 253–255], and lung cancer [11, p. 445–452]. AAT deficiency is characterised by misfolding of the AAT protein and belongs to a class of genetic diseases termed conformational disorders [12, p. 21–34].

The SERPINA1 gene is highly pleomorphic with over 100 alleles identified to date [13, p. 259–264].The most common mutation causing AATD is the Z mutation, with the S mutation weakly associated with lung disease. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. ATS/ERS guidelines advocate screening all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients[14, p. 313].

From a public health perspective, knowledge of the ATTD prevalence in every community is essential [15, p. 818–*855*]. The current study specifically attempts to determine the prevalence and number of subjects carrying the most common defective alleles, PI*S and PI*Z, in Asian countries. The present study estimates the total number of ZZ, SZ and MZ individuals in 20 Asian countries, and goes beyond earlier publications, in which only the gene frequencies for PI*M, PI*S, and PI*Z were reported for individual cohorts in individual cities or geographic regions [15, p. 818–*855*].

AATD is widespread throughout the world, with significantly high prevalence in countries through out the continent of Asia. It also is clear that α_1-antitrypsin deficiency is not just a disease of Caucasians (or whites), but is prevalent in many different races throughout the world[16,p. 1091-1099].

In Kazakhstan, the number of patients with COPD has increased more than twofold for the last 10 years, constituting 321 humans out of 100 thousand people in 2011. To compare, one of the most common diseases, diabetes is found among 158.3 people per 100 thousand. In fact, the number of patients with COPD is several times as many [17,p. 13-15].

MATERIALS AND METHODS

Study design and subjects

A case-control study was conducted in east Kazakhstan in 2014. In total, 133 subjects (22 healthy subjects, 111 subjects with COPD) participated in this study (Figure1). Study was performed in AAT Lab of Marburg University clinic, Germany).Collection of blood samples and the genetic investigations were performed after appropriate written informed consent of the subjects involved and approval by the ethical committees of the institutions involved(Emergency Hospital of Semey, Kazakhstan).

Capillary blood samples were collected on filter paper (DBS – dried blood spot) from133 subjects.

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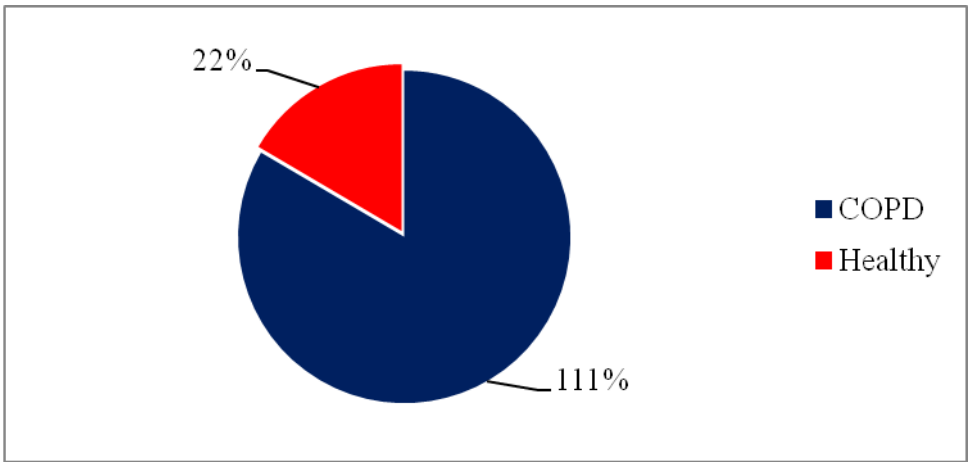

Figure 1. Percentage distribution of 133 subjects

According to the protocol of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) [1,p. 1256–1276], diagnosis of COPD was confirmed by spirometry with $FEV_1/FVC < 0.7$, where FEV_1 = forced expiratory volume in one second and FVC = forced vital capacity.Pre-bronchodilator spirometric tests were performed according to ERS guidelines with a rolling seal spirometer followed by post bronchodilator spirometric tests after inhalation of 400 mg salbutamol [18,p. 5–40].

Subjects were male or female and aged between 18–65 yrs with no clinically significant or uncontrolled cardiac, hepatic, renal, gastrointestinal, endocrine, metabolic, neurological or psychiatric disorder(table 1).

Table 1.Characteristics and lung function parameters of the study population

Parameter	Healthy Absolute*	COPD patients Absolute*	% predicted
Subjects n	22	111	-
Sex (M/F)	10/12	65/46	-
Age (years)	45.5±8.5	60.09±13.12	
Body mass index(kg/m^2)	0.23±0.04	0.23±0.06	
Inhaled steroids (yes/no)	-	40/71	
Smoking status (current/ex/never)	-	49/48/14	
Smoking history (pack years)	-	32.91±26.25	
FEV1 (l)		1.93±1.3	61.52±15.9
CAT test	0	27.56±9.4	
*Mean (SD) values. FEV1 = forced expiratory volume in 1 second; CAT - COPD Assessment Test			

Capillary blood samples were collected on filter paper (DBS – dried blood spot) from 132 subjects.

Quantification of AAT

The AAT level measurements were performed on dried blood spot (DBS) samples by a rate immune nephelometric method (Dade-Behring BN II) using a goat antihuman AAT antibody [19, p. 899–901].

AAT levels were measured by nephelometry (Dade-Behring BN II).

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Phenotyping

Qualitative detection and characterisation of AAT phenotypes was carried out using the Hydrasys electrophoresis platform (Sebia) and the Hydragel 18 A1AT Isofocusing kit (Sebia) [20, p. 260–263]. This isoelectric focusing (IEF) method on agarose gel has an added immunofixation step which utilises a specific antibody to AAT, rendering it superior to traditional IEF techniques.

Genotyping

Genotyping was performed on a MyCycler (Bio Rad) with specific primers designed for the PiZ(rs 28929474) and PiS (rs17580) mutations [21,p. 814–817].

Data elaboration and statistical analysis

Data were analysed using the Statistical Package for the Social Sciences version 20, (SPSS, USA, Chicago, IL).Data was analysed by descriptive statistics, percentage distribution, chi-square tests and other contingency parameters, as appropriate. Statistical significance was assumed at two-tailed $p < 0.05$, unless stated otherwise.

RESULTS

The level of alpha-1 antitrypsin in patients with COPD 51 below the norm, while in the control group-9 patients. Patients with mutations identified alpha1-antitrypsin level significantly lower than in COPD patients without the mutation. A total of 133(111 patients COPD, 22 control group) individuals for AATD identified 2 MZ heterozygotes, 1 MS heterozygote, 2 MI heterozygotes, and 1unknown mutation with allele frequencies of 0.9 for the MS mutation and 1.8 for the MZ mutation and 1.8 for the MI mutation in the targeted population. The distribution of the differentphenotypes and the corresponding mean of a1AT for each phenotype are shown in Table I.

Table 1. Distribution of phenotypes and alAT levels from patients with COPD

Phenotype	Genotype	Number	PercentageIncidence	Age	FEV1	alAT Level
MM	PI*MM	105	94.6	60.8(12.1)	60.7(15.2)	1.7 (0.4)
MS	PI*MS	1	0.9	62	40	1.3
MZ	PI*MZ	2	1.8	70.5(7.7)	70.0(2.8)	1.03(0.4)
MI	PI*MI	2	1.8	51.0(2.8)	60.5(3.5)	1.35(0.1
Unknown		1	0.9	39	35	2.01
Total		6	100			1.7(0.5)

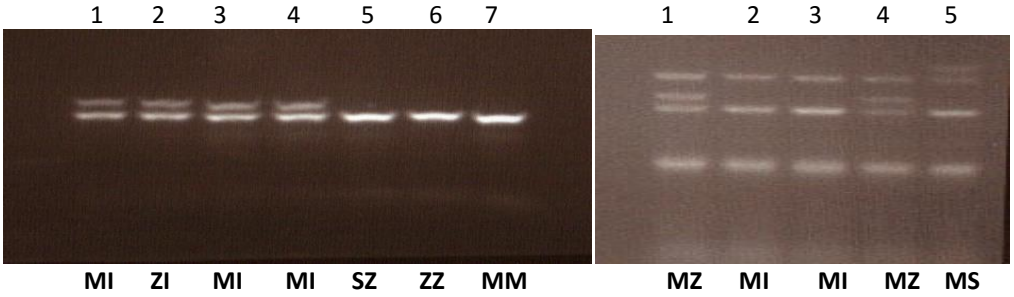

Figure 2A.Genotiping with primer I; 1-2 control probes MI,3-4 patients with MI mutations, 5- SZ, 6-ZZ, 7-MM; B. Genotiping with primers S and Z in our patients. 1- MZ, 2- MI, 3- MI, 4-MZ, 5-MS.

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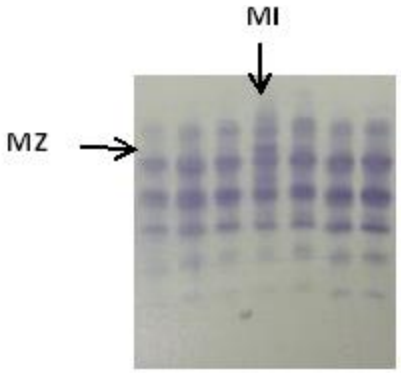

Figure 3. Phenotyping

Table 2.Distribution of phenotypes and aIAT levels from control group

Phenotype	Number	Percentage incidence	Age	FEV1	alAT Level
MM	22	100	45,5 (8.5)*	99 (4.3)	1.6 (0.3)*
Mean* - Significance for Shapiro-Wilk p>0,05					

The effect of smoking on the level of AAT shown in table 3.

AAT		Smoking		Total
		current/ex	never	
AAT<1,7 mg/dl	frequency	20	23	43
	AAT %	46,5%	53,5%	100,0%
	Smoking %	40,0%	38,3%	39,1%
AAT>1,7 mg/dl	frequency	30	38	68
	AAT %	44,8%	55,2%	100,0%
	Smoking %	60,0%	61,7%	60,9%
Total	frequency	50	61	111
	AAT %	45,5%	54,5%	100,0%
	Smoking %	100,0%	100,0%	100,0%
RR-1.126, 95%CI 0.525-2.415				

This result indicates a strong relationship between smoking and the level of AAT in our patients.

Table 4. Prevalence of MRC (Medical Research Council) according to phenotypes

Phenotypes		MRC*					Total
		1	2	3	4	5	
MM	frequency	14	35	22	20	14	105
	%	11.8%	31.8%	20.0%	18.2%	12.7%	94.5%
MZ	frequency	1	1	-	-	-	2
	%	0.9%	0.9%	-	-	-	1.8%
MI	frequency	-	1	1	-	-	2
	%	-	0.9%	0.9%	-	-	1.8%
MS	frequency	-	-	-	1	-	1
	%	-	-	-	0.9%	-	0.9%
Unknown	frequency	-	-	-	-	1	1
	%	-	-	-	-	0.9%	0.9%
Total	frequency	15	37	23	21	15	110
	%	12.7%	33.6%	20.9%	19.1%	13.6%	100.0%
Mean * Medical Research Council dyspnoea scale. 1- Not troubled by breathlessness except on strenuous exercise; 2- Short of breath when hurrying or walking up a slight hill; 3- Walks slower than contemporaries on the level because of breathlessness, or has to stopfor breath when walking at own pace; 4- Stops for breath after about 100 m or after a few minutes on the level; 5- Too breathless to leave the house, or breathless when dressing or undressing. $x^{2=}$15.875, df=16, p=0,462							

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The percentage with previous bronchitis or emphysema was greatest in the Pi MZ group but the differences were not statistically significant.

Table 5. Prevalence of CAT (COPD Assessment Test) according to phenotypes

Phenotypes		CAT*				Total
		0	1	2	3	
MM	frequency	6	24	29	46	105
	%	5.5%	20.9%	26.4%	41.8%	94.5%
MZ	frequency	-	1	1	-	2
	%	-	0.9%	0.9%	-	1.8%
MI	frequency	-	-	1	1	2
	%	-	-	0.9%	0.9%	1.8%
MS	frequency	-	-	-	1	1
	%	-	-	-	0.9%	0.9%
Unknown	frequency	-	-	-	1	1
	%	-	-	-	0.9%	0.9%
Total	frequency	6.0	25.0	31.0	49.0	111.0
	%	5.5%	21.8%	28.2%	44.5%	100.0%
Mean * COPD Assessment Test. 0- Mild 0-10; 1-Moderate 10-15; 2-Severe 15-25; 3- Very Severe 25-40. $x^{2=}$5.481, df=12, p=0.94						

The percentage with CAT severe and very severe degrees were greatest in the Pi MI, Pi MS and unknown mutations but the differences were not statistically significant.

Table 6. Prevalence of X-ray according to phenotype

Phenotypes		X-ray		Total
		Chronic bronchitis	Emphysema	
MM	frequency	65	40	105
	%	58.2%	36.4%	94.5%
MZ	frequency	2	2	2
	%	1.8%	1.8%	1.8%
MI	frequency	2	2	2
	%	1.8%	1.8%	1.8%
MS	frequency	1	1	1
	%	0.9%	0.9%	0.9%
Unknown	frequency	1	1	1
	%	0.9%	0.9%	0.9%
Total	frequency	65	46	111
	%	58.2%	41.8%	100.0%
$x^{2=}$8.829, df=4, p=0.066				

The percentage with emphysema was greatest in the Pi MZ, Pi MS, PiMI and unknown mutations but the differences were not statistically significant.

Table 8. Prevalence of groups COPD according to phenotypes

Phenotypes		COPD			Total
		Group A	Group B	Group D	
MM	frequency	2	78	25	104
	%	1.8%	70.0%	22.7%	94.5%
MZ	frequency	-	2	0	2
	%	-	1.8%	0.0%	1.8%
MI	frequency	-	2	0	2
	%	-	1.8%	0.0%	1.8%
MS	frequency	-	-	1	1

	%	-	-	0.9%	0.9%
Unknown	frequency	-	-	1	1
	%	-	-	0.9%	0.9%
Total	frequency	2	81	27	110
	%	1.8%	73.6%	24.5%	100.0%
x^2=7.60, df=8, p=0.474					

The percentage with group B and group D COPD were greatest in the all mutations but the differences were not statistically significant

DISCUSSION

The results of the study concluded that deficient AAT alleles are no infrequent in Kazakhstan. The low level of alpha-1 antitrypsin in patients with COPD and control groups is possible with the fact that we conducted a study of the dried drop of blood, so the results were less accurate. But in all patients with mutations in the gene alpha 1-antitrypsin level of alpha-1 antitrypsin profit significantly lower than in patients with COPD without mutations. In our study were identified six mutations, of which 2 cases of MI rare mutations.

According to the Irish scientists, the I mutation (Arg39Cys) was present at a relatively high frequency (0.0038) in a targeted population, with over 40 cases identified [23, p. 313]**.**

In neighboring Kyrgyzstan conducted a similar study in 2008. The result of this study of 100 patients with COPD have been identified 3mutations: 1-ZZ, 2-MZ mutations[22,p. 302]. It is clear from the data presented here that the statement "AATD is not a rare disease but a disease that is rarely diagnosed" is particularly apt in the Irish setting [24,p. 1851–1854]. The continuing lack of awareness and under-diagnosis of this condition is alarming considering the high numbers of individuals at risk due to deficient SERPINA1 mutations. The advantages of early and accurate diagnosis of AATD are manifold and include closer observation and management of affected individuals, especially regarding pulmonary and liver health; family member testing; aggressive smoking cessation efforts; consideration of occupational hazards and environment exposures; and significant economic benefits arising from the reduced burden on healthcare providers [25,p. 981–988,26,p. 1989–1994].

Our results correspond to the literature data. Emphysema is a chronic progressive lung disease characterised by abnormal permanent enlargement of airspaces as a result of destruction of alveolar walls [27,p. 1419–24]. Emphysema usually develops by the third to fourth decade in affected individuals who smoke cigarettes and may appear in the fifth or sixth decade in individuals who have never smoked[28,p. 16-18].

In summary, the findings of our study have significant consequences. The results of the study concluded that deficient AAT alleles are no infrequent in Kazakhstan. Therefore, further research is needed to allow the finding of deficient, even rare, mutations in our country. Today Kazakhstan health care facilities cannot offer diagnostic and management service to COPD patients having symptoms resembling AATD. The reasons include poor awareness of doctors, especially on the primary healthcare level, and lack of diagnostic equipment and skills. AATD was traditionally considered a disorder mainly affecting Caucasian population. However, this study has shown world-wide prevalence of the disorder. In future research is needed mutation alpha1-antitrypsin in large groups.

CONCLUSION

This study demonstrates that the I allele frequencies in Kazakhstan are quite high. The importance of an early diagnosis of AATD cannot be over-emphasised as the resulting appropriate medical follow-up and lifestyle changes can help prevent or at least postpone the development of the lung disease associated with this condition.

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