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Pulmonary Langerhans cell histiocytosis — insight into the incidence of alfa-1-antitrypsin deficiency (A1ATD) alleles

The authors declare no financial disclosure

Abstract

Introduction: The alpha-1 antitrypsin deficiency (A1ATD) 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. There is no data regarding the prevalence of main, clinically most important A1ATD alleles PI*Z and PI*S in patients with pulmonary Langerhans cell histiocytosis (PLCH). PLCH is not only strongly linked to the cigarette smoking, but is also characterised by polycystic lung lesions. The goal of the study was to assess the incidence of A1ATD alleles in patients with PLCH.

Material and methods: Blood samples were collected from 34 adult patients (14 women and 20 men), with histologically confirmed PLCH. AAT serum concentration was assessed by nephelometry and PI-phenotype, identified by isoelectrofocusing. The PI*S and PI*Z alleles were confirmed by genotyping using real-time PCR.

Results: Deficiency alleles PI*Z and PI*S were detected in 3 patients (one woman and 2 men), in 5.88% and 2.94% respectively. The estimated incidence of deficiency alleles was 29.4/1000 (95% CI; 10–69.5) for PI*Z and 14.7/1000(95%CI; 13.9–43.3) for PI*S. According to our previous reports, the expected prevalence of PI*Z and PI*S alleles in the general Polish population was 13.7/1000 (95% CI 5.8–21.5), and 7,6/1000 (95% CI 1.7–13.5) respectively.

Conclusions: The incidence of main A1AT deficiency alleles in patients with PLCH seems higher than in the general Polish population. The study is ongoing.

Key words: alpha-1 antitrypsin deficiency, A1ATD, polycystic lung diseases, pulmonary Langerhans cell histiocytosis

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Introduction

The alpha-1 antitrypsin deficiency (A1ATD) is one of the three most common genetic disorders in Caucasians [1–4]. A1ATD considerably increases the risk of progressive obstructive lung diseases, mostly chronic obstructive pulmonary disease. Cystic lung lesions are observed as an associated feature of this deficiency [1–4]. It has also been well documented that A1ATD deficient subjects exposed to cigarette smoke and/or occupational hazard are particularly prone to develop lung pathology relatively early in their lifetime [5, 6].

Langerhans cell histiocytosis is a rare disease caused by clonal proliferation of bone marrow derived Langerhans cells. LCH lesions are observed either in one organ, usually skin, bone, lymph nodes, lungs, or in multiple organs [7]. Pulmonary Langerhans cell histiocytosis (PLCH) is a disease of usually young adults, and it is strongly linked to cigarette smoking. It is characterised by the nodular and polycystic lung lesions [7]. The imbalance in protease and atiprotease activity may take a part in development of cystic changes [8, 9].

There is no data regarding prevalence of main, clinically most important alpha-1 an-

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Table 1. Estimated frequency for main ATT deficiency alleles and genotypes in PLCH patients and control population

	Estimated frequency of deficiency alleles (per 1,000)		Estimated genotype frequency (1/Hardy-Weinberg)					
	PI*Z (95% CI)	PI*S (95% CI)	MM	MZ	ZZ	MS	SS	SZ
PLCH N = 34	29.4 (–10–69.5)	14.7 (–13.9–43.3)	1/1.09	1/18	1/1156	1/36	1/4624	1/1156
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

titrypsin deficiency alleles PI*Z and PI*S, in patients with pulmonary Langerhans cell histiocytosis. While both disorders, A1ATD and PLCH are considered rare, relatively high number of PLCH patients was included in the current study aiming at the assessment of PI*Z and PI*S alleles in this highly selected group.

Material and methods

Blood samples were collected from 34 adult patients (14 women and 20 men) with PLCH. The diagnosis of PLCH was established on the basis of clinical picture of PLCH, characteristic chest CT and histological findings. The mean age of patients was 34.06 ± 13.66 years, and all of them were smokers. At the time of examination 10 were current smokers and 24 stopped smoking from 1 to 6 months before.

A1AT serum concentration was measured by rate immune nephelometry method (Immage 800 Immunochemistry System Beckaman-Coulter, USA) with commercially available reagents including goat anti-human A1AT antibody (Beckman-Coulter, USA).

The A1AT phenotype was identified by isoelectrofocusing(IEF) on polyacrylamide gel with pH of 4.2–4.9 using Multiphor II Electrophoresis System (GE Heatlth Care Bio-Sciences AB, Uppsala, Sweden), and was assessed by visual inspection and comparison with control M1M2, MS, MZ and ZZ samples.

Commercially available kit Extract-N-Amp Blood PCR Kits (Sigma Aldrich) was used for genomic DNA extraction. Genetic material eluted from dried blood spots at filter paper was used for A1AT genotyping. The most common mutations of the A1AT gene (Z, S variants) were identified in a single reaction by real-time PCR method using hydrolysing probes connected with fluorescent dyes (VIC or FAM) complementary to mutant variants (PI*S or PI*Z). According to the routine lab protocol as well as the international guidelines sequencing of the A1AT gene was performed in case of discrepancies between serum A1AT concentration, geno- and phenotyping or in case of irregularities in any of these tests. None of them was observed in the analysed group of patients. The method was previously described by Struniawski *et al.* [10].

The Hardy-Weinberg equation was applied to assess the prevalence of variant alleles Z and S as well as the A1AT genotypes.

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Results

Deficiency alleles PI*Z and PI*S were detected in 3 patients (one woman and 2 men) out of 34 (8.82%) in 5.88% and 2.94% respectively. The mean age of patients with MM genotype was 31 ± 10.84 years, one man with MZ genotype was diagnosed at the age of 33, and the woman and the other man with MS genotype were at the time of diagnosis 55 and 63 respectively.

The estimated incidence of deficiency A1AT alleles was calculated as 29.4/1000 (95% CI; 10–69.5) for PI*Z and 14.7/1000(95%CI; 13.9–43.3) for PI*S (Table 1).

Meanwhile, their prevalence in the general Polish population is 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 as demonstrated by the preliminary data from our ongoing large scale population study [11]. The differences between both groups did not reach statistical significance, most probably due to the considerable disproportion in their size.

Mean A1AT serum concentration in PLCH patients with normal A1AT MM phenotype was 158.5 mg/ml (95% CI: 143.8–173.1), while in MZ heterozygotes 83 mg/ml (95% CI 6,7–159.2) and 107 mg/ml in one MS heterozygote patient (Table 2).

Discussion

Alpha-1 antitrypsin (A1AT) also known as alpha-1 proteinase inhibitor (PI) or SERPINA1

Table 2. AAT serum concentration in patients with PLCH according to ATT phenotype

Phenotype	Median (mg/dl)	Standard deviation	95% CI	Min.	Max.
MM	158.5	37.8	143.8–173.1	93	248
MZ	83	8.5	6.7–159.2	77	89
MS	107	–	–	107	107

(Serine Protease Inhibitor, clade A, member 1), is a 394-amino acid, circulating glycoprotein with a molecular weight of 52-kDa [1–3].

Hepatocytes are the main places of A1AT production, next to monocytes, macrophages, pancreatic islets, lung alveolar cells and colonic enterocytes. A1AT is present in the lungs, liver, guts and all biological fluids [1–5].

It is an acute-phase glycoprotein, one of the major protease inhibitors ensuring the protease-antiprotease homeostasis. A1AT is involved in the inhibition of neutrophil elastase and proteinase-3, caspases 1 and 3, myeloperoxidase and cathepsin G from neutrophils, tryptase and chymase from mast cells, TACE (tumour necrosis factor alpha converting enzyme), calikrenine 7 and 14 [1–4].

Studies into cells, animal models, and humans have provided initial evidence for the efficacy of A1AT in emphysema, asthma, panniculitis, granulomatosis and polyangitis, diabetes mellitus, rheumatic arthritis, organ transplant rejection and other possible inflammatory diseases [1, 3]. For instance, A1AT by inhibition of matryptase overexpression — enzyme localised on the external part of the cellular wall, can regulate the sodium transport and mucus production, particularly in chronic obstructive pulmonary disease and mucoviscidosis. On the other hand, A1AT can regulate neutrophils chemotaxis and adhesions by influence on TACE — the most important enzyme involved in the creation of active TNF alpha [1–5].

The A1AT gene has two alleles, normal alleles are called M, and normal individuals have genotype MM. There are two most common deficiency alleles Z and S however also null variant has been noticed. In humans the different genetic variants of these alleles have been observed, which are translated into the wide range of serum A1AT concentration and activity. The most common deficiency variants might be detected due to low alpha-1 antitrypsin serum concentration. Dysfunctional variant identification is possible only by means of pheno- or genotyping by direct sequencing [1–3].

Pulmonary Langerhans cell histiocytosis is a strongly linked to smoking and over 95% of patients are active smokers [7]. Cigarette smoke induces inflammation, oxidative stress, and lung injury, but also the number, distribution, and activity of macrophages and Langerhans cells [7].

Activated Langerhans and other inflammatory cells produce matrix metalloproteinases (MMPs) causing bronchial wall destruction and airway remodeling in PLCH cases [7, 8]. Immunohistochemical staining of lung biopsies has shown strong reactivity to MMP2 and MMP9, particularly in dendritic and Langerhans cells and macrophages [8, 9]. Also another glycoprotein with cytokine-like properties, such as osteopontin is highly up-regulated in patients with smoking-related interstitial lung diseases [12]. Prasse *et al.* [12] reported that nicotine induces osteopontin and GM-CSF (granulocyte-macrophage colony stimulating factor), that enhances cell survival, promotes macrophage accumulation and fibrotic lung tissue remodelling. It might be hypothesised that imbalance between proteolytic and antiproteolytic enzymes can take a part in development of cystic pulmonary lesions in the course of PLCH [7–9, 13, 14].

Further on, the strong proinflammatory properties of mutated AAT variants have been well documented [3]. Similarly, the conformational changes in the normal MM variant, upon exposure to cigarette smoke and therefore oxidative stress are rendering AAT inactive [5].

However, to the best of our knowledge, except for speculations, there are no research data on the potential role of inherited or functional AAT deficiency in PLCH. We have demonstrated the considerable difference in PI*Z and PI*S frequencies in the PLCH group in comparison to the general Polish population. Still neither reached statistical significance.

The study is ongoing. The PLCH group will be extended.

Conflict of interest

The authors declare no conflict of interest.

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