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Identification of a new defective *SERPINA1* allele (*PI*Z*$_{la\ palma}$) encoding an alpha-1-antitrypsin with altered glycosylation pattern

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ABSTRACT

Background: Alpha-1-antitrypsin (AAT) deficiency is a genetic condition that arises from mutations in the *SERPINA1* gene and predisposes to develop pulmonary emphysema and, less frequently, liver disease. Occasionally, new defective *SERPINA1* alleles are detected as an outcome of targeted-screening programs or case-findings.
Methods: This study began with a female patient showing bronchial hyperreactivity. Serum level and phenotype for AAT was analysed by immunonephelometry and isoelectric focusing electrophoresis. The *SERPINA1* gene of the proband was genotyped by PCR amplification and DNA sequencing. Analysis of AAT deficiency was extended to the proband's family.
Results: An abnormal AAT variant that migrated to a more cathodal position than PiZ AAT was detected in the proband's serum. Genetic analysis demonstrated that proband is heterozygous for a new defective *SERPINA1* allele (*PI*Z*$_{la\ palma}$) characterized by the c.321C > A (p.Asn83Lys) mutation in the M1Val213 background. This mutation abolishes the N-glycosylation site in position 83 of the mature AAT. Eight relatives of the proband are carriers of the *PI*Z*$_{la\ palma}$ allele and four of them have shown symptoms of bronchial asthma or bronchial hyperreactivity. The mean α1AT level in the serum of *PI*MZ*$_{la\ palma}$ individuals was 87.1 mg/dl.
Conclusion: The reduction in circulating AAT levels associated to the *PI*Z*$_{la\ palma}$ allele was similar to that of *PI*Z* allele, representing a risk of impairment in lung function.

1. Introduction

Alpha-1-antitrypsin (AAT) is a 52 kDa serine protease inhibitor (Pi) encoded by the *SERPINA1* gene in humans, which is primarily expressed in hepatocytes [1]. After being released to the bloodstream by the liver, AAT reaches concentrations that normally exceed 1 g/l, representing the most abundant Pi in human serum. The main function of AAT is to inactivate the elastase secreted by neutrophils, protecting lower respiratory tract of lungs against the attack of this protease. A variety of additional functions have been described for AAT, including immunomodulatory and anti-inflammatory properties independent of its antiprotease activity [1].

The AAT deficiency (AATD) is a genetic condition characterized by decreased levels and/or function of circulating AAT. This autosomal hereditary trait (OMIM #613490) is associated with a higher risk of suffering pulmonary emphysema and, depending on the involved defective *SERPINA1* allele, liver disease [1]. Although the AATD is one of the most common hereditary disorders among Caucasians, with a prevalence similar to that of the cystic fibrosis, it remains an underdiagnosed condition [1–3]. The deficient *SERPINA1* alleles more common in Caucasians populations are *PI*S* and *PI*Z*, which are segregating at frequencies of 5–10% and 1-3%, respectively [1]. In North America and European countries, more than 95% of patients with clinical symptoms related to AATD have a *PI*ZZ* genotype, while the remaining clinical cases are usually *PI*SZ* individuals or other compound heterozygous for *PI*Z* and a rare defective allele. Here in, we described a rare mutation affecting *SERPINA1* gene that reduces the number of glycosyl chains of AAT and decreases the serum level of this antiprotease.

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2. Methods

2.1. Subjects

The index case was a 57-year-old woman from La Palma Island (Canary Islands, Spain) with no smoking history. Unproductive cough, after a respiratory tract infection, was the main symptom referred by this patient. Her IgE serum level was normal. Evaluation of pulmonary function by spirometry with a negative bronchodilator response, together with a normal level of exhaled nitric oxide (5 ppb), indicated that patient had normal lung function and did not suffer from asthmatic disease. Therefore, the diagnosis of nonspecific bronchial hyperreactivity was made. After informed consent, peripheral blood samples were taken from this patient and several members of her family, both in collection tubes without anticoagulant and in Whatman™ 903 paper cards, in order to conduct an analysis in the context of AATD. The study was approved by the Ethical Committee of the hospital.

2.2. Protein studies

Serum samples were processed for AAT phenotyping by isoelectric focusing (IEF) and immunofixation [4]. IEF migration patterns were contrasted with various control samples of known AAT phenotypes. Serum AAT concentrations were quantified by immunonephelometry and compared to the reference ranges (95% confidence intervals) for different AAT phenotypes [5]. In order to discard the influence of inflammatory conditions on AAT concentration, C-Reactive Protein levels were measured in patients. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpetidase (GGT) were measured by automated standard procedures.

2.3. DNA analysis

Genomic DNA (gDNA) was extracted from dried blood samples by an alkaline lysis method [6]. Genotyping and haplotyping assays were performed by amplicon sequencing after conventional PCR or allele specific PCR (AS-PCR), respectively. Oligonucleotides used in this assays are detailed in Table 1. Amplification reactions (20 μl) contained 2 μl of gDNA sample, 0.2 μM of each amplification primer, 200 μM of each dNTP, 1× reaction buffer (1.5 mM Cl_2Mg) and 0.2 μl of Phire® II DNA polymerase (Finnzymes). Following an initial denaturation at 98 °C for 30 s, 35 amplification cycles were applied with the thermal profile indicated in Table 1. Selectivity of the AS-PCR was controlled with a control sample from an individual homozygous for the wild-type variant. Amplicons were checked by electrophoresis in 1.5% agarose gels, purified with a commercial kit (Omega Bio-tek) and finally sequenced with the Big Dye Dydeoxy Terminator Cycle Sequencing Kit and an ABI 3730 Genetic Analyzer (Applied Biosystems).

3. Results

3.1. Characterization of the $PI^*Z_{la\ palma}$ allele

Respiratory symptoms observed in the index case made us to consider the inclusion of this patient in a detection program of AATD developed in our hospital. In this sense, the mean AAT concentration in the patient's serum was 75.6 mg/dl, a low value compared with the reference range for the normal *PI*MM* genotype (100-273 mg/dl) [5]. A high-resolution computed tomography (HRCT) was performed and the result was normal. In addition, the 6-min walking test (6MWT) was made and oximetry desaturation was not observed.

Interestingly, AAT phenotyping of proband by IEF showed the five normal PiM bands [4] and three additional bands that focused more proximal to the cathode, two of them even more than PiZ isoforms (Fig. 1A). In order to identify the genetic mutation responsible for this abnormal IEF pattern, exons 2-5 and corresponding introns of *SERPINA1* gene were amplified from the proband's gDNA as two overlapping PCR fragments. After nucleotide sequencing of coding exons and exon-intron boundaries, a new genetic variant (c.321C > A) was detected in heterozygous state (Fig. 1B) with two potential allelic backgrounds, M1Val213 or M1Ala213. To resolve this ambiguity, the *SERPINA1* allele containing the mutation in c.321 position was selectively amplified by AS-PCR and sequenced. This analysis revealed that the c.321C > A mutation is placed in the M1Val213 background (Fig. 1C). Attending to the position of altered AAT in the IEF gel, and according to the birthplace of the proband, the new deficiency *SERPINA1* allele was named $PI^*Z_{la\ palma}$.

3.2. Family study

Analysis of AATD was extended to 12 relatives of the proband, spanning two generations of the pedigree (Fig. 2). In addition to the index case, 8 family members were found to have a $PI^*MZ_{la\ palma}$ genotype, and 4 of them have manifested lung symptoms like bronchial asthma or bronchial hyperreactivity. The HRCT analysis and 6MWT were normal in this last subgroup of subjects. Overall, carriers of the mutant allele showed an important reduction of circulating AAT (mean = 87.1 mg/dl; SD = 16.8), comparable to that of *PI*MZ* individuals (95% range = 61-156 mg/dl) [5]. All PI^*MZ_{la}

Table 1
Oligonucleotides used for genotype and haplotype determinations.

Purpose	Amplification primers[b]	Amplicon size	Sequencing primers[b]	PCR thermal profile	
				Amplification	Final extension
SERPINA1 gene sequencing in proband	F: CTCTGGCTTTGGTTTCTTCATC R: TGTGTGGCTCATGTTTAAG	3232 bp	F: CTCTGTCTTGCAGGACAATG F: CAGGGAGCCTTAGACAGA R: AAGCTCCTTGACCAAATC	98 °C, 10 s 55 °C, 5 s 72 °C, 2 min	72 °C, 5 min
	F: GGGTGCTGCTGATGAAATAC R: TGGGAGGGATTTACAGTCACA	2664 bp	F: ATTTCAGCTAAAGATGACACT R: CCTGGCAGTGACCTTCACA		
c.321C > A genotyping in other subjects	F: TCATCATGTGCCTTGACTC R: AAGCTCCTTGACCAAATC	654 bp	F: CTCTGTCTTGCAGGACAATG	98 °C, 5 s 55 °C, 5 s 72 °C, 15 s	72 °C, 1 min
Molecular Haplotyping in proband[a]	F: GGAGGGCCTGAATTTCAA**A** R: TTGGGGAATCACCTTCTGTC	2077 bp	R: TTGGGGAATCACCTTCTGTC	98 °C, 10 s 66 °C, 20 s 72 °C, 1.5 min	72 °C, 3 min

[a] The nucleotide that allows specific amplification of the allele containing the c.321C > A mutation is highlighted in bold.
[b] F: Forward; R: Reverse.