Prevalence of alpha-1 antitrypsin deficiency and hereditary hemochromatosis gene mutations in Algarve, Portugal

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Introduction

Alpha-1 antitrypsin (AAT) deficiency and hereditary hemochromatosis (HH) are two of the most fatal genetic disorders in adult life, affecting million individuals worldwide. They are often under-diagnosed conditions and diagnosis is only made when the patient is already in the advanced stages of disease. AAT deficiency results from mutations in one highly pleiomorphic gene located on the long arm of chromosome 14, *SERPINA 1*, being *Z* and *S* mutations the most clinically relevant . These mutations, resulting from single nucleotide exchanges, create an abnormal folding of the AAT protein, that will not be secreted, compromising the lungs protection from proteolytic attack by neutrophil elastase . Consequently, AAT deficit originates emphysema, chronic bronchitis, asthma or even chronic obstructive pulmonary disease (COPD) and it is also strongly associated with various liver diseases [1]. On the other hand, HH disorder results from mutations in *HFE* gene, located in the short arm of chromosome 6, that encodes a transmembrane protein involved in intestinal absorption of dietary iron. Particularly, *C282Y* and *H63D* mutations in *HFE* gene are reported to be the most significantly associated with HH disorder resulting in increased iron absorption. This excessive iron progressively deposites in various tissues triggering cirrhosis, hepatic fibrosis, diabetes mellitus, arthropathy or even hepatocarcinoma [2].

Objectives

Given the insufficient population-based information about the prevalence of these gene variants in the Portuguese population, the aims of this study were to:

• Determine the prevalence of *Z, S, C282Y* and *H63D* mutations and their genotype combinations in a population sample from São Brás de Alportel, in the South of Portugal;

• Determine the association of these mutations with related chronic diseases.

Materials and Methods

• Sampling

Random sampling of participants was performed based on the National Health System card number of the S. Brás de Alportel Health Centre users, that covers over 99% of the total population. All participants were given a brief description of the objectives of the study, after which they signed a consent form. Each participant has answered an exhaustive questionnaire including health related questions and a sample of blood was collected.

• DNA extraction and genotyping

Genomic DNA was isolated from whole blood containing EDTA using standard methods. The selected SNPs (rs28929474, rs17580, rs1800562, rs1799945) were typed by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. All the information about PCR conditions, primers sequences and restriction enzymes is presented in Table 1.

Table1. Primer sequences and PCR reaction conditions for the used PCR-RFLP method.

Gene	SNP ID	Allele	Primers sequence (5´→3´)	Annealling temp. (ºC)	Product size (bp)	Restriction enzyme
SERPINA1	rs 28929474	Z	F: ATAAGGCTGTGCTGACCATCGTC R: TTGGGTGGGATTCACCACTTTTC	58	179	TaqI
	rs 17580	S	F: TGAGGGGAAACTACAGCACCTCG R: AGGTGTGGCAGCTTCTTGGTCA	59	121	TaqI
HFE	rs1800562	C282Y	F: ACGTTGGATGTACCCCAGATCACAATGAGG R: ACGTTGGATGTGGATAACCTTGGCTGTACC	65	117	RsaI
	rs1799945	H63D	F: ACGTTGGATGTCTACTGGAAACCCATGGAG R: ACGTTGGATGTTGAAGCTTTGGGCTACGTG	65	114	BclI

Analysis

Allele and genotype frequencies were determined by gene counting and the respective confidence intervals were calculated. Statistical analysis was performed using SPSS Software. Hardy-Weinberg equilibrium was tested for the genotype distribution of all investigated SNPs.

Results

In this study, participated 208 adult subjects, including 118 females and 90 males, with ages ranging from 26 to 91 years old (mean age: 58 years). Figure 1 shows genotype frequencies found in the studied population (n=208). Regarding AAT deficiency, we found a relative frequency of 4,3% MZ (95% CI: 0-17,6), 0,5% SS (95% CI: 0-14,0) and 15,4% MS (95% CI: 2,9-27,9) genotypes (Figure 1A). About HH, we found a relative frequency of 1,4% C282Y/H63D (95% CI: 0-14,9), 2,4% H63D/H63D (95% CI: 0-15,8), 5,8% C282Y/wt (95% CI: 0-19,0) and 23,6% H63D/wt (11,7-35,4) genotypes (Figure 1B).

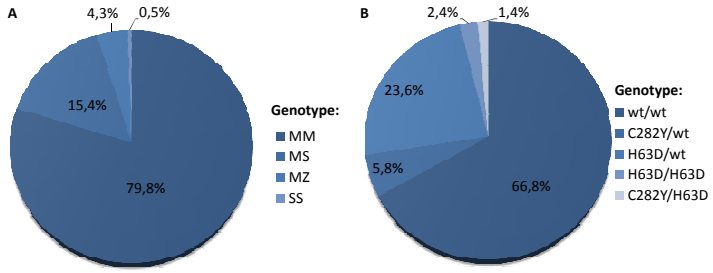

Figure1. A- Frequencies of the AAT associated genotypes; **B-** Frequencies of the HH associated genotypes (n=208; M represents non-S and non-Z alleles and wt represents Non-C282Y and non-H63D alleles).

Table 2 shows the calculated allelic frequencies, based on the genotype frequencies, and the respective 95% CI. All the observed allelic frequencies were in Hardy-Weinberg Equilibrium.

Table2. Observed allele frequencies and confidence interval (CI) on São Brás de Alportel population (n=208; zero was assumed since lower limit of CI was a negative value).

AAT Deficiency			HH disorder		
Allele	**Frequency (%)**	**CI 95%**	**Allele**	**Frequency (%)**	**CI 95%**
Z	2,2	0-11,7	**C282Y**	3,6	0-13
S	8,2	0-17,4	**H63D**	14,9	6-23,8
M	89,6	86,6-92,8	**wt**	81,5	77,4-85,6

In general, our results corroborate previously published studies confirming a considerable prevalence of the involved allelic mutations in the Portuguese population [3,4]. However we have found a higher prevalence for both *Z* and *C282Y* alleles, strongly associated with AAT and HH disorders. For the Z allele we found a similar value (2,2%) reported recently for Madeira Island (2,5%) that are similar to several Northern European countries [5,6]. In relation to the *C282Y* allele, its higher frequency (3,6%) is not consistent with the north–south decreasing gradient that has been found in mainland Portugal [4]. However, we have to consider the large amplitude of confidence intervals associated. Moreover, we found no association between the analysed gene variants and their related chronic diseases, including chronic bronchitis, asthma, COPD, cirrhosis, diabetes mellitus and arthropathy, as reported by the participants likely due to the small sample available.

Conclusions

Given the variability of the prevalence of these mutations among different populations , the data presented here is a contribution to better understand *SERPINA1* and *HFE* mutations distribution in Portugal.

Moreover, this kind of studies is crucial to estimate the number of individuals at risk of developing the associated diseases allowing to early diagnosis mechanisms to reach asymptomatic patients. Future studies involving a larger sample size will be necessary to evaluate the penetrance of the studied gene mutations and to assess gene-environment interactions that influence disease risk, contributing to reduce the burden of these diseases which can have a great public health impact.

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