Alpha-1-antitrypsin deficiency in Madeira (Portugal): The highest prevalence in the world

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Summary
Alpha-1-antitrypsin (AAT) deficiency is a common genetic disease which affects both lung and liver. Early diagnosis can help asymptomatic patients to adjust their lifestyle choices in order to reduce the risk of Chronic Obstructive Pulmonary Disease (COPD). The determination of this genetic deficiency prevalence in Madeira Island (Portugal) population is important to clarify susceptibility and define the relevance of performing genetic tests for AAT on individuals at risk for COPD.

Two hundred samples of unrelated individuals from Madeira Island were genotyped for the two most common AAT deficiency alleles, PI*S and PI*Z, using Polymerase Chain Reaction-Mediated Site-Directed Mutagenesis.

Our results show one of the highest frequencies for both mutations when compared to any already studied population in the world. In fact, PI*S mutation has the highest prevalence (18%), and PI*Z mutation (2.5%) was the third highest worldwide. The frequency of AAT deficiency genotypes in Madeira (PI*ZZ, PI*SS, and PI*SZ) is estimated to be the highest in the world: 41 per 1000.

This high prevalence of AAT deficiency on Madeira Island reveals an increased genetic susceptibility to COPD and suggests a routine genetic testing for individuals at risk. © 2009 Elsevier Ltd. All rights reserved.

Introduction

α1-Antitrypsin (AAT) deficiency, first described in 1963, is a common genetic disease that affects lung and liver and results from mutations on Protease Inhibitor (PI)
locus located in the long arm of chromosome 14. AAT, a glycoprotein, is a protease inhibitor that belongs to the family of serpins and its prime function is the inhibition of neutrophil elastase enzyme. Neutrophil elastase is an important protease that has as substrate some components of extra-cellular matrix and is associated to the action of neutrophil. Because excessive presence of neutrophil elastase results in the destruction of pulmonary parenchyma the correct expression of PI locus is essential in the protection of lungs. AAT is expressed mainly in liver cells (hepatocytes) and discharged to plasma where this glycoprotein should be kept at sufficient high levels to protect lung elastin from degradation by neutrophil elastase. AAT deficiency leads to low plasma and alveolar concentration of this protective glycoprotein, situation associated to a progressive, severe, and life-threatening pulmonary early-onset emphysema and chronic obstructive pulmonary disease (COPD). Accumulation of abnormal alpha-1-antitrypsin in hepatocytes is also responsible for liver disease. AAT deficiency is most prevalent in European populations but it has been also reported in almost all regions of the world. AAT deficiency is under recognized and under diagnosed but early diagnosis in the asymptomatic patient helps modify lifestyle choices to reduce the risk of COPD, namely eliminating smoking habits.

PI*M is the normal and most common allele but more than 100 different genetic variants of AAT locus were described worldwide. The two most common mutations, causing clinically relevant deficiency of the protein alpha-1-antitrypsin, are PI*S and PI*Z. PI*S allele result from a single base-pair substitution in exon III from Thymine to Adenine (Blu264GAA → Val GTA) that occur at 279–470 generations ago, probably in Iberian peninsula where it is more prevalent. PI*Z allele result also from a single base-pair substitution in exon V from Adenine to Guanine (Glu342 GAG → Lys AAG) that probably arose in Northern Europe at 107–135 generations ago, where presently it reaches higher frequencies.

PI*ZZ, PI*SZ, and PI*ZZ are considered deficiency genotypes and PI*MZ and PI*MZ' carriers of the anomaly. Individuals with deficiency allele combinations have been estimated to be 3.4 millions worldwide and carriers of the anomaly about 116 millions, despite there are clear ethnic differences on the prevalence of these alleles. PI*ZZ are at higher risk for COPD than PI*SZ subjects, despite, in smokers, both genotypes represents high risk. Carriers of the defective allele PI*Z (PI*MZ) have also an increased risk of COPD that manifest later in life and is dependent on the multifactorial interaction of genetic, smoking and environmental factors.

Madeira Island (Portugal), located in the North Atlantic Ocean, was uninhabited when the settlement process began by the Portuguese in the 15th century. Settlers were mainly Portuguese but included also people from Africa, brought as slaves, and from different European proveniences (Spain, Italy, France, and England) involved in North Atlantic trade. Since the 18th century, Madeira Island has been known all over the Europe as a place with good climatic conditions to treat pulmonary disease and for a long time many people moved to this island searching a cure for their pulmonary injuries, same time as a prescription from their physicians. Some studies have been done in order to characterize the Madeirean population genetic profile and they showed that, besides the greater influence of Caucasians, sub-Saharans also left an important genetic imprint on the population.

Despite several population studies have been done in order to characterize S and Z mutations prevalence on Protease Inhibitor locus, Madeira Island has never been included. The aim of the present study on Madeira Island population was to assess for the first time the unbiased frequencies of PI*S and PI*Z alleles and calculate the frequencies of individuals with deficiency allele combinations and the number of carriers of both defective alleles in order to clarify genetic susceptibility to COPD and defines the importance on a general application of genetic tests on citizens at risk.

Materials and methods

The samples, collected after informed consent, consisted of 200 unrelated males with ages ranging from 18 to 23 years old whose parents and grandfathers were born and living in Madeira Island. The main goal of this study consisted in the characterization of PI*S and PI*Z allele mutations prevalence in Madeira Island population and predominance of AAT deficiency. The selection of 200 individuals allowed us to detect rare mutations (frequencies lower than 0.005) with a probability of 99%.

Genomic DNA was isolated from whole blood containing EDTA using a phenol-chloroform procedure and frozen at −20 °C until use. The two most common AAT deficiency alleles, PI*S and PI*Z, were genotyped using PCR-MSDM (Polymerase Chain Reaction-Mediated Site-Directed Mutagenesis) as previously described with some modifications. PCR mixtures had a total volume of 50 μl, composed by 100 ng of genomic DNA, 0.2 U of Taq polymerase (FirePol™), buffer 1X (Solis BioDyne), 3.5 mM of MgCl₂ (Solis BioDyne), 0.16 mM of each dNTP (Promega) and 20 pmol of each primer described by Tazelaar and colleagues. To genotype each sample for S and Z mutation, two different reactions with the specific primers were carried out in a Biometra thermal cycler with the following temperature cycling conditions: initial 2 min denaturation at 94 °C, 40 cycles of 20 sec at 95 °C, 20 sec at 56 °C, 30 sec at 72 °C, and 7 min of final extension at 72 °C. We digested 5 μl of each PCR product with 20 U of Taq‘I restriction enzyme (New England Biolabs, Beverly, MA) for 1 h and 30 min at 65 °C, and 20 min at 80 °C. Digested DNA was subjected to electrophoresis in a 4% agarose gel for 1 h and 45 min at 40 Volts.

Analysis

Allele and genotype frequencies were determined by gene counting and respective confidence intervals through Exploratory Software for Confidence Intervals (ESCI JSMS, La Probe University, Melbourne, Australia) available online.

The expected numbers of AAT genotypes in Madeira Island population were extrapolated through observed genotype frequencies on the studied samples, except for PI*ZZ that was calculated from the Hardy–Weinberg
equilibrium formula. We also assessed the prevalence of each genotype in the population dividing the number of estimated genotypes by the total population number.

Gene diversity, Hardy–Weinberg equilibrium and Ewens–Watterson tests of selective neutrality were estimated with Arlequin version 3.1.24 The same software was used to perform an analysis of molecular variance (AMOVA), based on Euclidean distances, with Madeira and other 36 worldwide populations from the literature: Portugal, Spain, France, Italy, Belgium, Finland, Germany, Netherlands, Norway, Latvia, Denmark, Estonia, Poland, Russia, Sweden, Switzerland, United Kingdom, New Zealand, Japan, China, India, Iran, Pakistan, Jordan, Saudi Arabia, Tunisia, Zaire, Botswana, Congo, Gambia, Mali, Mozambique, Namibia, Nigeria, South Africa, and Somalia.8,25,26 The total genetic variation between Madeira Island and other populations, namely mainland Portugal, was estimated and the correspondent Fst values used to evaluate if there were significant differences between them. Variance components were tested for significance by nonparametric randomisation tests using 10,000 permutations under the null hypothesis of no population structure. The population genetic software Arlequin v3.11 was employed in all the above analyses.

**Results**

In the studied samples (n = 200), 57 heterozygote MS, 8 heterozygote MZ, 6 homozygote SS, and 2 heterozygote SZ were found. Therefore, the frequency of PI*M (non-S, non-Z) allele was 635 (95% CI: 568–702) per 1000, PI*S allele was 180 (95% CI: 127–233) per 1000; and PI*Z was 25 (95% CI: 127–233) per 1000 (Table 1).

Based on allele frequencies, the AAT deficiency prevalence in Madeira Island population was 1/1600 for ZZ, 1/33 for SS and 1/100 for SZ, and the prevalence of carriers was 1/25 for MZ and 1/4 for MS. In Madeira population we may expect around 158 (95% CI: 0–1.039) subjects with severe AAT deficiency (ZZ). Details on the expected numbers of AAT genotypes are shown in Table 2.

Madeira Island population was in Hardy–Weinberg equilibrium for AAT locus and its gene diversity was the highest in the world (0.34), followed by Portugal and Spain (0.25 and 0.22 respectively). Ewens–Watterson neutrality test revealed that AAT locus in Madeira population was not submitted to selective pressures, similarly to the other 36 studied populations.

Analysis of molecular variance (AMOVA), plotting Madeira with the 36 other worldwide populations from literature, revealed that most genetic variance at AAT locus is due to differences within each population (93%) and only 7% of total genetic variance comes from differences among them.

AAT allele frequencies in Madeira population had statistically significant differences (P < 0.005) when compared to any of the 36 world populations previously stated, including Portugal, which did not reveal statistically significant differences from Spain (P = 0.23).

**Discussion**

The determination of AAT allelic frequencies in different populations is crucial to estimate the number of subjects at risk of developing the associated diseases. This information is of much importance since AAT deficiency is under diagnosed all over the world. If healthcare systems knew the real extent of deficiency alleles in the population they will take action implementing early diagnosis mechanisms to reach asymptomatic patients.

The present study describes for the first time the prevalence of the two most common AAT deficiency alleles, PI*S and PI*Z, in Madeira Island population showing one of the highest frequencies found worldwide for both mutations. In fact, PI*S mutation in Madeira Island population (18%) has the highest prevalence comparing to any world population already studied, including Portugal (12.9%) and Spain (10.4%) in the Iberian peninsula, the place of origin of this mutation. Even PI*Z mutation reveals high frequencies in Madeira Island (2.5%), similar to several Northern European countries such as Denmark (2.26%), Estonia (2.45%), Poland (2.45%) and Sweden (2.3%) and only lower than Latvia (4.09%) and New Zealand (2.57%).8,25 The frequency of all the AAT deficiency genotypes in Madeira Island population (ZZ, SS, and SZ) was estimated as the highest in the world: 40.9 (95% CI: 14–68) per 1000.

The results obtained from this study were not expected since they combine high prevalence for both mutations (S and Z) that have distinct geographic distributions. Despite the small contribution of some European countries, other than Portugal, on the peopling of Madeira, there is none that justifies from itself the observed prevalence of Z mutation. The most likely explanation to this result is the influence of the many people that arrived to the Island since the 18th century from many places in Europe searching for good climatic conditions to treat pulmonary diseases. In fact, in the past two centuries many couples and families arrived in Madeira Island searching for a warmer climate where they could found better conditions to treat their pulmonary injuries. Many of them, experiencing improvements in their health, decided to stay and their descendants are now integrated and mixed in the population.15,16 Based on their health condition, we hypothesise that many of them, individuals and families, could be AAT deficient or carriers of the mutations. Once integrated with the Island population they may have contributed to a higher prevalence of S and Z mutations. The prevalence of S mutation in Madeira could also be explained as a consequence of the known Portuguese main contribution in the settlement, together with founder effect and genetic drift mechanisms.

Despite the well-known sub-Saharan genetic influence due to the contribution of slaves in the settlement of Madeira Island, revealed through mtDNA, Y-chromosome
and HLA studies, our results didn’t reveal any evidence of this contribution.15–20

The results obtained on this study recommend that the Madeira Island healthcare system adopt mechanisms of genetic testing to achieve early diagnosis on AAT deficiency, especially in people with higher risks, namely smokers and COPD patient relatives. This approach enables the implementation of prevention measures on genetic susceptible groups, reducing tobacco smoke, dust and pollution from occupational or environmental exposition.

The unexpected prevalence of AAT deficiency in Madeira Island highlights this population as an important case study to better understand the relationship between this genetic deficiency and the susceptibility to several diseases such as COPD, Asthma, lung cancer, liver disease and colorectal cancer. This high AAT deficiency prevalence can also allow us to study and clarify the influence of genetic and environmental factors in the development of pulmonary diseases. Future studies on Madeira Island population should address the association between AAT deficiency and pulmonary diseases development or even other pathologies.

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

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