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

Vânia Gaio1, Aida Fernandes2, Francisco Mendonça3, Filomena Orta Correia1, Alfredo Beleza4, Ana Paula Gil1, Mafalda Bourbon1, Astrid Vicente5, Carlos Dias3, Marta Barreto da Silva1
1.Instituto Nacional de Saúde Doutor Ricardo Jorge, Lisboa, Portugal; 2. Laboratório de Saúde Pública Dra. Laura Ayres, Faro, Portugal; 3. Administração Regional de Saúde do Algarve, Faro, Portugal

Introduction

Alpha-1 antitrypsin deficiency (AATD) and hereditary hemochromatosis (HH) are two of the most fatal genetic disorders in adults, affecting millions of individuals worldwide. They are often under-diagnosed conditions and only made when the patient is already in the advanced stages of disease. AATD deficiency results from mutations in one highly pleiomorphic gene located on the long arm of chromosome 14, SERPINA1, being Z and M the most clinically relevant. These mutations, resulting from single nucleotide exchanges, create an abnormal folding of the AAT protein, which will not be secreted, compromising the lungs protection from proteolytic attack by neutrophil elastase. Consequently, AATD 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 deposits 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 5, 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.

Table 1. Primer sequences and PCR reaction conditions for the used PCR-RFLP method.

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP ID</th>
<th>Allele</th>
<th>Primers sequence (5’→3’)</th>
<th>Annealing temp. (ºC)</th>
<th>Product size (bp)</th>
<th>Restriction enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERPINA1</td>
<td>rs 28929474</td>
<td>Z</td>
<td>F: TAAAGGCTGTGGACACACGCTC R: TGGTGCTGGATGATCAGCTTTT</td>
<td>58</td>
<td>179</td>
<td>TaqI</td>
</tr>
<tr>
<td></td>
<td>rs 17580</td>
<td>S</td>
<td>F: TGGGGGAGCAACTGAGGCTG CG AG</td>
<td>59</td>
<td>121</td>
<td>TaqI</td>
</tr>
<tr>
<td>HFE</td>
<td>rs1800562</td>
<td>C282Y</td>
<td>F: AGTTGAGATGCGACGATGACG R: AGTGGGAGCCAAGCAGGGG</td>
<td>65</td>
<td>117</td>
<td>BglII</td>
</tr>
<tr>
<td></td>
<td>rs1799945</td>
<td>H63D</td>
<td>F: AGTTGAGATGCGACGATGACG R: AGTGGGAGCCAAGCAGGGG</td>
<td>65</td>
<td>114</td>
<td>BglII</td>
</tr>
</tbody>
</table>

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% M2 (95% CI: 0-17.6), 0.5% S5 (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 (11.7-37.4) genotypes (Figure 1B).

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.

Table 2. 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).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Frequency (%)</th>
<th>CI 95%</th>
<th>Allele</th>
<th>Frequency (%)</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>2.2</td>
<td>0-13.57</td>
<td>S</td>
<td>8.2</td>
<td>0-17.42</td>
</tr>
<tr>
<td>S</td>
<td>8.2</td>
<td>0-17.42</td>
<td>C282Y</td>
<td>3.6</td>
<td>0-13</td>
</tr>
<tr>
<td>M</td>
<td>89.6</td>
<td>86.6-92.8</td>
<td>H63D</td>
<td>14.9</td>
<td>6-23.8</td>
</tr>
</tbody>
</table>

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.

Bibliography