GENETIC POLYMORPHISMS ASSOCIATED WITH THE ONSET OF ARTERIAL HYPERTENSION IN A PORTUGUESE POPULATION

Ana Célia SOUSA,1,2 Roberto Palma dos REIS3, Andreia PEREIRA,1 Sofia BORGES,1 Ana Isabel FREITAS,1 Graça GUERRA,1 Sara GOUVEIA,1 Teresa GOIŚ,1 Lino NÓBREGA,1 Mariana RODRIGUES,1 Eva HENRIQUES,1 Sônia FREITAS,1 Ilídio ORNELAS,1 Décio PEREIRA,1 António BREHM1, Maria Isabel MENDONÇA1


ABSTRACT

Introduction: Arterial hypertension is a complex, multifactorial disease, controlled by genetic and environmental factors.

Objective: Evaluate the genetic susceptibility for developing arterial hypertension and its association with the traditional risk factors in the outbreak of this pathology.

Material and Methods: Case-control study with 1712 individuals, mean age of 51.0 ± 7.9 years (860 hypertensive patients and 852 controls). Biochemical and traditional risk factors, and genetic variants were evaluated: ACE I/D rs4340, ACE A2350G rs4343, AGT T174M rs4762, AGT M235T rs699 AGTR1 A1166C rs5186, CYP11B2 -344 C/T rs1799998, ADRB1 R389G rs1801253, ADRB2 R166 rs1042713, ADD1 G460W rs4961, SCNN1G G173A rs5718, GNB3 C825T rs5443, ATP2B1 A/G rs2681472, CYP17A1 T/C rs11191548, SLC4A2 C/T rs2303934. The risk of each gene for hypertension was estimated by the dominant, recessive, co-dominant and multiplicative models. By logistic regression, variables associated with hypertension were evaluated. ROC curves were first performed with traditional risk factors and then adding the genetic variants associated with hypertension. Data were analyzed by SPSS for Windows 19.0 and MedCalc v. 13.3.3.0.

Results: The genetic variants ADD1 G460W, GNB3 C825T, ACE I/D, ACE A2350G were associated with hypertension. ROC curve with traditional risk factors and these variants showed an increase in the predictive capacity of hypertension ($p = 0.018$).

Discussion: According to the results of our study, the genetic variants found to be associated with hypertension were: ACE I/D rs4340, ACE A2350G rs4343, ADD1 G460W rs4961 and GNB3 C825T rs5443. The first two variants are associated with hypertension by interfering with the renin-angiotensin-aldosterone system, which plays an important role in regulating blood pressure. It should be noted that genes encoding the components of renin-angiotensin-aldosterone system are natural candidates for the development and progression of hypertension. In our population alpha-aducin polymorphism (ADD1 G460W rs4961) was also associated with hypertension. In a Portuguese population, known to have high salt intake, it makes sense that this polymorphism which is relevant in salt and water management may consequently be relevant in the onset of hypertension. The genetic variant GNB3 C825T rs5443 that affects intracellular signalling was also found to be a strong risk candidate for hypertension. Initially, with the elaboration of the ROC curve and calculation of the AUC using only with traditional risk factors and later by adding the variants ADD1 G460W, GNB3 C825T, ACE I/D and ACE A2350G to the traditional risk factors, we verified that genetic polymorphisms increased the predictive risk of hypertension, when compared to the risk given only by traditional risk factors, with statistical significance ($p = 0.018$). This suggests that hypertension is a multifactorial disease that results from the interaction of environmental, genetic and lifestyle factors that interact with each other and lead to the advent of this important pathology.

Conclusion: In our study, the hypertension-associated polymorphisms are linked to the renin-angiotensin-aldosterone axis (ACE I/D, ACE A2350G), as well as to salt and water management (ADD1 G460W, GNB3 C825T). Through a multivariate analysis, it was concluded that these two last genetic variants together with four of the traditional risk factors (smoking, alcohol consumption, obesity and diabetes) are associated in a significant and independent way with essential hypertension. In a predictive model of hypertension, the introduction of genetic variants slightly increases the predictive value of the model.

Keywords: Hypertension; Polymorphism, Genetic; Portugal; Risk Factors

RESUMO

Introdução: A hipertensão arterial é uma doença complexa, multifatorial, controlada por fatores genéticos e ambientais.

Objetivo: Avaliar a susceptibilidade genética no aparecimento de hipertensão arterial e sua associação com os fatores de risco tradicionais na eclosão desta patologia.

Material e Métodos: Estudo caso-controle com 1712 indivíduos, idade média de 51.0 ± 7.9 anos (860 hipertensos e 852 controles). Avaliaram-se os fatores tradicionais, bioquímicos e as variantes genéticas: ACE I/D rs4340, ACE A2350G rs4343, AGT T174M rs4762, AGT M235T rs699 AGTR1 A1166C rs5186, CYP11B2 -344 C/T rs1799998, ADRB1 R389G rs1801253, ADRB2 R166 rs1042713, ADD1 G460W rs4961, SCNN1G G173A rs5718, GNB3 C825T rs5443, ATP2B1 A/G rs2681472, CYP17A1 T/C rs11191548, SLC4A2 C/T rs2303934. Calculámos o risco de cada gene para a hipertensão, pelos modelos dominante, recessivo, co-dominante e multiplicativo. Através da regressão logística, avaliamos as variáveis associadas à hipertensão. Elaborámos-se curvas ROC com os fatores tradicionais e posteriormente adicionando as variantes genéticas associadas com hipertensão. Analisámos os dados através do SPSS for Windows 19.0 e MedCalc v. 13.3.3.0.

Resultados: As variantes genéticas ADD1 G460W, GNB3 C825T, ACE I/D e ACE A2350G associaram-se à hipertensão. A curva ROC


* Autor correspondente: Ana Célia Sousa. anacleria.bett@gmail.com

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INTRODUÇÃO
High blood pressure (also referred to as HBP or hypertension) is a relevant public health concern, affecting more than one thousand million people worldwide and a risk factor for myocardial infarction, stroke and chronic kidney disease. A 42% prevalence has been found in Portuguese adult population (44.4% in male and 40.2% in female). The complexity and heterogeneity of the disease are the main constraints to understanding human HBP pathogenic mechanisms. In fact, even though its clinical presentation seems quite uniform, different polygenic and environmental factors may become involved with its onset. Most environmental factors with an influence in blood pressure have already been identified, while genetic factors are still not clearly understood. Different studies have confirmed the multigenic nature of blood pressure in general and particularly of essential hypertension. An estimated 30% of blood pressure variation have been attributed to genetic factors in different populations.

Essential hypertension has been comprehensively studied with the emergence of genome-wide association studies (GWAS). A total of 13 loci associated with systolic blood pressure (SBP), diastolic blood pressure (DBP) and HBP have been found in 2009 in two GWAS involving more than 25,000 patients of European ancestry. Associations between different genes and systolic blood pressure have been shown in another study involving a group of Afro-American patients. A total of 29 independent genetic variants with an influence in blood pressure were recently described in a GWAS involving patients of European ancestry carried out by the International Consortium for Blood Pressure (ICBP).

The identification of underlying candidate hypertension susceptibility genes is still ongoing, particularly using association studies in humans and physiological and pharmacological intervention studies in animal models and in genetically-modified strains. Advances in the understanding and study of genes encoding the components of biological, physiological and cell-function pathways in blood pressure regulation have therefore been produced by these new approaches.

Biologically plausible polymorphisms at candidate genes for HBP were included in the present study, namely: ACE I/D rs4340, ACE A2350G rs4343, ADD1 G460W rs4961, GNB3 C825T. Através de uma análise multivariada, concluiu-se que estas duas últimas variantes genéticas conjuntamente com quatro dos fatores tradicionais (tabagismo, hábitos alcoolicos, obesidade e diabetes) se associam de forma significativa e independente à hipertensão arterial essencial. Num modelo preditivo de hipertensão arterial, a introdução das variantes genéticas aumenta ligeiramente o valor preditivo do modelo.

Palavras-chave: Factores de Risco; Hipertensão; Polimorfismo Genético; Portugal

MATERIAL AND METHODS
Study population
This study was carried out in Portuguese Archipelago of Madeira with a population of around 300,000 people, involving a group of white patients born in Madeira from white parents and grandparents who were also born in the island.

The study was approved by the Ethics Committee of the Hospital Central do Funchal and was carried out according to the Helsinki Declaration. A written informed consent has been obtained from each participant, with a specific consent for biochemical and DNA analyses as well as for relevant data collection.

This was a case-control study involving 1,712 patients with an average age of 51.0 ± 7.9 years (51% male), selected from the Family Medicine and Internal Medicine outpatient clinics at the Hospital Central do Funchal. Two groups of patients were established and (i) 860 cases (average age 51.4 ± 8; 53.3% male) and (ii) 852 controls (mean age 50.7 ± 7.7; 48.7% male) were included in the study. Controls
were matched with cases according to gender and age.

**Definition of high blood pressure**

The presence of HBP was considered when patients were already diagnosed with HBP and/or were on anti-hypertensive drugs for at least three months at study inclusion or were diagnosed with SBP and DBP ≥ 140/90 mmHg measured in at least three occasions.14

Normotensive patients were never treated and presented with SBP and SDP < 140/90 mmHg.

Blood pressure measurement was taken in the right arm with the patient seated upon a 10-minute resting time and using a Welch Allyn standard sphygmomanometer (phases I to V). The mean value of three measurements taken two minutes apart was considered.14

Pregnant mothers and patients with secondary HBP, multiple organ failure, mental disorders, chronic inflammatory disease or on medication for other pathologies with drugs that might have affected blood pressure were excluded from the study.

**Data collection**

A standard questionnaire was completed by all the participants, with reference to patient’s age, gender, lifestyle, smoking, alcohol misuse and medication.

Patient’s height was measured in centimetre and body weight in kilogram. Body mass index (BMI) was obtained by using the formula (BMI = weight, kg/height, m²). Obesity was defined as BMI ≥ 30 kg/m².15

The presence of diabetes mellitus was defined with fasting glucose level ≥ 126 mg/dL or glucose ≥ 200 mg/dL and/or with a history of diabetes therapy.16

The presence of physical inactivity was considered when patients did not regularly follow any moderate-intensity physical activity program of at least 150 minutes per week.17

Smoking and alcohol misuse were considered when these were found at study inclusion.

**Biochemical analysis**

Biochemical analyses were carried out at the central laboratory of the Hospital, according to standard techniques.

Blood samples were collected upon a 14-16-hour fasting period and plasma was prepared for the quantification of biological profiles. Glucose, total cholesterol, triglyceride, HDL-C (high-density lipoprotein cholesterol) and LDL-C (low density lipoprotein cholesterol) levels were measured by use of standard methods at the central laboratory of the Hospital.

**Genetic analysis**

Peripheral blood leukocyte genomic DNA was collected by use of the salting-out standard method and genotypic analysis was carried out by use of oligonucleotide probes marked with specific fluorescence for each of the alleles in a trial combining conventional PCR (polymerase chain reaction) technique and the TaqMan (Applied Biosystems) technique. Primers and probes were those pre-established by suppliers (TaqMan SNP Genotyping Assays, Applied Biosystems) for the 14 genetic polymorphisms ACE I/D rs4340, ACE A2350G rs4343, AGT T174M rs4762, AGT M235T rs699, AGTR1 A1166C rs5186, CYP11B2 -344 C/T rs1799998, ADRB1 R389G rs1801253, ADRB2 R16G rs1042713, ADD1 G460W rs4961, SCNN1G G-173A rs5718, GNB3 C825T rs5443, ATP2B1 A/G rs2681472, CYP17A1 T/C rs11191548, SLC4A2 C/T rs2303934. Oligonucleotides were synthesized and FAM and VIC fluorogenic markers were coupled to the extremities 5’ of the probes in order to reach allelic discrimination. The two-step polymerisation process consisted of 40 denaturation cycles of 15 seconds at 92°C and primer annealing and extension of 1 minute at 60°C and was carried out with a 7300 Real-Time PCR System (Applied Biosystems) platform. Genotypes were determined with the 7300 System SDS Software (Applied Biosystems) with no previous knowledge on the corresponding clinical data.

**Statistical analysis**

Continuous variables with a normal distribution were represented by mean ± standard deviation in variables or, whenever this was not the case, by median (range). Student’s t-test or Mann-Whitney’s test were used to compare these variables between cases and controls. Categorical variables were represented by frequency and percentage and chi-square test was used for comparisons.

A logistic regression was carried out in multivariate analysis, in order to look for an independent association between the following variables and HBP: obesity, diabetes mellitus, smoking and alcohol misuse, physical inactivity and the genetic variants that were significantly associated with HBP in univariate analysis: ADD1 G460W rs4961, GNB3 C825T rs5443, ACE I/D rs4340 and ACE A2350G rs4343. The Forward Wald method was used for the selection of the order of entry of variables into the model.

ROC (receiver operating characteristic) curves and AUC (area under the curve) were obtained, in order to assess the predictive risk of HBP, initially involving the traditional risk factors (TRF) and subsequently adding the ADD1 G460W, GNB3 C825T, ACE I/D and ACE A2350G variants to these. The two curves were compared by use of the Delong test.18 Models were calibrated by the Hosmer Lemeshow test.

Statistical data were analysed by use of the SPSS (Statistical Package for the Social Sciences) software, version 19.0) (IBM, Armonk, NY, USA) and MedCalc software (version 13.3.3.0).

A significance threshold of \( p < 0.05 \) has been considered and bilateral analyses were carried out.

**RESULTS**

**General characteristics of our group of patients**

A case group of 860 patients with HBP (average age 51.4 ± 8; 53.3% male) and 852 controls with no HBP (average age 50.7 ± 7.7; 48.7 male) were included in the study.
Baseline characteristics of our group of patients are shown in Table 1. Patient’s gender and age were similar on both groups by adjusting the populations to the study methodology. When both groups were compared (Table 1), significantly higher values were found in cases when compared to controls, regarding the following variables: physical inactivity ($p = 0.018$), alcohol misuse ($p = 0.019$), diabetes mellitus ($p < 0.0001$), obesity ($p < 0.0001$), systolic blood pressure ($p < 0.0001$), diastolic blood pressure ($p < 0.0001$) and heart rate ($p = 0.001$). A higher prevalence of smoking has been found in the group of controls when compared to cases and this was a statistically significant difference ($p = 0.001$).

Significant differences as regards biochemical variables (Table 2) were found between both groups, including haemoglobin level, leukocyte count, fasting glucose and high-sensitivity C-reactive protein (hs-CRP) levels, which were higher in cases vs. controls ($p < 0.0001$). Lower HDL-C levels were found in cases vs. controls ($p < 0.0001$) while no significant differences were found between both groups as regards total cholesterol, LDL-C and platelet count levels.

Genetic variants associated with the development of HBP

An association with the development of HBP was found regarding four from a total of 14 genetic variants that were studied (Table 3), namely polymorphisms of (i) alpha-aden- ducin $ADD1$ G460W rs4961 under recessive ($p = 0.003$) and co-dominant models ($p = 0.004$); (ii) $G$ protein $\beta3$ subunit ($GNB3$ C825T rs5443) under dominant ($p = 0.004$) and multiplicative models ($p = 0.044$); (iii) angiotensin-converting enzyme ($ACE$ I/D rs4340) under recessive ($p = 0.032$) and multiplicative models ($p = 0.025$) and (iv) $ACE$ A2350G rs4343 under recessive ($p = 0.036$), multiplicative ($p = 0.023$) and co-dominant models ($p = 0.028$).

A logistic regression analysis of the four genetic variables associated with HBP and with TRF (including obesity, diabetes mellitus, smoking, alcohol misuse and physical inactivity) was subsequently carried out (Table 4). The variables that remained in the equation and that were significantly and independently associated with the development of HBP included (i) obesity ($p < 0.0001$), (ii) diabetes mellitus ($p < 0.0001$), (iii) alcohol misuse ($p = 0.013$) and (iv) WW genotype of the $ADD1$ gene ($p = 0.013$) and CT genotype of $GNB3$ ($p = 0.010$).

Smoking habit was a protective variable regarding the development of HBP ($p = 0.001$).

A ROC-curve and AUC were initially obtained regarding the TRF and subsequently adding the genetic polymorphisms that were associated with HBP in univariate analysis (Fig. 1). The inclusion of these four genetic polymorphisms to the TRF added a predictive value (AUC) from 0.688 to 0.681, with a statistically significant difference, according to the Delong test ($p = 0.018$).

DISCUSSION

This study has shown that, with the univariate analysis, genetic variants $ADD1$ G460W rs4961, $GNB3$ C825T rs5443, $ACE$ I/D rs4340, $ACE$ A2350G rs4343 related to HBP and variants $ADD1$ G460W rs4961, $GNB3$ C825T rs5443 were significantly and independently associated with this pathology in our group of patients.

Two variants, $ACE$ I/D and $ACE$ A2350G, have had an interference on the renin-angiotensin-aldosterone system (RAAS), which has an important role in the regulation of renal haemodynamic and volemia.19

Therefore, genes encoding the components of the RAAS are natural candidates for the onset and progression of HBP.20

Human $ACE$ I/D gene, located on chromosome 17q23, is an insertion/deletion (I/D) polymorphism of a 287-base pair in intron 16 and has been extensively used as a genetic marker.21 Angiotensin-converting enzyme (ACE) plays and essential role in the generation of angiotensin II and degradation of bradykinin and, therefore, affecting

Table 1 — Demographic and clinical characteristics of our group of patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n = 1,712)</th>
<th>Cases (n = 860)</th>
<th>Controls (n = 852)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 ± 7.9</td>
<td>51.4 ± 8</td>
<td>50.7 ± 7.7</td>
<td>0.069</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>873 (51)</td>
<td>458 (53.3)</td>
<td>415 (48.7)</td>
<td>0.060</td>
</tr>
<tr>
<td>Physical inactivity, n (%)</td>
<td>915 (53.4)</td>
<td>484 (56.3)</td>
<td>431 (50.6)</td>
<td>0.018</td>
</tr>
<tr>
<td>Alcohol misuse, n (%)</td>
<td>634 (37)</td>
<td>342 (39.8)</td>
<td>292 (34.3)</td>
<td>0.019</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>392 (22.9)</td>
<td>169 (19.7)</td>
<td>223 (26.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>203 (11.9)</td>
<td>163 (19)</td>
<td>40 (4.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Obesity, n (%)</td>
<td>457 (26.7)</td>
<td>330 (38.4)</td>
<td>127 (14.9)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7 ± 4.8</td>
<td>29.1 ± 5.2</td>
<td>26.2 ± 4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134.1 ± 20.5</td>
<td>147.5 ± 19</td>
<td>120.7 ± 11</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84.2 ± 12.2</td>
<td>91 ± 11.9</td>
<td>77.2 ± 7.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>72 ± 11.7</td>
<td>72.9 ± 12.2</td>
<td>71 ± 11.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; continuous data represented by mean ± standard deviation; significant values for $p < 0.05$. 


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morbidity and reactivity of the vascular wall, as well as renal function. Individual ACE plasma levels in healthy populations are stable and reproducible, even though with a wide inter-individual variability. The polymorphism of the gene ACE I/D strongly modulates the plasmatic level of ACE and around half of this variability may be explained by this polymorphism. Some studies have described the allele D as a risk factor for HBP in different populations. An association of DD genotype with an increased DBP in men, although not in women, has been found in a group of 3,095 participants within the Framingham Heart Study. The ACE locus was associated with DBP and with mean arterial pressure in another population study involving adolescents with an average age of 15. DD genotype of the ACE I/D polymorphism was associated with HBP with a risk of 1.61 in a meta-analysis by Ji LD, Zhang LN et al. and positive associations were also found under dominant, recessive and multiplicative genetic models. However, this association was not found in other studies.  

Therefore, the concept that DD genotype of the ACE gene is significantly associated with HBP in a Portuguese group of patients was enhanced by this study. A relatively low odds ratio of 1.2 was found, suggesting an isolated and relatively moderate influence, which may explain the fact that this difference had not been significant in other studies, namely involving smaller groups.  

One other ACE polymorphism, a G/A transition at base 2350 in exon 17 (A2350G) was studied by Zhu et al. in a group of 1,343 Nigerian patients and was associated with higher plasma concentration of ACE and with blood pressure. Saeed et al. have also found that GG genotype of the ACE gene was associated with the development of HBP with an average age of 15. The polymorphism was associated with HBP with a risk of 1.61 in a meta-analysis by Ji LD, Zhang LN et al. and positive associations were also found under dominant, recessive and multiplicative genetic models. However, this association was not found in other studies.  

Table 2 — Biochemical characteristics of our group of patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n = 1,712)</th>
<th>Cases (n = 860)</th>
<th>Controls (n = 852)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.2 (9.6 - 18.2)</td>
<td>14.4 (9.6 - 18.2)</td>
<td>14.2 (10.1 - 17.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Platelet (10^3/µL)</td>
<td>229 (23 - 664)</td>
<td>233 (23 - 664)</td>
<td>228 (65 - 544)</td>
<td>0.099</td>
</tr>
<tr>
<td>Leukocyte (10^3/µL)</td>
<td>6.4 (2.1 - 16.6)</td>
<td>6.7 (2.9 - 14.8)</td>
<td>6.3 (2.1 - 16.6)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>206 (100 - 370)</td>
<td>206.5 (100 - 346)</td>
<td>206 (107 - 370)</td>
<td>0.776</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>48 (17.2 - 111.7)</td>
<td>47 (17.2 - 104)</td>
<td>49.0 (20.8 - 111.7)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>129 (37.7 - 269)</td>
<td>128.4 (37.7 - 269)</td>
<td>130 (42 - 260)</td>
<td>0.082</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>111 (21 - 1098)</td>
<td>122.5 (29 - 1098)</td>
<td>100 (21 - 688)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Hs-CRP (mg/dL)</td>
<td>0.22 (0.01 - 19.62)</td>
<td>0.23 (0.02 - 19.62)</td>
<td>0.2 (0.01-18.51)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>96 (66 - 364)</td>
<td>99 (70 - 360)</td>
<td>94 (66 - 364)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

HDL-C: High-density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol; hs-CRP: High-sensitivity C-reactive protein; data are represented by median (range); significant values for p < 0.05.

Table 3 — Genetic variants associated with the onset of high blood pressure

<table>
<thead>
<tr>
<th>Genes</th>
<th>Cases (n = 860)</th>
<th>Controls (n = 852)</th>
<th>Genetic Models</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dominant</td>
</tr>
<tr>
<td>ACE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4340</td>
<td>II</td>
<td>110 (12.8)</td>
<td>128 (15)</td>
</tr>
<tr>
<td></td>
<td>ID</td>
<td>368 (42.8)</td>
<td>389 (45.7)</td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>382 (44.4)</td>
<td>335 (39.3)</td>
</tr>
<tr>
<td>ACE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4343</td>
<td>AA</td>
<td>143 (16.6)</td>
<td>167 (19.6)</td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>424 (49.3)</td>
<td>435 (51.1)</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>293 (34.1)</td>
<td>250 (29.3)</td>
</tr>
<tr>
<td>ADD1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4961</td>
<td>GG</td>
<td>600 (69.8)</td>
<td>617 (72.4)</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>230 (26.7)</td>
<td>224 (26.3)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>30 (3.5)</td>
<td>11 (1.3)</td>
</tr>
<tr>
<td>GNB3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs5443</td>
<td>CC</td>
<td>286 (33.3)</td>
<td>341 (40)</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>440 (51.2)</td>
<td>378 (44.4)</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>134 (15.6)</td>
<td>133 (15.6)</td>
</tr>
</tbody>
</table>

ACE: Angiotensin-converting enzyme; ADD1: Alpha-adducin; GNB3: G-protein β3 subunit.
bility to HBP, even though conflicting results have been found. Adducin is a cytoskeletal heterodimeric protein found in different tissues, consisting of α, β and γ subunits involved in cell-cell contact, ionic transportation across the cell membrane and cell signal transduction. It is one of the proteins involved in the regulation of the Na+−K+−ATPase. An influence of ADD1 460W genetic variant in the superficial expression and the maximum velocity of the Na+−K+−ATPase and subsequently in a quicker renal tubular sodium reabsorption and blood pressure increase has been found, leading to a changed adducin. A significant risk of HBP, with a 2.8 OR, associated with alpha-adducin polymorphisms, both under recessive and co-dominant models, has been found in this study. The relevant influence of these polymorphisms in salt and water management and therefore in the onset of HBP makes sense in populations known for its high salt intake.

An association of the G protein β3 subunit C825T polymorphism with HBP has been described by different authors even though this was not confirmed by other studies. The gene of G protein β3 subunit is located on chromosome 12p13. GNB3 C825T polymorphism stems from an alternative splicing of the exon 9 with the deletion of 41 amino acids from the protein and is therefore associated with the expression of a new truncated variant (G3-s) correlated with G protein activation. This truncated variant is a functional protein, even though producing an increased G-protein activity and making intracellular signalling easier. In addition, an increased G protein activity leads to an increased Na+/H+ exchange and is associated with higher sodium and lower potassium plasmatic levels, showing the role of these proteins in HBP pathogenesis.

### Table 4 — Logistic regression analysis* for the prediction of high blood pressure

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>0.662 (0.518 – 0.845)</td>
<td>0.001</td>
</tr>
<tr>
<td>Alcohol misuse</td>
<td>1.306 (1.058 – 1.613)</td>
<td>0.013</td>
</tr>
<tr>
<td>Obesity</td>
<td>3.138 (2.469 – 3.989)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3.510 (2.404 – 5.126)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>ADD1</strong></td>
<td>------</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>GW</strong></td>
<td>1.048 (0.834 – 1.317)</td>
<td>0.687</td>
</tr>
<tr>
<td><strong>WW</strong></td>
<td>2.527 (1.219 – 5.239)</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>GNβ3</strong></td>
<td>------</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>CT</strong></td>
<td>1.339 (1.073 – 1.671)</td>
<td>0.010</td>
</tr>
<tr>
<td><strong>TT</strong></td>
<td>1.283 (0.947 – 1.739)</td>
<td>0.108</td>
</tr>
</tbody>
</table>

* Forward Wald method (SPSS v. 19.0), under which the following variables were removed from the equation: physical inactivity, ACE8 and ACE ID genes. CI: Confidence interval; statistically significant for p < 0.05.

![AUC curve comparison](attachment:auc_curve.png)

Figure 1 — ROC curves for TRF and TRF + genetic variants

Delong test, p = 0.018

AUC = 0.668

AUC = 0.681
shown a moderate influence of these polymorphisms in the onset of HBP, which was statistically significant under dominant and multiplicative models, with risks ranging between 1.2 and 1.3.

In addition, upon logistic regression analysis, our results have shown that variables including obesity (OR = 3.138), alcohol misuse (OR = 1.306), diabetes mellitus (OR = 3.510) and CT genotype of the ADD1 rs4961 (OR = 2.527) and CT genotype of the GNB3 rs5443 (OR = 1.339) were significantly and independently associated with the onset of HBP.

It is worth mentioning that smoking emerged as a protective factor, i.e. more controls than cases were smokers (26.2% vs. 19.7%), an apparently conflicting result that may be explained by the fact that patients with HBP were already attending an outpatient clinic and, within a general approach to the cardiovascular risk, many had already quit smoking.

A statistically significant (p = 0.018) increase in the prediction of the risk of HBP when compared to the risk associated with TRF has been shown by ROC curves and AUC considering TRF initially and subsequently adding ADD1 G460W, GNB3 C825T, ACE I/D and ACE A2350G variants.

These results have suggested that HBP is a multifactorial disease, caused by environmental, genetic and lifestyle factors with an interaction between these and leading to the onset of this pathology.

Strengths and limitations of the study

This is the first case-control study carried out with a population from Madeira, a Portuguese population, genetically homogeneous and relatively isolated, in which the association previously established by genetic linkage studies or by GWAS of genetic variants with an increased susceptibility to essential hypertension has been analysed.

Our group of patients, with the abovementioned characteristics, represents an advantage to the mapping of rare diseases and, in addition, according to different authors, to the study of culturally and genetically isolated populations with similar lifestyle, eating habit and natural environment, which may reduce the environmental variation.

However, a limited number of genetic variants associated with HBP were assessed in our study and, therefore, the inclusion of more genetic variants will enhance or add further results.

CONCLUSION

The polymorphisms associated with the onset of HBP in our study are related to the renin-aldosterone-angiotensin system, namely the ACE I/D and ACE A2350G, as well as the polymorphisms related to the management of salt and water, such as the polymorphisms of the alpha adducin (ADD1 G460W) and of G protein β3 subunit (GNB3 C825T).

A significant association between the genetic variants ADD1, GNB3 and the traditional risk factors of HBP regarding the ability to predict the risk of HBP was found in multivariate analysis. The complexity of HBP has been shown by this study in addition to the relevant association between behavioural and genetic factors with the onset of HBP.

HUMAN AND ANIMAL PROTECTION

The authors declare that the followed procedures were according to regulations established by the Ethics and Clinical Research Committee and according to the Helsinki Declaration of the World Medical Association.

DATA CONFIDENTIALITY

The authors declare that they have followed the protocols of their work centre on the publication of patient data.

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in writing this manuscript.

FINANCIAL SUPPORT

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