Relationship between ADD1 Gly460Trp gene polymorphism and essential hypertension in Madeira Island

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Abstract

Essential hypertension (EH) is a complex disease in which physiological, environmental, and genetic factors are involved in its genesis. The genetic variant of the alpha-adducin gene (ADD1) has been described as a risk factor for EH, but with controversial results.

The objective of this study was to evaluate the association of ADD1 (Gly460Trp) gene polymorphism with the EH risk in a population from Madeira Island.

A case-control study with 1614 individuals of Caucasian origin was performed, including 817 individuals with EH and 797 controls. Cases and controls were matched for sex and age, by frequency-matching method. All participants collected blood for biochemical and genotypic analysis for the Gly460Trp polymorphism. We further investigated which variables were independently associated to EH, and, consequently, analyzed their interactions.

In our study, we found a significant association between the ADD1 gene polymorphism and EH (odds ratio 2.484, P = .01). This association remained statistically significant after the multivariate analysis (odds ratio 2.548, P = .02).

The ADD1 Gly460Trp gene polymorphism is significantly and independently associated with EH risk in our population. The knowledge of genetic polymorphisms associated with EH is of paramount importance because it leads to a better understanding of the etiology and pathophysiology of this pathology.

Abbreviations: ADD1 = alpha-adducin gene, BMI = body mass index, CI = confidence interval, DBP = diastolic blood pressure, EH = essential hypertension, GENHYMAPE = Genes and Hypertension in Madeira, Gly = glycine, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, MDR = multifactorial dimensionality reduction, OR = odds ratio, PCR = polymerase chain reaction, SBP = systolic blood pressure, Trp = tryptophan.

Keywords: ADD1 Gly460Trp gene polymorphism, case-control study, essential hypertension

1. Introduction

Essential hypertension (EH) affects one-quarter of adults worldwide, and is estimated to increase to one-third by 2025.[1] Its overall prevalence in the Portuguese adult population is about 42%, of which 44.4% is in men and 40.2% in women.[2]

Several meta-analyses have demonstrated consistently and linearly that elevated blood pressure (BP) is a powerful risk factor for cardiovascular disease and progression to renal failure, independently of other vascular risk factors. Persistently high BP values lead to cardiac, vascular, and renal disease.[3,4]

Essential hypertension is a complex and multifactorial disorder resulting from environmental and genetic factors, and their interactions.[5] Epidemiological studies have shown that 20% to 40% of BP variation is genetically determined.[6,7] In the last decades, many efforts have been made to clarify the pathophysiological mechanisms of hypertension.

Several genetic variants have been studied and associated with the development of EH.[8,9] However, the molecular genetics of EH remains unclear.

Cusi et al[9] first described a genetic variant at alpha-adducin (ADD1) gene level, which resulted from a glycine (Gly) to tryptophan (Trp) substitution at amino acid position 460 (Gly460Trp).[9] This genetic variant Gly460Trp modulates the overall ability of tubular epithelial cells to transport ions, leading to an increased Na+/K+-ATPase pump activity and thus to a greater sodium reabsorption at the proximal renal tubule, increasing, consequently, the BP.[9–11]

The association of this genetic variant with hypertension was confirmed in some studies,[12–15] but not in others.[16–19]

The purpose of this study was to evaluate the association between the ADD1 Gly460Trp polymorphism and EH in a
cohort from Madeira Island. The identification of these causal and/or associated variants promotes the development of approaches for EH prediction and prevention.

2. Material and methods

2.1. Study population

All individuals were selected from Genes and Hypertension in Madeira (GENHYMAPE) study, an ongoing Madeira population-based study which started in 2006, and consists of adults aged 30 to 75 years. It is a hospital-based case-control study, with patients enrolled from Cardiology consultations and controls selected from the Internal Medicine, on condition that they were normotensive and matched for sex and age.

Its purpose is to identify determinants of essential hypertension in Madeira Island (Portugal). This Island is located on the African plate, in the Atlantic Ocean, between 30° and 33° north latitude, 978 km southwest of Lisbon, about 700 km west of the African coast.

Participants of this study include native inhabitants and residents in Madeira Island for at least 3 generations with no migration history, from both rural and urban origins. This population is considered a genetically fairly homogeneous South European Caucasian population.

2.2. Study design

This case-control study was performed with a total of 1614 individuals with a mean age of 50.6 ± 8.2 years (50.1% male), namely 817 patients diagnosed with EH (mean age 50.8 ± 8.1 years; 51.5% male) and 797 normotensive controls (mean age of 50.1 ± 8.3 years; 48.6% male).

This study was approved by the ethics committee of the Funchal Hospital Center, and was performed in conformity with the guidelines outlined in the Declaration of Helsinki statement. Written informed consent was obtained from each participant, including explicit permission for the DNA analyses and the collection of relevant clinical data.

2.3. Data collection

Data were collected from all subjects in a standardized file comprising demographic, clinical characteristics, and traditional risk factors (sex, age, sedentary lifestyle, smoking and alcohol habits, body mass index [BMI], diabetes, and dyslipidemia). The definition of the traditional risk factors was based on the standard criteria, as previously reported.

Sedentary lifestyle was considered when individuals practiced less than 150 min/wk of moderate activity. Alcohol consumption was considered as a risk factor when individuals consumed 70 g of alcohol per week for more than 1 year. “Smoking status” refers to current smokers or subjects with less than 5 years of smoking cessation. BMI was defined as body mass divided by the square of the body height, universally expressed in units of kg/m², with obesity defined as a BMI ≥ 30.

Subjects were classified as being diabetic when taking oral antidiabetic medication or insulin, or if their fasting plasma glucose was higher than 7.0 mmol/L or 126 mg/dL.

2.4. Definition of essential hypertension

Blood pressure was measured by an Internal Medicine specialist, after 10 minutes of resting, in the right arm, using a standard Welch Allyn sphygmomanometer (phases I through V). The average of 3 readings taken 2 minutes apart was recorded.

Essential hypertension was considered when patients, at the entry into this study, were already diagnosed and/or had been on antihypertensive medication for more than 3 months or newly diagnosed hypertensives with systolic BP (SBP)/diastolic BP (DBP) ≥ 140/90 mm Hg measured on at least 3 occasions.

The normotensive controls had never been treated with antihypertensive medications, and presented with SBP/DBP < 140/90 mm Hg. In addition, subjects with secondary hypertension, pregnant and lactating females, and those receiving medications for other indications that could affect BP were excluded from this study. Participants with multiple organ failure, a mental disorder, or chronic inflammatory disease were also excluded from this study.

2.5. Biochemical analyses

Blood samples were extracted after 14 to 16 hours of fasting. Biochemical analyses were performed in the Central Laboratory of the Hospital, according to the usual techniques.

To determine total cholesterol and triglycerides, blood samples were placed in dry tubes and centrifuged half an hour later at 3500 g, and subsequently quantified by an enzymatic technique using a Hitachi 911 auto analyzer.

2.6. Genotype analysis

Genomic DNA was extracted from 80 μL of peripheral blood using a standard phenol-chloroform method. To identify ADD1 genotypes, a TaqMan allelic discrimination assay was performed using labelled probes and primers pre-established by the supplier (TaqMan SNP Genotyping Assays, Applied Biosystems).

The genotyping reaction was amplified and detected on an Applied Biosystems 7300 Real-Time PCR System, and genotypes were determined by using the 7300 System SDS Software (Applied Biosystems, Foster City, CA) without any prior knowledge of individual clinical data.

2.7. Statistical analysis

Deviation from Hardy–Weinberg equilibrium for genotypes at individual locus was assessed using the chi-square test. Comparisons of characteristics between cases and controls were analyzed by chi-square test for categorical variables and Student t test or Mann–Whitney U test for continuous variables, as appropriate. No missing data were observed.

The case-control matching procedure was based upon the distributions of the characteristics among the cases, named “frequency-matching.”

Genotypic frequencies were determined from observed counts and compared by chi-square analysis. Three statistical genetic models were tested, namely additive (distribution of Trp allelic frequency of ADD1), recessive (TrpTrp vs GlyGly+GlyTrp), and dominant (GlyGly vs GlyTrp+ TrpTrp). The odds ratio (OR) and corresponding 95% confidence interval (CI) were determined.

A multivariate logistic regression model was performed using forward Wald method to examine associations between ADD1 genotypes and to account for significant risk factors as described (sex, age, smoking status, alcohol consumption, BMI, diabetes, sedentary lifestyle). The environmental risk factors were further studied for their interaction with ADD1 polymorphism by using multifactorial dimensionality reduction (MDR) 3.0.2 software.
The best combination pattern was searched on the principle that both cross-validation consistency and test balance accuracy were maximized to evaluate the interaction of the genetic polymorphism with the environmental risk factors, in relation to EH.

Statistical analysis was done using SPSS software version 19.0 (IBM, Armonk, NY) and R version 3.2.0. 

3. Results

3.1. Baseline characteristics of the population

The baseline characteristics of the study subjects are shown in Table 1. This study included a total of 1614 individuals, namely 817 patients diagnosed with EH and 797 normotensive controls. The case-control matching procedure was based upon the “frequency-matching.” Specifically, using chi-square test, we obtained a male frequency of 51.5% for cases and 48.6% for controls, without statistical significance (P = .05), meaning that variable age is correctly adjusted for cases and controls (Table 1).

Sedentary lifestyle, total cholesterol, and low-density lipoprotein cholesterol (LDL-C) showed no significance between cases and controls (all P > .05). However, SBP and DBP, BMI, alcohol consumption, diabetes, dyslipidemia, triglycerides, and fasting glucose were significantly higher in the hypertensive group than in controls (P < .05).

Smoking and high-density lipoprotein cholesterol (HDL-C) were higher in the control group, with statistical significance (P = .001 and P < .001, respectively) (Table 1).

3.2. Genotype distribution of ADD1 (Gly460Trp) gene polymorphism among cases and controls, and EH risk

The genotype distributions of ADD1 are shown in Table 2. In our population, genotype distributions were in Hardy–Weinberg equilibrium (P > .05). The wild genotype (GlyGly) was more prevalent in controls than in cases (71.8% vs 70.1%). On the contrary, the mutated genotype (TrpTrp) was more frequent in cases than in controls (3.1% vs 1.2%) with statistical significance (P = .01) (Table 2).

Risk prediction was evaluated under 3 genetic models of inheritance (Table 2). The alpha-2-aducin Gly460Trp gene polymorphism showed a statistical significant increase in EH risk, under the recessive genetic model (odds ratio [OR] 2.484, 95% confidence interval [CI] 1.185–5.207, P = .01). No significant association was found using a dominant genetic model (OR 1.083, 95% CI 0.873–1.342, P = .47) or additive model (OR 1.143, 95% CI 0.943–1.386, P = .17) (Table 2).

A multivariate analysis (logistic regression) was performed to determine which variables were independently associated with EH risk (Table 3). ADD1 Try/Try genotype remained in the equation as a significant and independent risk factor for EH (OR 2.548, 95% CI 1.159–5.601, P = .02). Additionally, diabetes, alcohol consumption, and BMI were also significantly associated with EH risk (Table 3).

Remarkably, smoking habits appeared in the equation as a significant protective variable to EH development (Table 3).

### Table 1
**Baseline characteristics of study subjects.**

| Variables | Total (N = 1614) | Hypertensives (n = 817) | Controls (n = 797) | P |
|-----------|------------------|------------------------|-------------------|---|
| Male sex, n (%) | 808 (50.1) | 421 (51.5) | 387 (48.6) | .252 |
| Age, y | 50.6 ± 8.2 | 50.8 ± 8.1 | 50.4 ± 8.3 | .252 |
| SBP, mm Hg | 134.7 ± 20.4 | 148.1 ± 18.6 | 121 ± 10.8 | <.001 |
| DBP, mm Hg | 84.7 ± 12 | 91.6 ± 11.6 | 77.7 ± 7.4 | <.001 |
| BMI, kg/m² | 27.8 ± 4.0 | 29.3 ± 5.2 | 26.2 ± 4 | <.001 |
| Sedentary lifestyle, n (%) | 881 (54.6) | 460 (66.3) | 421 (52.8) | .16 |
| Alcohol consumption, n (%) | 628 (38.8) | 340 (41.6) | 286 (35.9) | .02 |
| Smoking status, n (%) | 320 (19.8) | 135 (16.5) | 185 (23.2) | <.001 |
| Diabetes, n (%) | 169 (10.5) | 133 (16.3) | 36 (4.5) | <.001 |
| Dyslipidemia, n (%) | 1336 (82.8) | 715 (87.5) | 621 (77.9) | <.001 |
| Total cholesterol, mg/dL | 207 (107–370) | 209 (115–344) | 206 (107–370) | .08 |
| HDL-C, mg/dL | 48 (17.2–111.7) | 46.9 (17.2–103) | 49 (20.8–111.7) | <.001 |
| LDL-C, mg/dL | 131.1 (37.7–269) | 131.1 (37.7–269) | 131 (42–260) | .94 |
| Triglycerides, mg/dL | 110 (21–1098) | 122 (31–1098) | 98 (21–698) | <.001 |
| Fasting glucose, mg/dL | 95 (66–364) | 96 (70–360) | 93 (66–364) | <.001 |

Statistically significant for P < .05.

BMI = body mass index, DBP = diastolic blood pressure, HDL-C = high-density lipoprotein cholesterol, LDL-C = low-density lipoprotein cholesterol, SBP = systolic blood pressure.

### Table 2
**Genotype distribution of ADD1 gene polymorphism among hypertensive subjects and controls and essential hypertension risk.**

| ADD1 Gly460Trp | Cases (n = 817) | Controls (n = 797) | Dominant | Recessive | Additive |
|----------------|----------------|-------------------|----------|-----------|---------|
| Gly/Gly, n (%) | 573 (70.1) | 572 (71.8) | 1.083 | 2.484 | 1.143 |
| Trp/Trp, n (%) | 219 (26.8) | 215 (27) | (0.873–1.342) | (1.185–5.207) | (0.943–1.386) |
| Trp/Gly, n (%) | 25 (3.1) | 10 (1.2) | P = .47 | P = .01 | P = .17 |

Statistically significant for P < .05.

ADD1 = alpha-2-ducin gene, CI = confidence interval, Gly = glycine, OR = odds ratio from the genetic risk models, with the recessive (n Italics) showing the best results, Trp = tryptophan.
Table 3

Logistic regression analysis with variables independently associated with essential hypertension.

| Variables | Odds ratio (95% CI) | P   |
|-----------|---------------------|-----|
| Diabetes  | 3.028 (2.026–4.526) | <.001|
| Alcohol consumption | 1.238 (0.996–1.538) | .05 |
| BMI       | 1.163 (1.132–1.195) | <.001|
| Smoking status | 0.713 (0.544–0.935) | .01 |
| ADD1      | 0.163 (0.132–1.195) | <.001|
| ADD1 (Gly/Trp) | 0.965 (0.761–1.225) | 0.177|
| ADD1 (Trp/Trp) | 2.548 (1.150–5.661) | .02 |

Method: forward Wald (SPSS version 19.0).
ADD1 = alpha-adducin gene; BMI = body mass index; CI = confidence interval; Gly = glycine; Trp = tryptophan.
Not remained in the equation: age, sex, and sedentary lifestyle.
Statistically significant for P < .05.

The MDR software was finally employed to analyze the interaction between the nongenetic variables and the ADD1 polymorphism (Table 4). The best association model included diabetes, BMI, and ADD1 polymorphism, which showed a maximal testing accuracy of 0.641 and a cross-validation consistency of 10 out of 10 (Table 4). These results indicate that ADD1 interacts with diabetes and BMI in a synergistic way, with a 3.77-fold greater risk of EH (Table 4).

4. Discussion
In our population from Madeira Island, subjects who were carriers of the mutated ADD1 Trp460Trp genotype had an increased risk of EH. This association was further strengthened in the multivariate analysis, reinforcing the importance of this variant in the susceptibility to EH in our population. Cusi et al.[9] published the first Caucasian study that has proved a significant linkage between ADD1 Gly460Trp gene polymorphism and EH. Similar results have also been found in other ethnicities, such as Asians[12,13] and Africans.[14] Nevertheless, the association of the ADD1 polymorphism with the susceptibility to EH was not always clearly demonstrated.[15–19,26] The lack of association did not exclude the involvement of this genetic variant with renal sodium-handling abnormalities.

The ADD1 polymorphism results in the amino acid substitution of glycine by tryptophan (Gly460Trp), which is reported to be associated with a salt sensitive form of hypertension patients. Manunta et al.[11] have studied the involvement of ADD1 polymorphism in abnormalities of renal function in hypertensive patients, and have concluded that the fraction of excretions of lithium and uric acid, markers of proximal renal tubular function, was reduced in patients with the alpha-adducin variant TrpTrp. This supports the hypothesis that this variant is related to an increase in proximal Na+ reabsorption at the kidney level[16] by an increase in the Na+ pump activity and a higher number of Na+ pump units expressed on the cell surface.[27]

Cusi et al.[9] exposed hypertensive subjects to an acute salt sensitivity test and investigated the effect of ADD1 polymorphism on the response of acute BP to sodium changes. Authors concluded that heterozygotes (Gly460Trp) had a greater decrease in BP and significantly lower plasma renin activity than homozygotes (Gly460Gly).[9]

This leads us to conclude that alpha-adducin 460Trp polymorphism is associated with an increase in BP, not in all hypertensive individuals,[14] but in the specific hypertensive phenotype—salt-sensitive and low renin hypertension.

Other investigators have found decreased renin activity levels in subjects carrying the 460Trp allele.[12,28]

Grant et al.[29] showed that this genetic variant modifies sodium homeostasis and associates with intermediate phenotypes of salt sensitivity and low renin hypertension. This may be explained by the fact that a deficient alpha-adducin function leads to increased tubular sodium reabsorption, producing sodium retention and intravascular volume expansion with suppression of plasma renin activity.[29]

The genetic variance of 1 or more of these genes may justify the increased salt sensitivity of some individuals; hence the importance of their knowledge to individualize EH control and therapeutic measures.

In addition to ADD1 Trp460Trp genotype, other variables such as BMI, alcohol habits and diabetes remained in the equation as independent risk factors of EH. According to our results, smoking status appeared to be a protective factor. A likely explanation of these apparent protective effects of smoking on hypertension might be related to the fact that hypertensive patients are most likely to have been advised by their physicians to quit smoking, so that the proportion of smoking hypertensives is lower than the proportion of nonsmoking ones. MDR analyses further showed the interaction between ADD1 polymorphism, BMI, and diabetes as the best model for EH susceptibility. Since BMI plays a key role in development of high BP and ADD1 is 1 of the most important targets for salt sensitivity and expanded plasma volume, it is not surprising that their interaction may play an important role in the susceptibility to hypertension. On the contrary, it is now established that insulin resistance, which predicts type 2 diabetes, also has a role in the development of hypertension.[30] Indeed, hypertension and diabetes substantially share common pathways such as obesity, inflammation, oxidative stress, insulin resistance, and mental stress.

Table 4

Best models to analyze gene–environment interactions by multifactor dimensionality reduction.

| Best model | Training balanced accuracy | Training odds ratio | Training P | Testing balanced accuracy | Testing odds ratio | Testing P  | Testing cross validation consistency |
|------------|----------------------------|--------------------|------------|--------------------------|-------------------|-----------|-----------------------------------|
| BMI        | 0.622                      | 3.71 (2.38–4.77)   | <.001      | 0.622                    | 3.71 (1.73–7.92)  | <.0005    | 1/10                               |
| Diabetes; BMI | 0.638                      | 3.76 (2.96–4.76)   | <.001      | 0.638                    | 3.76 (1.84–7.66)  | <.0002    | 1/10                               |
| Diabetes; BMI; ADD1 | 0.641                      | 3.77 (2.98–4.77)   | <.001      | 0.641                    | 3.77 (1.87–7.62)  | <.0001    | 1/10                               |
| Diabetes; BMI; smoking; ADD1 | 0.642                      | 3.96 (3.12–5.03)   | <.001      | 0.629                    | 3.44 (1.70–7.0)   | <.0005    | 1/10                               |

Statistically significant for P < .05.
ADD1 = alpha-adducin gene; BMI = body mass index.
* Overall best combination model with highest training balanced accuracy, highest testing accuracy, and best cross-validation (CV) consistency.
Essential hypertension is a multifactorial disease caused by environmental, genetic, and individual lifestyle interactions that lead to the onset of this pathology. Further studies are needed to study gene–gene and gene-environment interactions and/or the diversity of individual genetic and environmental characteristics that lead to the development of EH.

4.1. Study strengths and limitations

We must point out that this is the first study done with a population from Madeira Island, a genetically homogeneous and isolated Southern European population. This has been especially valuable for mapping rare recessive disorders, but many researchers believe this could be a solution for more complex disorders as well because of the relatively uniform genetic background of the population. Some culturally and genetically isolated populations have a more similar way of living, eating habits, and natural environment, which reduce environmental variation.\footnote{23}

A significant limitation of the study is the lack of biochemical data on plasma renin, salt intake, and salt sensitivity, and other confounders such as stress/anxiety levels and insomnia to explain the specific physiological role of alpha-adducin polymorphism in our population.

Another limitation is that hypertensives are included in the study after their diagnosis and are already taking antihypertensive therapy, for ethical reasons. In this study, only 38 hypertensive individuals were not medicated, which limits the baseline BP assessment of individuals with and without this genetic variant. It would be very interesting to measure the impact of gene polymorphisms in relation to SBP and DBP.

5. Conclusions

In summary, the present study shows that the ADD1 Gly460Trp gene polymorphism is independently and significantly associated with the EH risk amongst Madeira Island’s population. The knowledge of the genetic polymorphisms associated with EH could be widely used in clinical practice for diagnosis, treatment, and prognosis of patients with essential hypertension.

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