CYP2D6 1846 G/A gene polymorphism in association with Hemorrhagic Stroke/Aneurysm in patients from the Federal District (Brazil)

Polimorfismo do gene CYP2D6 1846 G/A em associação com o Acidente Vascular Encefálico/Aneurisma em uma população do Distrito Federal (Brasil)

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RESUMO

Objetivo: Investigar uma possível associação do gene CYP2D6 1846 G/A em pacientes que possuem AVEH/Aneurisma, residentes no Distrito Federal (Brasil) além de cruzar as informações obtidas com as manifestações clinicas e o prognóstico. **Método:** Tratou-se de um estudo caso-controle de base populacional, envolvendo 81 casos com AVEH e/ou Aneurisma. Para a genotipagem dessas amostras utilizou-se a técnica laboratorial PCR-RFLP e adotou-se o nível de significância de 5%. **Resultados:** Ao associar as frequências genotípicas e alélicas em relação ao AVEH/Aneurisma, percebeu-se que não houve diferença estatística. Em relação à média de glicemia, participantes com genótipo GG, apresentavam índices elevados desse marcador no grupo caso quando comparado ao grupo controle. **Conclusão**: A presença do polimorfismo CYP2D6 1846 G/A deve ser amplamente estudada em pesquisas futuras, pois é necessário esclarecer se tais polimorfismos representam associações ao AVEH/Aneurisma.

Descritores: Acidente Vascular Cerebral; Polimorfismo Genético; Citocromo P-450 CYP2D6; Hipertensão; Diabetes Mellitus.

ABSTRACT

Objective: To investigate a possible association of the CYP2D6 1846 G/A gene in HS/aneurysm patients residing in the Federal District (Brazil), as well as to crosscheck the information obtained with clinical manifestations and prognosis. **Method:** The investigation was a population-based case-control study involving 81 cases with HS/aneurysm. The samples genotyping employed the PCR-RFLP technique with a significance level of 5%. **Results:** The attempt to associate genotypic and allelic frequencies to the HVA/Aneurysm resulted in no statistical difference. Nevertheless, in patients with the GG genotype and hypertension, the risk for HS increased 44 times. Participants with GG genotype also had statistically higher glycemia mean levels in the case group. **Conclusion:** The presence of the CYP2D6 1846 G/A polymorphism should be extensively, as it is necessary to clarify whether such polymorphisms represent associations with HS and Intracerebral Aneurysm.

Descriptors: Stroke; genetic polymorphism; Cytochrome P-450 CYP2D6; Hypertencion; Diabetes Mellitus.

ORIGINAL

Introduction

Stroke or cerebrovascular accident (CVA) corresponds to an acute neurological dysfunction caused by the disruption of blood flow in brain areas, which may lead to several disabilities, and the overall impairments may interfere with the daily activities in the patient's life.[1] More than one million people have a stroke each year in Europe and, by 2025, this estimate is expected rising to 1.5 million due to population aging.[2]

This pathology can be subdivided into two categories that present similitudes, but differ as to their etiology, pathophysiology, medical, and surgical treatment.[3]

Hemorrhagic stroke (HS) is one of the leading causes of mortality worldwide, accounting for 10-15% of all stroke cases.[4] It involves the rupture of an intracerebral artery that bleeds into the surrounding brain, thus forming a hematoma.[5] HS is a multifactorial disease that emerges from the combination of genetic and environmental factors, which contribute to the increased risk of HS occurrence.[6-7]

The cytochrome p450 family 2, subfamily D, member 6 (CYP2D6) is located in the long arm of chromosome 22 (22q13.1) and is responsible for the metabolism of several drugs, such as beta-blockers.[8] Although the CYP2D6 enzyme is extensively studied due to its clinical importance, the CYP2D subfamily evolution is still not fully understood.[9] CYP2D6*4 G/A polymorphism limits its capacity to metabolize substances; this reduction prolongs the exposure time to neurotoxins and may lead to the occurrence of stroke.[10]

In brief, this study aimed to investigate possible associations of the CYP2D6 1846 G/A gene in patients with a cerebrovascular accident, an intracerebral aneurysm, or both, residing in the Federal District (Brazil), and crosscheck with the information obtained from the clinical manifestations and prognosis.

Material and Methods

Subjects

As an observational, descriptive case-control study, the research participants were divided into two groups.

The case group consisted of 81 patients of both sexes, aged over 18 years, and had the diagnosis of hemorrhagic stroke (HS), intracerebral aneurysm or both. All patients were residents of the Federal District (Brazil) and had their pathology confirmed by computed tomography. This group consisted of 48 patients (59.3%) with HS and 33 (40.7%) with an aneurysm.

Whereas, 81 individuals without the questioned pathology of both sexes, over 18 years old and not related to the case group, formed the control group. For inclusion, the participants in the control group should have performed some biochemical tests at least 15 days prior, such as serum glucose.

Notably, in this study, most research participants were women, both in the case group (59.3%) and the control group (53.1%). Regarding the age of the population studied, the case group had an average of 53.49 years (± 5.94), while the control group had an average of 52.27 years (± 5.75).

FEPECS Research Ethics Committee approved this study (number: 0095/2010), and all participants had a detailed clinical history and clinical developments followed.

Laboratory Methods

Each participant, after signing the (free and) informed consent form (ICF), had 10 mL of their venous blood collected. The DNA (Deoxyribonucleic acid) was then extracted from blood collected using Invitek's Invisorb Spin Blood Mini Kit (250) (catalog # CA10-0005, lot # 1031100300) with an average concentration of 20 ng/µL.

CYP2D6 polymorphism genotyping adopted a combination of the polymerase chain reaction technique with the restriction fragment length polymorphism based analysis (PCR-RFLP). The primers used in the CYP2D6 1846 G/A amplification were the sense 5'-GCC TTC GCC AAC CAC TCC -3 'and the antisense 5'-AAA TCC TGC TCT TCC GAG GC -3'. Amplification employed the following thermocycling conditions: 94˚C for 5 minutes (initial denaturation), followed by 30 cycles of denaturation at 94˚C for 30 seconds, succeeded by 60˚C for 15 seconds and 72˚C for 20 seconds for oligonucleotide annealing. The final extension occurred at 72ºC for 7 minutes.[11]

Finally, the 355 bp PCR products were incubated for 90 minutes at 60 ° C with the BseBI enzyme (Jena, Germany). Allele A, or mutated allele, was not cleaved by the enzyme, thus remaining as a 355 bp fragment. Allele G presented two DNA fragments, one of 106 bp and the other of 250 bp. The fragments were visualized on a 4% agarose gel with ethidium bromide and exposed to ultraviolet light.

Statistical analysis

The control group was considered within the Hardy-Weinberg equilibrium, and the frequency of genotypes and alleles in patients with HS, aneurysm, or both, was estimated using the SPSS version 23.0 program. The chi-square test was used to compare the frequency distribution of the two groups, and associations with probabilities lower than 5% ($p < 0.05$) were considered.

The characteristics frequencies of the research participants were estimated, namely: sex, smoking, alcoholism, presence of systemic arterial hypertension (SAH), and diabetes. Alternately, the quantitative variables age and glycemia were described in terms of their summary statistics (mean and standard deviation).

Results

Table 1 displays the allele and genotype frequencies of the CYP2D6 1846 G/A polymorphism. Most of the case group (74.1%) and the control group (71.6%) presented the GG genotype. There was no statistical difference in the other genotypic frequencies (P = 0.930). Regarding the allele frequency, 86.4% of the patients with HS/Aneurysm had the G allele, compared to the 83.3% of the control group participants with the same allele. Such a difference was not statistically significant (P = 0.764).

Table 1. Genotypic and allelic distribution of CYP2D6 polymorphism in case and control groups.

CYP2D6 1846 G/A	Groups						
	HS/Aneurism		Control				
	N	%	N	%	P	OR	CI
GG	60	74,1	58	71,6			
GA	17	21,0	19	23,5	0,930	NA	NA
AA	4	4,9	4	4,9			
Total	81	100,0	81	100,0			
GG	60	74,1	58	71,6			
GA+AA	21	25,9	23	28,4	0,729	1,13	0,56-2,27
Total	81	100,0	81	100,0			
G	137	86,4	135	83,3			
A	25	15,4	27	16,7	0,764	1,09	0,60-1,98
Total	162	100,0	162	100,0			

NA = does not applied; chi-square test.

Table 2 presents the evaluation of the difference in the proportion of individuals according to their clinical characteristics (systemic arterial hypertension - SAH, Diabetes, Smoking, and Alcoholism) associated with the CYP2D6 1846 G/A polymorphism. However, there was no association between the genotypes and SAH (P = 0.867), diabetes (P = 1.00), smoking (P = 0.938), and alcoholism (P = 0.332).

Table 2. Distribution of clinical characteristics SAH, diabetes, smoking, and alcoholism according to **CYP2D6** polymorphism in the research participants.

		CYP2D6 1846 G/A						
		GG		GA+AA				
		N	%	N	%	P	OR	CI
SAH	Yes	50	42,4%	18	40,9%	0,867	1,062	(0,526 – 2,146)
	No	68	57,6%	26	59,1%			
Diabetes	Yes	2	1,7%	1	2,3%	1	0,741	(0,066 – 8,386)
	No	116	98,3%	43	97,7%			
Smoking	Yes	41	34,7%	15	34,1%	0,938	1,029	(0,496 – 2,135)
	No	77	65,3%	29	65,9%			
Alcoholism	Yes	33	28,0%	9	20,5%	0,332	1,510	(0,655 – 3,482)
	No	85	72,0%	35	79,5%			

chi-square test.

Table 3 measures the association between clinical characteristics and stroke occurrence in different genotypes. 76.7% of the participants in the case group with the GG genotype had SAH, compared to 6.9% in the control group. Among participants with the GG genotype, the risk for stroke increased 44 times in the case group with SAH (P <0.001; OR = 44; 13.65 - 144.17). 66.7% of the GA + AA participants in the case group were hypertensive, compared to 17.4% in the control group with the same genotype. The risk for HS/Aneurysm in patients with SAH and the GA + AA genotype increased 10-fold compared to the control group (P = 0.002; OR = 10; 2.32 - 38.87).

Table 3. Clinical characteristics associated with the occurrence of HS/Aneurysm by GG and GA + AA genotype of CYP2D6 polymorphism.

		***CYP2D6* 1846 G/A**											
		GG						**GA+AA**					
		Groups						**Groups**					
		HS/Aneurism		**Control**				**HS/Aneurism**		**Control**			
		N	**%**	**N**	**%**	**P**	**OR (CI)**	**N**	**%**	**N**	**%**	**P**	**OR (CI)**
SAH	Yes	46	76,7	4	6,9	<0,001*	44,36 (13,65 - 144,17)	14	66,7	4	17,4	0,002*	9,50 (2,32 - 38,87)
	No	14	23,3	54	93,1			7	33,3	19	82,6		
	Total	60	100,0	58	100,0			21	100,0	23	100,0		
Diabetes	Yes	2	3,3	0	0,0	0,496	2,0 (1,67 - 2,40)	1	4,8	0	0,0	0,477	2,15 (1,56 - 2,96)
	No	58	96,7	58	100,0			20	95,2	23	100,0		
	Total	60	100,0	58	100,0			21	100,0	23	100,0		
Smoking	Yes	24	40,0	17	29,3	0,223	1,61 (0,75 - 3,46)	8	38,1	7	30,4	0,592	1,41 (0,40 - 4,92)
	No	36	60,0	41	70,7			13	61,9	16	69,6		
	Total	60	100,0	58	100,0			21	100,0	23	100,0		
Alcoholism	Yes	17	28,3	16	27,6	0,928	1,04 (0,46 - 2,32)	5	23,8	4	17,4	0,716	0,71 (0,34 - 6,48)
	No	43	71,7	42	72,4			16	76,2	19	82,6%		
	Total	60	100,0	58	100,0			21	100,0	23	100,0%		

After verifying the genotypic and allelic frequencies and the association of CYP2D6 polymorphism with the clinical manifestations of the disease, Table 4 evaluates the difference in the mean blood glucose level between the study groups according with the different genotypes. Assessing the GG genotype, the mean blood glucose level in the case group was 106 mg/dL compared to 96 mg/dL in the control group. These mean differences were statistically different (P = 0.004); consequently, the mean glucose level was higher in the case group than in the control group.

Table 4. Glycemic analysis in the study groups according to the CYP2D6 polymorphism genetic profile.

	CYP2D6 1846 G/A									
	GG					GA+AA				
	Groups					Groups				
	HS/Aneurism		Control			HS/Aneurism		Control		
	Mean	Standard deviation	Mean	Standard deviation	P	Mean	Standard deviation	Mean	Standard deviation	P
Glucose (mg/dL)	106	21	96	7	0,004*	107	27	96	7	0,126

*P<0.05.

Discussion

Stroke is the second leading cause of global mortality among cardiovascular diseases, especially in adults and the elderly. In Latin America, Brazil remains one of the countries with the highest risk of premature death due to stroke.[12] According to the WHO Health Report, 5.5 million people die each year as a result of a stroke. Worldwide, tremendous amounts of money and resources are funneled to the treatment of this disease. Nonetheless, the methods to identify people at high risk of stroke are limited, and, therefore, the molecular mechanisms involved in this work are fundamental to solve this problem.[13]

In this study, the incidence of HS, aneurysm, or both was slightly higher in females (59.3%, n = 48) than in males (40.7%, n = 33), and the mean age of the case group was of 53 years old.

One study analyzed the topographic and epidemiological aspects of the patients, as well as their severity level at hospital admission and release, and their clinical condition after one year of release. 77 (52%) of the 148 patients diagnosed with HS were male and 71 (48%) female. The age varied from 20 to 92 years old, with a higher number of cases in males. Of the total number of cases, there were 57 deaths (38.5%). The highest number of deaths was in the 40 to 59 age range with a slight difference between genders, with the mortality rate being higher for females (43.7%).[6]

Cytochrome P450 (CYP) represents a large family of hemoproteins. Biologically, they participate in the metabolism of some endogenous components such as hormones and cholesterol.[14] The CYP2D6 gene encodes the CYP2D6 enzyme that belongs to the cytochrome p450 system. It is expressed not only in the liver but also in the central nervous system (CNS), consequently playing a pivotal role in the catalysis of drug metabolites and biological toxins. The CYP2D6*4 mutation is caused by a transition from G to A, resulting in a lower capacity to metabolize substances. The decreased CYP2D6 activity may prolong neurotoxin exposure time, leading, therefore, to CNS cell damage. This suspected hypothesis may play a role in the pathogenesis of stroke, Parkinson's disease, and Alzheimer's disease.[15]

In the present study, CYP2D6*4 polymorphism exhibited no statistical difference regarding the different genotypes (Table 1). Nevertheless, among participants with GG genotype, the risk for stroke increased 44-fold in the case group with systemic arterial hypertension - SAH (P <0.001, OR = 44, 13.65-144,17). Whereas, among GA + AA genotype participants, the risk for

HS/Aneurysm in patients with SAH increased 10-fold (Table 3). Genotype seems to have influence when the study groups were divided (Table 3), but not when considering all research participants (Table 2). Notably, the dominant GG genotype seems to favor the risk of SAH more strongly.

Risk factors linked to stroke are SAH, diabetes mellitus, physical inactivity, heart disease, smoking, obesity, and genetic predisposition.[16] In SAH, despite the availability of antihypertensive drugs with different mechanisms of action, most of the treated hypertensive patients do not achieve optimal blood pressure levels. CYP2D6 polymorphism enables patients to be characterized as ultra-fast, intermediate, or slow drug metabolizers. The latter corresponds to 7% to 10% of the population of Caucasian individuals.[17]

In an Egyptian study, hypertension and control cases were compared regarding their genotypic allelic variants of the CYP2D6 gene. Hypertension cases showed a significantly larger dominant homozygous genotype when compared to the control cases (P = 0.01). This phenomenon also manifested itself among obese cases that had significantly smaller mutated homozygous forms than controls (P = 0.040), cases with cardiac lesions (P = 0.008), and cases with complications (P = 0.010).[18]

In this study, the mean glucose level was higher in the case group than the control group in both GG (P = 0.004) and GA + AA (P = 0.126) genotypes of the CYP2D6 polymorphism. In a case-control study, the CYP2D6 polymorphism associated with the development of type 2 diabetes mellitus in the population of Bosnia and Herzegovina was studied. This study found no relationship between the different genotypes and diabetes in the indicated population. The authors considered that the lack of association was due to research limitations, for instance, differences in ethnic characteristics, such as lifestyle, environmental factors, genetic background, besides the number of research participants, 37 participants in the case group and 44 in the control group.[19]

Conclusion

The presence of systemic arterial hypertension (SAH) is a risk factor for the HS/Aneurysm occurrence. Moreover, there is an increase in blood glucose levels in patients diagnosed with the studied pathology.

When the genotypic and allelic frequencies of the CYP2D6 1846 G/A polymorphism were assessed, it was noted no statistical difference with the distribution in association with the pathology.

No statistical difference was found when analyzing the polymorphism with the clinical characteristics (SAH, diabetes, smoking, and alcoholism) in the different genotypes. Yet, among the GG genotype participants, the risk of HS increased 44 times in the SAH group. Furthermore, the risk for HS in patients with SAH and GA + AA genotype increased 10-fold when compared with the control group.

Regarding the glycemia variable, its average was higher in the case group than in the control group, whereas in the GG genotype, the highest mean glucose level in the case group was statistically significant.

The presence of the CYP2D6 1846 G/A polymorphism should be extensively researched, and the results of this analysis may be useful in other gene-linked

disease studies. The possible influence on gene expression in this research remains ongoing, so further association studies should be conducted in different populations to clarify whether this polymorphism has associations with HS and Intracerebral Aneurysm.

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