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Association of the *CHGA* gene polymorphism in patients with hemorrhagic stroke and/or aneurysm

Associação do polimorfismo do gene CHGA *em pacientes com acidente vascular encefálico hemorrágico e/ou aneurisma*

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ABSTRACT

Introduction: Cerebrovascular diseases have been associated with several genes. Chromogranin A (*CHGA*) has been used as maker in cardiovascular disease. Therefore, evaluating the polymorphism and verifying its association with this pathology is very important to better understand this disease. **Objective:** The aim of this study was to identify the association between coding region polymorphism in -264 position of the *CHGA* gene (*Glu264Asp*) and hemorrhagic stroke (HS)/aneurysm in the Federal District, Brazil. **Methods:** This is a population-based case-control, involving 45 cases with HS and/or aneurysm. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method is used for genotyping these samples. A significance level of 5% was adopted. **Results:** The absence of the CC genotype the *Glu264Asp CHGA* polymorphism in the study participants and the significant presence of the GC heterozygote genotype were observed in this study. However, the distribution of genotypes did not differ statistically in the groups. **Conclusion:** The *Glu264Asp CHGA* polymorphism does not seem to contribute to the genesis of the *CHGA* protein expression in this patients group, but to understand whether or not there is a possible association of the pathology in question and whether the mutation will contribute in the gene therapy and thus to improve patients' quality of life.

Key words: genetic polymorphism; chromogranin A; hemorrhagic stroke.

RESUMO

Introdução: *Doenças cerebrovasculares têm sido ligadas a diversos genes. A cromogranina A (*CHGA*) é utilizada como um marcador em doenças cardiovasculares. Portanto, avaliar o polimorfismo e verificar a associação com essa patologia é muito importante para melhor compreensão dessa doença.* ***Objetivo:*** *O foco do estudo foi identificar a associação entre o polimorfismo na região codante posição -264 do gene* CHGA *(*Glu264Asp*) e o acidente vascular encefálico hemorrágico (AVEH)/aneurisma no Distrito Federal, Brasil.* ***Métodos:*** *Estudo caso-controle de base populacional, envolvendo 45 casos com AVEH e/ou aneurisma. Para a genotipagem dessas amostras, utilizou-se a técnica laboratorial reação em cadeia da polimerase-polimorfismo de comprimento de fragmento de limitação (PCR-RFLP). Nível de significância de 5% foi adotado.* ***Resultados:*** *A ausência do genótipo CC do polimorfismo* Glu264Asp CHGA *nos participantes do estudo e a presença significativa do genótipo heterozigoto GC foram verificadas. No entanto, a distribuição dos genótipos não diferiu estatisticamente nos grupos.* ***Conclusão:*** *O polimorfismo* Glu264Asp CHGA *parece não contribuir para a gênese da expressão da proteína* CHGA *nesse grupo de pacientes, mas revela se existe ou não uma possível associação da patologia em questão e se a mutação contribuirá para a terapia gênica e melhorará a qualidade de vida dos pacientes.*

Unitermos: *polimorfismo genético; cromogranina A; acidente vascular cerebral.*

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RESUMEN

Introducción: *Enfermedades cerebrovasculares han sido vinculadas a diversos genes. La cromogranina A (CgA) es utilizada como un marcador en enfermedades cardiovasculares. Por consiguiente, evaluar el polimorfismo y verificar la asociación con esa patología es muy importante para la mejor comprensión de la enfermedad.* ***Objetivo:*** *El enfoque del ensayo fue identificar la asociación entre el polimorfismo en la región codificante en la posición -264 del gen* CHGA *(*Glu264Asp*) y el accidente cerebrovascular hemorrágico (ACVH)/aneurisma en Distrito Federal, Brasil.* ***Métodos:*** *Estudio de caso-control de base poblacional, involucrando 45 casos con ACVH y/o aneurisma. Para el genotipaje de las muestras, se utilizó la técnica de laboratorio reacción en cadena de la polimerasa-polimorfismos en la longitud de los fragmentos de restricción (PCR-RFLP). Nivel de significación elegido: 5%.* ***Resultados:*** *Ausencia del genotipo CC del polimorfismo* Glu264Asp CHGA *en los participantes del ensayo y presencia significativa del genotipo heterocigoto GC. Sin embargo, la distribución de los genotipos no difirió estadísticamente en los grupos.* ***Conclusión:*** *El polimorfismo* Glu264Asp CHGA *parece no contribuir para la génesis de la expresión de la proteína CgA en este grupo de pacientes, pero revelar si existe o no una posible asociación de la patología en cuestión y si la mutación contribuirá para la terapia genética y mejorará la calidad de vida de los pacientes.*

Palabras clave: *polimorfismo genético; cromogranina A; accidente cerebrovascular.*

INTRODUCTION

Cerebrovascular accident (CVA), also known as stroke, consists of an acute neurological dysfunction of vascular origin, followed by sudden occurrence of signs and symptoms related to impairment of focal areas in the brain, where symptoms persist for more than 24 hours[1]. According to the Brazilian Society of Cerebrovascular Diseases, 2016, this pathology is the leading cause of death in Brazilians: each year, 17 million individuals have a stroke worldwide; 6.5 million die; and 26 million are permanently incapacitating[2].

Cerebral aneurysm can be defined as an abnormal arterial dilation at a point in the arterial wall of the circle of Willis. In the United States, 27,000 new cases of subarachnoid hemorrhage occur from aneurysm rupture, and 5% to 15% develop into hemorrhagic stroke (HS)[3, 4].

In general, stroke has as its main feature the lack of blood supply in a particular brain region, which may be caused by a blockage of an artery or when a blood vessel bursts. It is divided into two categories: ischemic and hemorrhagic, which have similarities, but differ in physiology, etiology and treatment[5].

The ischemic stroke (thrombosis) is characterized by interruption of blood flow in a particular brain region. This interruption usually occurs due to the presence of a thrombus, that is, an inappropriate clotting process in the artery or vein, with bacterial aggregates and inflammatory cells[6]. The HS is the result of vascular fragility that can lead to rupture of a vessel and consequently to blood leakage in brain regions[7]; it presents systemic arterial hypertension (SAH) as the main associated pathology[8].

Currently, the diagnosis of this pathology is based on patient's clinical history and the aid of computed tomography (CT). Symptoms may vary and are related to different areas of the brain. It is common that neurological disorders associated interfere with speech, vision, cognition, sensitivity, balance and movement[9]. As well as the various chronic non communicable diseases (NCDs) commonly found in the world population, the hemorrhagic stroke is a multifactorial disease that is related to a number of factors that influence its environmental and genetic development[10, 11]. Therefore, identifying the causes, with the help of some health technologies, is important to understand such pathology and to provide better quality of life to the patient, since this disease requires some physical dependence. This type of dependence generates social, emotional and economic changes, and isolation, causing neuropsychiatric disorders[12, 13].

Chromogranin A (CHGA) is an acid secreting glycoprotein located on chromosome 14q32.12. This glycoprotein has been used as a biomarker of tumors in neuroendocrine tissues, in addition to neurodegenerative and neuropsychiatric diseases, hypertension, cardiovascular diseases, and renal and liver failure[14]. Evaluating the polymorphism of any gene is a major challenge, especially in relation to the *CHGA* gene, as there are few studies that relate its mutation to any pathology. Among the different regions of this gene, the G/A base exchange in *CHGA-264* has been shown to be a decreasing factor in the expression of this protein.

Based on the above, the objectives of this study are to verify the frequency of *CHGA-264* G/A polymorphism in a Brazilian

population sample of individuals with HS and/or cerebral aneurysm, as well as to investigate a potential association between *CHGA* G/A polymorphism, in the coding region, with different clinical manifestations. Additionally, to analyze this association in the prognosis of patients in relation to the pathology studied.

METHODS

Composition

The study of 97 patients was conducted at the Faculdade de Ceilândia, Universidade de Brasília (UnB), Brazil. The samples that comprise this case-control study were obtained at the Base Hospital for two years (January 2011 to December 2012). A total of 45 individuals diagnosed with HS and/or cerebral aneurysm (32 women and 13 men; mean age 52 ± 1 year) were recruited for this study. All patients presented the clinical signs described in the World Health Organization (WHO) definition of stroke; these signals were also confirmed by imaging [CT or magnetic resonance imaging (MRI)].

The control group consisted of 52 individuals (31 women and 21 men; mean age 51 ± 1 year) considered healthy and grouped by age and sex. All were volunteers who accompanied patients at the general outpatient clinic. The sample was calculated by estimating a 2% prevalence of HS/aneurysm in the adult population; 5% sampling error; 95% confidence interval (CI) in the 112 patients evaluated according to the criteria described above. A total of 24 participants were included. We considered the sample of 45 patients with HS to compensate for any loss.

The consent form was obtained from all participants before collecting the information. The Institutional Ethics Committee approved the study. Participants younger than 18 years with no diagnosis of HS and/or aneurysm, or those who presented any kind of blood relationship to the control group were excluded.

Clinical features

Detailed history and clinical evolution were tracked. Patients were asked if they had hypertension (blood pressure was measured) or diabetes, if they were smokers and consume alcohol. Scales were used to measure severity, disease stages and disability of patients: Rankin Scale (RS) to check motor functional capacity; Barthel index (BI) for mobility and personal care; and Glasgow Scale to evaluate patient's level of consciousness. In addition, biochemical tests, such as glucose, creatinine and platelets, were obtained through the patient's clinical records.

Laboratory methods

Each individual in this study, case and control, provided a 5 ml sample of venous blood. Deoxyribonucleic acid (DNA) was extracted from the blood collected using Invisorb Spin Blood Mini Kit (250) of the Invitek (catalog #CA10-0005, lot #1031100300). *CHGA-264* G/A polymorphism genotyping was performed using the polymerase chain reaction (PCR) technique combined with the restriction analysis of the fragment studied in the polymorphism. The primers used for *CHGA-264* G/A amplification were 5'-AGGGTGGCAGGCAAAGAG-3' sense and 5'-AAGGTGGAATGAGGTTATGG-3' antisense. Amplification was performed based on the following thermocycling conditions: 94ºC for 5 minutes (initial denaturation), followed by 30 cycles of denaturation at 94ºC for 1 minute, followed by 56ºC for 1 minute and 72ºC for 1 minute for primers annealing. Finally, the extension process was performed at 72ºC for 7 minutes. Two hundred thirty-five pb products of PCR were incubated for 90 minutes at 37ºC with the DPN1 enzyme. Allele G was not cleaved by the enzyme, thus yielding a 235 pb fragment. Allele A presented two DNA fragments, one 106 pb and one 129 pb. The fragments were visualized on 3% agarose gel with ethidium bromide and exposed to ultraviolet light.

Statistical analysis

The frequency of genotypes and alleles in patients with HS and/or aneurysm was compared with the control group using the Chi-square test in the recessive and dominant models. The Armitage test was performed to check for dosage effects on the risk allele for HS/aneurysm. The association with clinical characteristics and each genotype was analyzed by Chi-square, adopting a significance level of 5%. To analyze the difference in the mean of the laboratory exams in the different genotypes, a Student's *t* test was performed, since normality assumptions were observed.

RESULTS

In **Table 1**, genotypic frequencies were described. The absence of CC genotype in both groups was observed, but a high prevalence of heterozygous individuals (GC) in the sample was verified. Thus, it was observed that there was no significant association between the pathology studied with the *Glu264Asp CHGA* mutation. Regarding the association between the presence of the polymorphic allele and the HS, no relationship was verified either ($p > 0.05$).

TABLE 1 – Distribution of genotype and allele frequencies of *Glu264Asp CHGA* polymorphism in the different groups

Glu264Asp CHGA	Group						
	HS/aneurysm		Control				
	n	%	*n*	%	*p*	OR	CI
GG	10	22.2	9	17.3			
GC	35	77.8	43	82.7	0.543	1.37	0.5-3.73
CC	0	0	0	0			
Total	45	100	52	100			
G	55	61.1	61	58.7			
C	35	38.9	43	41.3	0.729	1.11	0.62-1.97
Total	90	100	104	100			

CHGA: chromogranin A; HS: hemorrhagic stroke; OR: odds ratio; CI: confidence interval.

Tables 2 and **3** describe the associations between the clinical laboratory characteristics and the prognosis of patients with the polymorphism studied. No association of mutation with the study variables was verified ($p > 0.05$).

TABLE 2 – Association between *Glu264Asp CHGA* polymorphism and clinical features/habits and patient prognosis measured on scales

Clinical features		*Glu264Asp CHGA*				
		GG		GC		
		n	%	*n*	%	*p*
SH	Yes	8	22.9	27	77.1	0.848
	No	2	20	8	80	
Diabetes	Yes	0	0	3	100	0.338
	No	10	23.8	32	76.2	
Smoking	Yes	3	20	12	80	0.8
	No	7	23.3	23	76.7	
Alcoholism	Yes	1	14.3	6	85.7	0.583
	No	9	23.7	29	76.3	
Glasgow	Intermediate coma	2	33.3	4	66.7	0.482
	Superficial coma	0	0	0	0	
	Normality	8	20.5	31	79.5	
Rankin	Asymptomatic	0	0	2	100	0.864
	Symptom with no disability	8	23.5	26	76.5	
	Slight disability	0	0	1	100	
	Moderate disability	0	0	2	100	
	Moderately severe disability	1	33.3	2	66.7	
	Severe disability	1	33.3	2	66.7	
Barthel index	Severe disability	2	33.3	4	66.7	0.371
	Moderate disability	0	0	0	0	
	Slight disability	7	25.9	20	74.1	
	Functional independence	1	8.3	11	91.7	

SH: systemic hypertension.

TABLE 3 – Summary statistics of blood glucose, creatinine and platelets according to the presence of *Glu264Asp CHGA* polymorphism in case group patients

Laboratory Variables	*Glu264Asp CHGA*						
	GG		GC		Total		
	Mean	Standard error of the mean	Mean	Standard error of the mean	Mean	Standard error of the mean	*p*
Glucose	112	9	109	4	110	4	0.79
Creatinine	0.9	0.1	1.4	0.4	1.3	0.3	0.439
Platelets	321	17	339	12	335	10	0.455

CHGA: chromogranin A.

DISCUSSION

Stroke is a disorder of great social impact that causes a neurological deficit of vascular origin; affects adults and the elderly. This disease is the leading cause of death in Brazil and is the second leading cause worldwide, right after the ischemic heart disease[15, 16]. In this study, the incidence of women (71.1%) – age approximately 52 years – was higher than that of men (28.9%) in the HS and/or aneurysm group.

Genetic research involving *CHGA* polymorphisms associated with some diseases has already been described in the literature. In this study, an analysis of the association between the presence of the *Glu264Asp CHGA* polymorphism and patients with HS and/or aneurysm who were undergoing treatment at a health facility in the Federal District, Brazil, was performed.

Genetic variation studies indicate that of all stroke cases, about 38% are hereditary. They also state that most of the genetic contribution to stroke is polygenic and reflects effects of multiple genes, each of them exerting small effects[17]. Regarding HS, few literature reviews have been performed, when compared with the ischemic outcome of the disease.

CHGA is part of a group of proteins present in various neuroendocrine tissues; it has been considered as a marker for several pathologies, such as hypertension, neuropsychiatric diseases, renal and hepatic failure[14, 18, 19]. In addition, this protein stands out in the heart failure (HF) marker group because it is included as part of the mechanism of neuroendocrine dysfunction. Braunwald Classification of Biomarkers for HF, grouped into seven categories, identifies chromogranins as biomarkers for prognostic and cardiovascular risk assessment[20]. Thus, studies on the *CHGA* genetic polymorphism may contribute to a better understanding of the pathogenesis and prognosis of cardiovascular diseases, such as HS. However, this study did not indicate any association of *CHGA-264* G/A polymorphism with the occurrence of HS and/or aneurysm. Few studies have identified this polymorphism in a group of individuals. The study

proposed by Salem *et al.* (2008)[21] identified that polymorphisms in the *CHGA* gene were associated with the presence of hypertensive kidney disease, but did not observe the relevance of *Glu264Asp CHGA* polymorphism for the pathogenesis of the disease[21].

Another study, by Kogawa *et al.* (2016)[22], found that the polymorphism was not associated with the presence of diabetes, the same was observed in the present study, when it was aimed to investigate this association between patients with diabetes in the case group and those in the control group.

Regarding the prognosis, in this study it was also not found that *CHGA* polymorphism was associated with patient profile measured by neurological assessment scales. It can be observed that HS and/or aneurysm is a broad-spectrum disease in the population. However, as a limitation of this study, the sample size was very small when considering the magnitude of the pathology in question. Therefore, further research on specific populations should be performed to help understanding the disease associated with the genetic mutation, as Brazil is represented by a group of miscegenated individuals.

Understanding the genes responsible for normal or pathological traits allows us to apply the foundations of genomic medicine that will modify gene therapy. The principle of this technology involves the application of genes responsible for proteins that may be beneficial to the individual in the patient with genetic diseases, i.e., the application of a normal gene may reverse the clinical condition of the carrier. This procedure could contribute to the improvement of the patients' quality of life and possibly to reduce the incidence rates, not only for this pathology, but also for several others[23].

CONCLUSION

The *Glu264Asp CHGA* polymorphism does not seem to contribute to the genesis of CHGA protein expression in this group of patients, so it is not the ideal basis for understanding the reasons why this protein is a HS biomarker.

Understanding the genetic mutations of this pathology contributes to the development of a gene therapy, a technology of great importance to health. Therefore, further studies should be carried out to improve the understanding of the disease and improve quality of life for patients. This study showed no association, but checking for potential association in populations from different countries would be an interesting investigation.

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