Evaluation of the TP53 Arg72Pro gene polymorphism and its relationship with hemorrhagic stroke in Brazilian patients

Avaliação do polimorfismo do gene TP53 Arg72Pro e sua Relação com Acidente Vascular Encefálico Hemorrágico em Pacientes Brasileiros

Evaluación del polimorfismo del gen TP53 Arg72Pro y su relación con el accidente cerebrovascular hemorrágico en pacientes brasileños.

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RESUMO

Objetivo: investigar a associação entre o polimorfismo TP53 Arg72Pro em pacientes diagnosticados com acidente vascular encefálico hemorrágico (AVEH) ou aneurisma intracerebral em uma amostra do Distrito Federal**. Método:** Tratou-se de um estudo caso-controle, com 162 indivíduos equitativamente divididos nos grupos, com anotações das características clínicas do prontuário e análise da genotipagem por meio da estratégia de PCR. As frequências genotípicas foram estimadas por contagem direta. O nível de significância adotado foi de 5%. **Resultados:** Foi verificado que a presença do alelo Arg do polimorfismo do TP53 Arg72Pro atuou como fator do risco para a ocorrência do AVEH/aneurisma intracerebral. A presença do genótipo Arg/Arg aumentou o risco para o prognóstico ruim (ERM >3) em pacientes portadores do AVEH/aneurisma (P<0,01; OR= 6,07). Não houve associação estatística entre HAS, diabetes, tabagismo e etilismo e a presença do polimorfismo TP53 Arg72Pro no grupo estudado**. Conclusão:** Concluiu-se que a presença do alelo Arg do polimorfismo do TP53 Arg72Pro está associada ao aumento do risco de ocorrência do AVEH/aneurisma intracerebral.

Descritores: Genes p53; Acidente Vascular Cerebral; Polimorfismo Genético.

ABSTRACT

Objective: to investigate the association between the TP53 Arg72Pro polymorphism in patients diagnosed with hemorrhagic stroke (HS) or intracerebral aneurysm in a sample from Distrito Federal. **Method:** This was a case-control study, with 162 individuals equally divided into groups, with notes on the clinical characteristics of the medical record and analysis of genotyping using the PCR strategy. Genotype frequencies were estimated by direct counting. The level of significance adopted was 5%.**Results:** we found that the presence of the Arg allele of the TP53 Arg72Pro polymorphism acted as a risk factor for the occurrence of HS/intracerebral aneurysm. The presence of the Arg/Arg genotype increased the risk for poor prognosis (ERM> 3) in patients with HS/aneurysm (P <0.01; OR = 6.07). There was no statistical association between SAH, diabetes, smoking and alcohol consumption and the presence of the TP53 Arg72Pro polymorphism in the studied group. **Conclusion:** It was concluded that the presence of the Arg allele of the TP53 Arg72Pro polymorphism is associated with an increased risk of occurring HS/intracerebral aneurysm.

Descriptors: Genes p53; Stroke; Polymorphism, Genetic.

RESUMEN

Objetivo: investigar la asociación entre el polimorfismo TP53 Arg72Pro en pacientes diagnosticados de accidente cerebrovascular hemorrágico (AVEH) o aneurisma intracerebral en una muestra del Distrito Federal. **Método:** Este fue un estudio de casos y controles, con 162 individuos igualmente divididos en grupos, con notas sobre las características clínicas de la historia clínica y el análisis de genotipado utilizando la estrategia de PCR. Las frecuencias de genotipo se estimaron por conteo directo. El nivel de significación adoptado fue del 5%. **Resultados:** Se encontró que la presencia del alelo Arg del polimorfismo TP53 Arg72Pro actuó como un factor de riesgo para la aparición de AVEH/aneurisma intracerebral. La presencia del genotipo Arg / Arg aumentó el riesgo de mal pronóstico (ERM> 3) en pacientes con AVEH/aneurisma (P <0.01; OR = 6.07). No hubo asociación estadística entre SAH, diabetes, tabaquismo y consumo de alcohol y la presencia del polimorfismo TP53 Arg72Pro en el grupo estudiado. **Conclusión:** se concluyó que la presencia del alelo Arg del polimorfismo TP53 Arg72Pro se asocia con un mayor riesgo de aparición de AVEH/aneurisma intracerebral.

Descriptores: Genes p53; Accidente Cerebrovascular; Polimorfismo Genético.

ORIGINAL

Introduction

One of the main causes of mortality today is chronic non-communicable diseases (NCDs), which are responsible for approximately 70% of all deaths. Among the most prevalent NCDs, neoplasms, diabetes and cardiovascular diseases stand out.[1] Among these diseases, the cerebrovascular accident (CA), which according to WHO is responsible for a significant number of hospitalizations and mortality, in addition to the great potential to cause permanent injuries and invalidate.[2]

Cerebrovascular accident can be defined as the interruption of blood flow in a certain brain region, which persists for at least 24 hours and causes a sudden loss of neurological function. Some visible signs are changes in the level of consciousness and impairment of sensory, motor, cognitive, perceptual and language functions.[3] Cerebrovascular accident can be divided into ischemic, when occlusion of an artery due to atheroma or emboli occurs[4], and hemorrhagic, when there is intraparaquentous hemorrhage or subarachnoid hemorrhage.[5] Some evidence suggests that genetic variations may be associated with cerebrovascular accident susceptibility and prognosis.[6]

The tumor suppressor gene TP53 is found in position 1.3 of the short arm of chromosome 17, consisting of 12 exons. This gene is responsible for encoding the transcription factor p53, which has antitumor property, as it participates in processes that mediate apoptosis.[7] The functional results after stroke can be explained by the presence of apoptotic neurons both in the ischemic penumbra and in the perihematoma area, indicating a late apoptotic neuronal death.[8-9]

The most studied polymorphisms in this gene are of the SNP type (single nucleotide polymorphism). Some studies have shown that the Arg72Pro polymorphism is related to different cerebrovascular accident prognosis.[10] There are data that indicate the association of the TP53 gene with neuroprotection after ischemia[11], probably because the transcription factor p53 would be shifted from cortisol to mitochondria in response to the ischemic stimulus.[10] However, the participation of genetic variations of TP53 in Hemorrhagic Stroke (HS) is still unknown, there are few studies evaluating the association of polymorphisms of this gene with HS.

In this context, this study aims to assess the association of the TP72 gene Arg72Pro polymorphism in individuals with HS. This research was approved by the research ethics committee of the State Health Department of the Federal District, opinion number: 0095/2010.

Method

This is an observational, prospective, case-control study in patients with a diagnosis of stroke or intracerebral aneurysm and in individuals without a record of the disease, all of whom live in the Federal District (Brazil). The personal data of the research participants were obtained by completing a specific identification form. The identification form made it possible to characterize the participants according to age, sex, presence of hypertension, diabetes, smoking, alcoholism, glycemia, creatinine, urea and platelet levels, score on the Modified Rankin Scale (MRS).

The case group had as inclusion criteria, patients: male and female; older than 18 years; with a diagnosis of stroke or intracerebral aneurysm with a confirmed diagnosis using computed tomography.

For the control group, the inclusion criteria for individuals were: both sexes; older than 18 years; without stroke and intracerebral aneurysm; unrelated to the patients in the case group. It was also considered as an inclusion criterion those individuals who underwent biochemical blood tests (serum glucose) less than 15 days ago and had a formal record of this result.

In total, 162 individuals were analyzed, with the case group consisting of 81 patients with stroke and / or intracerebral aneurysm with a mean age of 53.5 ± 5.9 years (48 women and 33 men). The control group with no history of stroke and / or intracerebral aneurysm was composed of 81 people included, with a mean age of 52.3 ± 5.7 (43 women and 38 men).

Approximately 5 mL of venous blood were collected by means of peripheral vein puncture. The DNA was extracted from peripheral blood using the Invisorb Spin Blood Mini Kit (250) from Invitek (catalog # CA10-0005, lot # 1031100300, Germany). The average concentration of the obtained DNA was estimated by the spectrophotometer (NanoDrop 2000 / 2000c - Thermo Fischer Scientific). The average concentration achieved was 20 ng / µL.

Genotyping was performed using the qualitative PCR technique. The following oligonucleotide sequences were used:

Foward 5`-TCCCCCTTGCCGTCCCAA -3`
Reverse 5`-CGTGCAAGTCACAGACTT -3`

The thermocycling conditions were 94˚C for 2 minutes (initial denaturation), followed by 35 cycles of denaturation at 94˚C for 30 seconds, followed by 60˚C for 45 seconds, for the annealing of oligonucleotides and 72˚C for 30 seconds for the length of the fragments. The final extension was performed at 72˚C for 10 minutes. The equipment used was a Thermocycler Techne model TC-512.

In each reaction, 4.0 µL of genomic DNA was used at a concentration of 2.5 ng / µL; 2.5 µL of 10x buffer (10 mM Tris and 50 mM KCl); 0.5 µL of MgCl2 (Ferments), 0.5 µL of dNTPs (2.5mM; LGC); 0.5 µL of Taq Polymerase (Fermentas, 5U / µL); 1.5µL of each oligonucleotideofoward and reverse (10 µM); completing with Milli-Q water to a final volume of 25 µL per reaction. The product of this PCR is a 279 bp fragment.

PCR products were subjected to enzymatic digestion with BstUI enzyme (New EnglandBiolabs, Inc. Beverly, MA, USA). Allele 1 (G) creates a new restriction site, and the 279 bp fragment is cleaved into two 160 bp and 119 bp; and allele 2 (C) is not cleaved by the enzyme. To assemble the digestion system, 10.0 µL of the PCR were used; 2.0µL of 10x NEB4 buffer (Biolabs); 1 µL of BstUI enzyme (10U / µL), making up with Milli-Q water for a final volume of 20 µL per reaction. The system was maintained at 60˚C for 2 hours.

The digestion products were submitted to an electrophoretic run in a 3% agarose gel, with ethidium bromide at a power of 100W for 20 minutes.

Genotypic frequencies were counted using direct counting, using the SPSS version 20.0 program. The comparison of the distributions of these frequencies was done through the application of the chi-square tests, in order to detect

possible associations of the genotypes between the groups evaluated, case group and control group. Associations were considered statistically significant when those p-values were less than 5% ($p < 0.05$).

The frequencies of certain clinical characteristics of the study subjects were estimated, considering sex and the quantitative age variables described in terms of their summary statistics (mean and standard deviation). Subsequently, the clinical characteristics of the control group were described statistically, according to the modified Rankin Scale.

For the aforementioned variables, the comparison of frequency distributions was performed using the Chi-square and OddsRatio (OR) test. Associations were considered statistically significant when those p-values were less than 5% ($p < 0.05$).

Results

The characteristics of the individuals in the study are described in Table 1, in which they were grouped and analyzed according to sex and age, with no statistical difference in their percentage distribution between the two groups (P = 0.429 and 0.185, respectively). We observed a higher prevalence of females among patients with Aneurysms/HS (59.3%) and in the control group (53.1%).

Table 1- Distribution of research participants according to sex and age in the Aneurysm/HS and control groups.

		Aneurysm/HS		Control		
Variable*		**N**	**%**	**N**	**%**	**P**
Sex	Female	48	59,3%	43	53,1%	0,429
	Male	33	40,7%	38	46,9%	
	Total	81	100,0	81	100,0	
Age		Mean	Standard Deviation	Mean	Standard Deviation	
		53,49	5,94	52,27	5,75	0,185

*Student T test

In Table 2, we observed that the frequency of the genotypes showed a statistically significant difference between the case group and the control group (P <0.001). The Arg / Arg genotype was found in 39.5% of the patients in the control group (n = 32) and in 13.6% of the case group (n = 11). Allele frequencies were statistically different between the HS/aneurysm and control groups (Arg allele: 64 versus 104 respectively, P <0.01; OR = 0.36). Thus, the presence of the Arg allele acted as a protective factor for the stroke and aneurysm occurrence. The control group was in Hardy-Weinberg equilibrium (P = 0.504).

Table 2- Study of the genotypic and allelic association of the TP53 Arg72Pro polymorphism in the case-control study.

TP53Arg72Pro	Groups						P	OR (CI 95%)
	Aneurysm/HS		Control		Total			
	N	%	N	%	N	%		
Arg/Arg	11	13.6	32	39.5	43	26.5	<0.01*	
Arg/Pro	42	51.9	40	49.4	82	50.6	<0.01*	NA
Pro/Pro	28	34.6	9	11.1	37	22.8		
Total	81	100	81	100	162	100		
Arg/Arg	11	13.6	32	39.5	43	26.5		
Arg/Pro + Pro/Pro	70	86.4	49	60.5	119	73.5	<0.01*	0.24 (0.11-0.52)
Total	81	100	81	100	162	100		
Arg	64	39.5	104	64.2	168	51.9		
Pro	9	60.5	58	35.8	156	48.1	<0.001*	0.36 (0.23-0.57)
Total	162	100	162	100	324	100		

NA: not applicable

It was possible to observe that the presence of the Arg / Arg genotype increases the risk for a poor prognosis (ERM> 3) in patients with Aneurysm / AVEH (P = 0.026; OR = 6.09), as shown in Table 3.

There was no statistically significant association between the presence of the TP53 Arg72Pro polymorphism and the patients' clinical characteristics.

Table 3- Association between TP53 Arg72Pro polymorphism and prognosis according to the modified Rankin Scale related to patients in the case group.

		TP53 Arg72Pro					
		Arg/Arg		Arg/Pro + Pro/Pro			
		N	%	N	%	P	OR (CI95%)
ERM	Poor prognosis	4	36.4%	6	8.6%	0.026	6.09 (1.38-26.95)
	Good prognosis	7	63.6%	64	91.4%		

Chi-Square Tests; MRS: Modified Rankin Scale

Table 4- Association between TP53 Arg72Proe polymorphism and clinical characteristics / habits related to patients in each group.

Groups			TP53 Arg72Pro					
			Arg/Arg		Arg/Pro + Pro/Pro			
			Count	% of the column	Count	% of the column	P	OR (CI95%)*
Aneurysm /HS	SAH	yes	6	54.5	54	77.1	0.142	0.36 (0.96-1.32)
		no	5	45.5	16	22.9		
	Diabetes	yes	1	9.1	2	2.9	0.353	3.4 (0.28-41.03)
		no	10	90.9	68	97.1		
	Smoking	yes	3	27.3	29	41.4	0.513	0.53 (0.12-2.17)
		no	8	72.7	41	58.6		

Control	Alcoholism	yes	2	18.2	20	28.6	0.718	0.56 (0.11-2.80)
		no	9	81.8	50	71.4		
	SAH	yes	4	12.5	4	8.2	0.706	1.6 (0.37-6.94)
		no	28	87.5	45	91.8		
	Diabetes	yes	0	0	0	0	NA	NA
		no	32	100	49	100		
	Smoking	yes	11	34.4	13	26.5	0.407	1.45 (0.55-3.81)
		no	21	65.6	36	73.5		
	Alcoholism	yes	6	18.8	14	28.6	0.431	0.58 (0.20-1.71)
		no	26	81.3	35	71.4		

*Chi-Square Test; NA: not applicable; SAH- Systemic Arterial Hypertension

Discussion

Alkhalaf et al. Investigated the polymorphism in the TP53 codon 72 gene in association with atherosclerotic disease and diabetes. They performed an association analysis in relation to the homozygous genotypes Pro / Pro, Arg / Arg and heterozygous Arg / Pro. The results showed that there was no significant difference between the degree of expression in any of the genotypes and the diseases studied, nor a significant association between the frequency of the Pro and Arg allele and atherosclerotic disease.[12]

Gomes-Sanchez and collaborators showed that regardless of the origin (ischemic or hemorrhagic), the presence of the TP53 Arg72Pro polymorphism determines the functional outcome after the stroke. The TP53 Arg / Arg genotype being a genetic marker of poor functional prognosis, while the Pro / Pro has a good prognosis.[13]

This last report corroborates the results found in this study, in which it was demonstrated that the Tp53 Arg / Arg genotype is a genetic marker of poor functional prognosis after HS. These results suggest that the Tp53 Arg/Arg genotype can be considered as a genetic marker that predicts a weak functional outcome after stroke. When comparing all patients with stroke and aneurysm we found that the proportion of patients with poor functional outcome was always higher in Arg / Arg patients when compared to Arg/Pro or Pro/Pro.

However, it is worth mentioning that the Arg / Arg genotype is associated with a good prognosis in anticancer treatments and Tp53 Arg 72 protects cells against neoplastic development.[13]

It has been observed that the presence of the GG genotype increases the risk for poor prognosis (ERM> 3) in patients with HS/aneurysm. Since the Arg genotype is a more potent inducer of apoptosis, the TP53 Arg72Pro polymorphism contributes to a worse outcome in the prognosis of HS/ aneurysm. Studies demonstrate that the presence of this polymorphism in the stroke contributes to the worsening of the patient's clinical condition.[13]

Finally, effects on apoptosis are important to be evaluated in the pathogenesis of HS, given that neurons present in the ischemic penumbra for several hours or days can suffer the phenomenon of cell death, leading to the spread of brain damage, thus preventing apoptosis in penumbra may be one of the objectives to limit the volume of cerebral infarction after clinical stroke.[8]

Conclusion

The data gathered indicate that there is an association between the presence of the TP53 Arg72Pro gene polymorphism and the occurrence of HS/ aneurysm.

Regarding the studied clinical characteristics, no statistical association was found between the TP53 Arg72Pro polymorphism and these resources, which rules out the possibility of a study and confirms the role of the polymorphism as a risk factor for the occurrence of HS/aneurysm.

However, the evaluation of the prognosis using the modified Ranking Scale demonstrated that the presence of the Arg / Arg genotype constituted a risk factor for the poor prognosis of HS / aneurysm, contributing to highlight the inducing role of arginine and, consequently, the worsening in outcome of the HS/aneurysm.

Bearing in mind that p53 is an important regulator of metabolic pathways, understanding the relationship between the polymorphism of the TP53 gene and its contribution to the outcome of the HS / aneurysm is essential. In addition, future studies should be carried out in order to elucidate the association of HS/aneurysm with such polymorphism, in addition to checking the possibility of using the TP53 gene as a therapeutic target in the studied pathologies and, consequently, improving the patients' prognosis.

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References

1. Malta, D. C. *et al.* Noncommunicable diseases and the use of health services: analysis of the National Health Survey in Brazil. *Rev. Saude Publica* **51**, (2017).
2. World Health Organization. *Global status report on noncommunicable diseases 2014 - Global Target 7: Halth the rise in diabetes and obesity*. (2014).
3. Baggi Prieto Alvarez, R., Rodrigues Pires, E. & Caramêz, R. Acidente Vascular Encefálico. *Rev. Unilus Ensino e Pesqui.* **11**, 88–89 (2014).
4. Schuster, R. Evaluation of the stroke patient ' s functionality Avaliação da funcionalidade de indivíduos acometidos por Acidente Vascular Encefálico. (2017).
5. Pontes-Neto, O. M. *et al.* Diretrizes para o manejo de pacientes com hemorragia intraparenquimatosa cerebral espont\ A¢nea. *Arq. Neuropsiquiatr.* **67**, 940–950 (2009).
6. Matarin, M., Singleton, A., Hardy, J. & Meschia, J. 102642^^The genetics of

ischaemic stroke. *J. Intern. Med.* **267**, 139 (2010).
7. Basu, S. & Murphy, M. E. Genetic Modifiers of the p53 Pathway. 1–14 (2016).
8. Broughton, B. R. S., Reutens, D. C. & Sobey, C. G. Apoptotic mechanisms after cerebral ischemia. *Stroke* **40**, (2009).
9. Qureshi, A. I. *et al.* Apoptosis as a form of cell death in intracerebral hemorrhage. *Neurosurgery* **52**, 1041–7; discussion 1047-8 (2003).
10. Gomez-Sanchez, J. C. *et al.* The human Tp53 Arg72Pro polymorphism explains different functional prognosis in stroke. *J. Exp. Med.* **208**, 429–437 (2011).
11. Ramos-Araque, M. E. *et al.* The Neuronal Ischemic Tolerance Is Conditioned by the Tp53 Arg72Pro Polymorphism. *Transl. Stroke Res.* **10**, 204–215 (2019).
12. Alkhalaf, M., Al-Bustan, S., Hamoda, H. & Abdella, N. Polymorphism of p53 gene codon 72 in Kuwaiti with coronary artery disease and diabetes. *Int. J. Cardiol.* **115**, 1–6 (2007).
13. Gomez-sanchez, J. C. *et al.* O polimorfismo humano TP53 Arg72Pro explica diferentes prognósticos funcionais no AVC. **72**, (2011).

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