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ORIGINAL ARTICLE

Endothelial nitric oxide synthase rs1799983 gene polymorphism is associated with the risk of developing intracranial aneurysm

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Abstract

Purpose The intracranial aneurysm (IA) rupture is associated with a subarachnoid hemorrhage. One third of patients die, and one third remain depend for daily activities. Genetic factors are crucial in the formation and clinical evolution of IAs. Multiple loci have been associated with AIs, much of them implicating multiple pathways related to vascular endothelial maintenance and extracellular matrix integrity. Thus, the aim of our study was to characterize whether polymorphisms in genes implicated in the vascular endothelial maintenance could modify the risk of developing IAs.

Subjects and methods We have studied 176 patients with IA recruited in the Service of Neurosurgery at the University Hospital of Valladolid (Spain) and a control group if 150 sex-matched healthy subjects. Clinical variables were collected from each patient. We have analyzed VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, endoglin rs3739817, and eNOS rs1799983 polymorphisms.

Results Our results showed that allele T of the eNOS rs1799983 polymorphism is correlated with decreased risk of developing the disease; thus, allele G of the eNOS rs1799983 polymorphism increased the risk of developing IA.

Conclusion The association of eNOS rs1799983 polymorphism with the risk to suffer IA reinforces the hypothesis that genetic variants in eNOS gene could be crucial in the pathogenesis of IA.

Keywords Intracranial aneurysm · Aneurysmal subarachnoid hemorrhage · Endothelial nitric oxide synthase · eNOS · Polymorphism

Introduction

Intracranial aneurysm (IA) is a pathological dilatation of cerebral vasculature that involves specific structural changes in the arterial wall. The IA incidence is 0.33–5% of adults worldwide [1, 10]. The IA rupture is associated with aneurysmal subarachnoid hemorrhage (aSAH) [30, 20]. The mortality associated with aSAH is one-third to one-half of patients, and the disability rate is 30% among survivors. Unruptured IA could be detected through cranial imaging for trauma or alterative pathologies, or by screening of high-risk individuals [26, 21, 25].

Despite the prevalence and impact of IAs, their pathogenesis remains largely unknown. Epidemiological studies have already identified specific risk factors such as age, presence of medical comorbidities, hypertension, smoking, and alcohol intake [7, 6]. It has been reported that in addition to environmental risk factors, genetic factors could be crucial in the formation and clinical evolution of IAs [18,

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29]. It was estimated that in the 4.1–6.1% of cases of IA formation and rupture, there was a heritable contribution [36, 3] and that the rest of cases could be explained to gene mutation-environment interactions that lead to sporadic IA [13]. It has been reported that first-degree relatives of aSAH patients have sevenfold increased risk of suffering IA in the general population [13, 5, 14]. Moreover, Broderick et al. established that there is 17 times more risk to aneurysm rupture in a familial IA patient than that in a patient with sporadic IA (matching for aneurysm size and location) [4]. Multiple loci have been associated with the pathogenies of AIs, much of them implicating multiple pathways related to vascular endothelial maintenance and extracellular matrix integrity [2]. Thus, the aim of our study was to characterize whether polymorphisms in genes implicated in the vascular endothelial maintenance could modify the risk of developing IAs. Therefore, in this study, we have analyzed the vascular endothelial growth factor A (VEGFA) rs833061, vascular endothelial growth factor receptor 2 (VEGFR2) rs2071559, endothelin rs5370, endoglin rs3739817, and endothelial nitric oxide synthase (eNOS) rs1799983 polymorphisms to evaluate the role of these genetic variants in modulating the formation and clinical evolution of IAs.

Subjects and methods

Subjects

We have studied 176 patients with IA recruited in the Service of Neurosurgery at the University Hospital of Valladolid (Spain) between 2015 and 2019. As a control group, 150 sex-matched healthy subjects over 40 years old were recruited, and cerebral angiography is not executed in this group by ethical reasons. Smokers and subjects with hypertension or family background of IA were excluded from the control group. Clinical variables such as gender, age at diagnosis, IA risk factors (hypertension, tobacco, alcohol, diabetes mellitus, and dyslipidemia), familiar history, diagnosis status (ruptured/unruptured), number of aneurysms, aneurysm location, Hunt and Hess score [15], Fisher score [8], aneurysm size, vascular territory involved, treatment, and evolution [11] (GOSe compressed in death (grade 1), severe disability (grades 2, 3, and 4), moderate disability (grades 5 and 6), and good recovery (grades 7 and 8)) were collected from each patient. In the familial cases, we have included only one affected patient of each family.

DNA insolation and polymorphism genotyping

Genomic DNA was extracted from peripheral blood leukocytes by standard phenol/chloroform procedure [9]. Genotyping of polymorphisms included in the study (Table 1) was performed using TaqMan 5′-exonuclease allelic discrimination assays that contain sequence-specific forward and reverse primers to amplify the polymorphic sequences and two probes labeled with VIC and FAM dyes to detect both alleles of each polymorphism. PCR reactions were carried out using TaqMan universal PCR Maxter Mix following instructions in a Step-One Plus Real-time PCR system [28]. To assess reproducibility, a random selection of 5% of the samples were re-genotyped; all these genotypes matched with the genotypes initially designated. We have selected VEGFA, VEGFR2, endothelin, endoglin, and eNOS genes because they code for proteins that are crucial in the vascular endothelial maintenance and extracellular matrix integrity. Initially, we selected those non-synonym polymorphisms with a population frequency of the minor allele higher than 10% in Caucasian population and located in sequences highly conserved throughout the evolution. This was the case of endothelin rs5370, endoglin rs3739817, and eNOS rs1799983 polymorphisms; they are missense mutations. VEGFA rs833061 and VEGFR2 rs2071559 are consequence of an intronic mutations, but we selected these polymorphisms because they were previously reported in the literature, the frequency of the minor allele in Caucasians was higher than 10%, and the polymorphisms involve changes in the recognition sites for transcription factors.

Statistical analysis

The healthy subject group was tested for conformity to the Hardy–Weinberg equilibrium using chi-squared test for each polymorphism. Odds ratios (ORs) and 95% confidence

Table 1 Polymorphisms analyzed in the study

Gene	SNP ID	Assay ID[a]	Change	Amino acid change	HWE[b]
VEGFA	rs833061	c_1647381_10	T > C	-	> 0.05
VEGFR2	rs2071559	c_15869271_10	T > C	-	> 0.05
Endothelin	rs5370	c_598677_1	G > T	K197N	> 0.05
Endoglin	rs3739817	c_27491008_10	C > T	T343T	> 0.05
eNOS	rs1799983	c_3219460_20	G > T	E298D	> 0.05

[a]All the assays were used commercially

[b]*HWE*, Hardy–Weinberg equilibrium in the control group

intervals (95% CIs) were estimated for each polymorphic variant using unconditional logistic regression models to evaluate the association with PDB risk. *p*-values were adjusted by sex and age at diagnosis. These statistical analyses were performed using SPSS software. For the analysis, differences with a p-value < 0.05 were considered as statistically significant.

Results

A total of 176 patients with IA and 150 healthy subjects were analyzed. The clinical variables of the patients are summarized in Table 2. The distribution of genotypes of VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, endoglin rs3739817, and eNOS rs1799983 polymorphisms in control samples was in Hardy–Weinberg equilibrium (Table 1).

The genotypic frequencies of the polymorphisms included in the study and the results of the association analysis between patients with IA and healthy subjects are summarized in Table 3. No significant differences were found in genotypic distribution for VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, and endoglin rs3739817 polymorphisms between patients and healthy subjects. However, we found statistically significant differences in genotypic distribution for eNOS rs1799983 polymorphism between patients with IA and healthy subjects. Being a carrier of the TT genotype of the eNOS rs1799983 polymorphism was associated with decreased risk of developing cerebral aneurysm (Table 3). No significant differences were found in the allelic distribution for VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, and endoglin rs3739817 polymorphisms between patients and healthy subjects (Table 4). We found statistically significant differences in allelic distribution for eNOS rs1799983 polymorphism. Allele T of the eNOS rs1799983 polymorphism confers a decreased risk of developing the disease. Thus, allele G of the eNOS rs1799983 polymorphism increased the risk of developing IA (Table 4). No significant differences were found in the analysis of the different clinical forms, clinical evolution and the genotypic distributions of VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, endoglin rs3739817, and eNOS rs1799983 polymorphisms in our cohort of IA patients.

Discussion

The IA pathogenesis remains largely unidentified. It is known that in addition to environmental and clinical risk factors, genetic factors could be crucial in the formation and clinical evolution of IAs [18], and multiple loci have been associated with the pathogenies of AIs, much of them

Table 2 Clinical characteristics of the patients included in the study

Clinical characteristics	Results
Male sex, *n* (%)	76 (43.2%)
Age at diagnosis, mean ± SD	58.16 ± 12.95
Cerebral aneurysm risk factors	
Hypertension, *n* (%)	123 (69.9%)
Tobacco, *n* (%)	72 (40.9%)
Alcohol, *n* (%)	44 (25%)
Diabetes mellitus, *n* (%)	17 (9.7%)
Dyslipidemia, *n* (%)	67 (38.1%)
Familiar history, *n* (%)	4 (2.3%)
Diagnosis	
Subarachnoid hemorrhage, *n* (%)	116 (65.9%)
Sentinel headache, *n* (%)	11 (6.3%)
Incidental, *n* (%)	49 (27.8%)
Number of aneurysms, mean ± SD	1.27 ± 0.63
Aneurysm location	
Internal carotid artery, *n* (%)	33 (18.8%)
Posterior communicating artery, *n* (%)	26 (14.8%)
Anterior communicating artery, *n* (%)	58 (33.0%)
Pericallosal artery, *n* (%)	5 (2.8%)
Anterior cerebral artery, *n* (%)	2 (1.1%)
Middle cerebral artery, *n* (%)	31 (17.6%)
Ophthalmic and choroid artery, *n* (%)	1 (0.6%)
Vertebral artery, *n* (%)	3 (1.7%)
Basilar artery, *n* (%)	12 (6.8%)
Posterior inferior cerebellar artery, *n* (%)	5 (2.8%)
Hunt and Hess score	
Grades I, *n* (%)	62 (35.22%)
Grade II, *n* (%)	50 (28.4%)
Grade III, *n* (%)	11 (6.25%)
Grade IV, *n* (%)	13 (7.38%)
Grade V, *n* (%)	40 (22.72%)
Fisher score	
Grades I, *n* (%)	0 (0%)
Grades II, *n* (%)	13 (7.38%)
Grade III, *n* (%)	44 (25%)
Grade IV, *n* (%)	119 (67.61%)
Aneurysm size	
< 3 mm, *n* (%)	14 (8%)
3–11 mm, *n* (%)	153 (86.9%)
11–25 mm, *n* (%)	5 (2.8%)
> 25 mm, *n* (%)	4 (2.3%)
Vascular territory involved	
Anterior, *n* (%)	156 (88.6%)
Posterior, *n* (%)	20 (11.4%)
Treatment	
Coils, *n* (%)	94 (53.4%)
Stent, *n* (%)	30 (17.0%)
Clip, *n* (%)	34 (19.3%)
None, *n* (%)	18 (10.2%)
Evolution	

Table 2 (continued)

Clinical characteristics	Results
Death (grade 1), *n* (%)	20 (11.4%)
Severe disability (grades 2, 3, and 4), *n* (%)	23 (13.06%)
Moderate disability (grades 5 and 6), *n* (%)	32 (18.18%)
Good recovery (grades 7 and 8), *n* (%)	101 (57.4%)

implicating multiple pathways related to vascular endothelial maintenance and extracellular matrix integrity [36, 2, 35]. Thus, the aim of our study was to characterize whether polymorphisms in genes implicated in the vascular endothelial maintenance could modify the risk of developing IAs in a Spanish patient sample. Therefore, we have analyzed the VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, endoglin rs3739817, and eNOSrs1799983 polymorphisms to evaluate the role of these genetic variants in modulating the formation and clinical evolution of IAs. It is important to note that the clinical aspects are crucial; for example, the branching site is more vulnerable to hemodynamic stress because of the deflection and oscillation of blood flow, and cerebral aneurysms occur preferentially at arterial bifurcations.

No significant differences were found in genotypic and allelic distributions in VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, and endoglin rs3739817 polymorphisms; nevertheless, these results do not exclude

Table 3 Genotypic frequencies of polymorphisms among patients and healthy subjects and the association with cerebral aneurysm risk (IA, intracranial aneurysm). *p*-values were adjusted by sex and age at diagnosis

SNP	Genotype	Controls	IA patients	*p*-value	OR (95%CI)
VEGFA rs833061	TT	41 (27.3%)	41 (23.3%)		
	TC	69 (46.0%)	93 (52.8%)	0.473	
	CC	40 (26.7%)	42 (23.9%)		
	TT	41 (27.3%)	41 (23.3%)	0.443	
	TC + CC	109 (72.7%)	135 (76.7%)		
	TT + TC	110 (73.3%)	134 (76.1%)	0.561	
	CC	40 (26.7%)	42 (23.9%)		
VEGFR2 rs2071559	TT	38 (25.3%)	36 (20.5%)		
	TC	82 (54.7%)	97 (55.1%)	0.462	
	CC	30 (20.0%)	43 (24.4%)		
	TT	38 (25.3%)	36 (20.5%)	0.353	
	TC + CC	112 (74.7%)	140 (79.5%)		
	TT + TC	120 (80.0%)	133 (75.6%)	0.339	
	CC	30 (20.0%)	43 (24.4%)		
Endothelin rs5370	GG	80 (53.3%)	107 (60.8%)		
	GT	57 (38.0%)	60 (34.1%)	0.266	
	TT	13 (8.7%)	9 (5.1%)		
	GG	80 (53.3%)	107 (60.8%)	0.175	
	GT + TT	70 (46.7%)	69 (39.2%)		
	GG + GT	137 (91.3%)	167 (94.9%)	0.202	
	TT	13 (8.7%)	9 (5.1%)		
Endoglin rs3739817	CC	134 (89.3%)	151 (85.8%)		
	CT	16 (10.7%)	25 (14.2%)	0.337	
	TT	0 (0%)	0 (0%)		
	CC	134 (89.3%)	151 (85.8%)	0.337	
	CT + TT	16 (10.7%)	25 (14.2%)		
	CC + CT	150 (100%)	176 (100%)	> 0.05	
	TT	0 (0%)	0 (0%)		
eNOS rs1799983	GG	55 (36.7%)	72 (40.9%)	/	1.00
	GT	59 (39.3%)	82 (46.6%)	0.809	1.06 (0.65–1.72)
	TT	36 (24.0%)	22 (12.5%)	0.019	0.46 (0.24–0.88)
	GG	55 (36.7%)	72 (40.9%)	0.494	
	GT + TT	95 (63.3%)	104 (59.1%)		
	GG + GT	114 (76.0%)	154 (87.5%)	/	1.00
	TT	36 (24.0%)	22 (12.5%)	0.008	0.42 (0.25–0.81)

Table 4 Allelic frequencies of polymorphisms among patients and healthy subjects and the association with cerebral aneurysm risk (IA, intracranial aneurysm). *p*-values were adjusted by sex and age at diagnosis

SNP	Allele	Controls	IA patients	*p*-value	OR (95%CI)
VEGFA rs833061	T	151 (50.3%)	175 (49.7%)	0.875	
	C	149 (49.7%)	177 (50.3%)		
VEGFR2 rs2071559	T	158 (52.7%)	169 (48.0%)	0.236	
	C	142 (47.3%)	183 (52.0%)		
Endothelin rs5370	G	217 (72.3%)	274 (77.8%)	0.104	
	T	83 (27.7%)	78 (22.2%)		
Endoglin rs3739817	C	284 (94.7%)	327 (92.9%)	0.354	
	T	16 (5.3%)	25 (7.1%)		
eNOS rs1799983	G	169 (56.3%)	226 (64.2%)	/	1.00
	T	131 (43.7%)	126 (35.8%)	0.004	0.71 (0.52–0.98)

an involvement for any of these genes in IA pathogenesis because we have only studied a selected polymorphism for each gene. Maderna et al. reported that aneurysm formation was associated with an alteration in the Vegf/Vegfr protein levels [17]. Endoglin protein was involved in the vascular development, and it had been reported the association between a polymorphism in endoglin gene with the rupture of IA among individuals of Chinese Han ethnicity [16]. Despite this, in our knowledge, this is the first time that VEGFA rs833061, VEGFR2 rs2071559, endothelin rs5370, and endoglin rs3739817 polymorphisms are studied in patients with IA.

Nitric oxide (NO) is mostly synthetized by the nitric oxide synthase (NOS) family enzymes [12]. There are three members of NOS family: neuronal (nNOS/NOS1), inducible (iNOS/NOS2), and endothelial (eNOS/NOS3). NO is involved in a large number of biological processes such as mediator in relaxing vascular smooth muscle, vasodilation maintenance of the structure of the vessel wall, and mediating the cell proliferation of the vascular smooth muscle, and it is also involucrated in the platelet and monocyte adhesion [23, 27, 19, 33]. Thus, it had been reported that NO-NOS pathway could participate in the pathogenesis of several vascular diseases such as carotid atherosclerosis, coronary vasospasms, hypertension, acute myocardial infarction, or aneurysm formation [22, 31] Our results showed statistically significant differences in genotypic and allelic distributions for eNOS rs1799983 polymorphism between patients with IA and healthy subjects. Being a carrier of allele G of the eNOS rs1799983 polymorphism was associated with an increased risk of developing IA. The rs1799983 polymorphism corresponds to G894T change in eNOS exon 7, and it encodes to a Glu > Asp amino acid change in the position 298. Wang et al. reported that rs1799983 polymorphism reduced eNOS enzyme activity [32]; therefore, it could be the cause of the increased risk of developing IA association. The hypothesis could be that the polymorphism modifies the NO synthesis; this could alter the vascular endothelial maintenance, and therefore, it could modify the risk of developing IA. Previously, the eNOS rs1799983 polymorphism has been already studied in the pathophysiology of IA; more specifically, it was been associated with the risk to aneurysm rupture and with the aSAH [34, 24]. In our study, it was associated with the risk of developing IA; the comparison between patients with aneurysm rupture and healthy subjects did not yield significant statistical association. Our work reinforces previously published that eNOS rs1799983 polymorphism was associated with the pathophysiology of IA. Our results did not show statistically significant differences between the genotypic and allelic distributions of the polymorphisms included in the study and the clinical characteristics of the patients.

The main strengths of our work are the cohort of patients drawn from a follow-up study; the functional polymorphism that has been associated in this study has a broad physiopathological base. However, one limitation of our study could be the size of the cohort; another limitation is the lack of the smoking and hypertension prevalence of the control group.

In conclusion, this report is showing the association of allele G of eNOS rs1799983 polymorphism with the risk to suffer IA, which reinforces the hypothesis that genetic variants in eNOS gene could be crucial in the pathogenesis of IA. In the future, more studies will be necessary in order to describe the specific role of eNOS enzyme in the cerebrovascular diseases and to define how genetic variants of eNOS gene could modulate the pathological process. The validation of our results could be of great clinical importance, as it will allow us to identify a new genetic risk factor associated to the formation and clinical evolution of IAs.

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Declarations

Ethics approval The experimental protocol was in accordance with the Declaration of Helsinki (2008) of the World Medical Association, approved by the University Hospital Clínico Hospital of Valladolid Ethics Committee (CINV 15–64) and in compliance with the Spanish data protection law (LO 15/1999) and specifications (RD 1720/2007). All who accepted to participate in the study signed a written consent.

Conflict of interest The authors declare no competing interests.

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