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Genetics

β2-Adrenergic Receptor Gene Polymorphisms and Risk of Ischemic Stroke

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Background: β2-adrenergic receptors (β2-AR) mediate vasorelaxation in response to adrenergic agents. Genetic polymorphisms of β2-AR were implicated in various cardiovascular and noncardiovascular traits.

Methods: We tested the role of the β2AR-16 and β2AR-27 gene variants in the susceptibility to the development of ischemic stroke in a genetically homogenous and clinically well-characterized case-control sample that included 294 cases and 286 controls from Sardinia, Italy. This population was shown to be an optimal study sample for carrying out genetic analyses.

Results: Age, hypertension, dyslipidemia, and atrial fibrillation were independent risk factors for stroke in this cohort. We found that the presence of the Glu27 allelic variant was associated with a significantly increased risk of stroke when assuming a recessive mode of inheritance (odds ratio [OR], 1.68; 95% confidence interval [CI], 1.17–2.41; $P = .005$). The same results were obtained for the subgroup of ischemic strokes of arterial origin ($n = 215$): OR, 1.71; 95% CI, 1.14–2.57; $P = .009$. Furthermore, haplotype analysis confirmed that the presence of the Glu27 allele increased the risk of cerebrovascular accidents.

Conclusions: Our data suggest that the Glu27 allelic variant of the β2-AR gene may be a determinant of ischemic stroke. Am J Hypertens 2007;20:657–662 © 2007 American Journal of Hypertension, Ltd.

Key Words: β2-adrenergic receptor, genetics, ischemic stroke, polymorphisms.

Genetic predisposition to common forms of stroke has been convincingly demonstrated over the last few years in a hypertensive animal model of stroke, as well as in humans.[1–6] However, the full dissection of stroke genes remains a complex and difficult task. Among other genes, the gene encoding β2-AR represents a potential candidate for ischemic stroke. This cell-surface receptor activates adenylyl cyclase by coupling to guanine nucleotide-binding proteins, is highly expressed throughout the cardiovascular system, mediates arterial vasodilation in response to adrenergic agents,[7] and stimulates both fibroblast and endothelial-cell proliferation.[8,9]

Human β2-AR is encoded by an intronless gene located on chromosome 5q31–32. Several polymorphisms were identified in the coding block of the β2-AR gene, resulting in amino-acid changes and alteration of the receptor function in vitro.[10] In particular, substitution of glycine for arginine at position 16 leads to enhanced agonist-induced desensitization, whereas substitution of glutamic acid for glutamine at position 27 associates with resistance to desensitization.[11] Recently, these polymorphisms were directly implicated, though with some controversy, in various cardiovascular and noncardiovascular traits, including obesity,[12–15] diabetes mellitus,[13,14] essential hypertension,[15–17] myocardial infarction,[18–20] congestive heart failure,[21] and asthma.[22] To date, only one study has investigated the relationship between β2-AR polymorphisms and the occurrence of ischemic stroke.[20]

We performed the present study to test the hypothesis that β2-AR gene variants might be associated with a modulatory effect on the risk of developing ischemic stroke in a genetically homogenous population from Sardinia, Italy.

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Materials and Methods

Study Population

The characteristics of our case-control study, including a description of selection criteria for both cases and controls, were previously described.[4,5] Briefly, patients were recruited at the Department of Neurology of the University of Sassari (Sassari, Sardinia, Italy). Of all consecutive patients with cerebrovascular events admitted between September 1998 and March 2003, we diagnosed 294 cases of ischemic stroke. The diagnosis of ischemic stroke was based on clear, unequivocal clinical parameters, with signs and symptoms persisting for more than 24 h, and confirmed by computed tomography scan or nuclear magnetic resonance imaging. Stroke subtypes were as follows: large-vessel disease (47%), small-artery disease (27%), embolic stroke (24%), and other origins and unknown (2%).

Unrelated control subjects ($n = 286$), drawn from the same geographical area and randomly selected among patients admitted to the same hospital, were included if they had vascular risk factors or a history of cardiovascular disease (myocardial infarction, previous coronary revascularization procedure, and peripheral vascular disease), but they were excluded if they had either current or previous cerebrovascular disease. Consanguineous subjects were excluded, based on an accurate and careful family history of each individual.

Body mass index (BMI) was defined as weight divided by height squared (kg/m^2). Hypertension was defined as present if subjects had been previously diagnosed according to World Health Organization/International Society of Hypertension guidelines and were routinely receiving antihypertensive therapy. Hypercholesterolemia was defined as a total cholesterol blood level >220 mg/dL. Smoking was categorized as either past (if subjects had stopped smoking >2 months before the study) or current. Alcohol consumption was defined as an intake of >30 g/day. The presence or absence of diabetes, and a history of myocardial infarction and peripheral arteriopathy, were recorded. The study protocol was approved by the local ethics committee, and all participants gave informed, written consent.

DNA Isolation and β2-AR Genotyping

Genomic DNA was isolated from peripheral blood, using a commercially available kit (Qiagen, Chicago, IL). β2-AR genotyping was performed by using standardized procedures.[12] Briefly, polymerase chain reactions were carried out with specific sets of oligo, processed on an MJR Thermocycler (PTC100; MJ Research, Waltham, MA), and finally subjected to digestion with the appropriate restriction enzyme: *Nco*I (New England Biolabs, Boston, MA) for the β2AR-16 polymorphism, and *Bbv*I (New England Biolabs) for the β2AR-27 polymorphism. The restriction digests were resolved on agarose gels and visualized by ethidium bromide staining and ultraviolet illumination.

Statistical Analysis

Age is reported as a median value with interquartile range (IQR). Body mass index is reported as mean ± SD. Differences in sex, BMI, and cardiovascular risk factors were assessed using the χ^2 test or Fisher's exact test, when necessary. Continuous variables were analyzed using the Wilcoxon rank-sum test, since the age distribution was skewed toward older ages.

Genotype and allele frequencies were computed for each locus, and their distribution in cases and controls was analyzed by χ^2 test with two degrees of freedom and one degree of freedom. Concordance to the frequency predicted by the Hardy-Weinberg equilibrium (HWE) was assessed by χ^2 test with one degree of freedom. The risk associated with each genotype in the occurrence of ischemic stroke was estimated by logistic regression analysis under the assumption of an additive effect (ie, fitting the three genotypes, assuming a one-step increase in odds per mutated allele) or codominant effect (ie, fitting the model

Table 1. Characteristics of study population

	Ischemic strokes ($n = 294$)	Controls ($n = 286$)	*P*
Median age in years (IQR)*	75 (68–83)	73 (62–79)	.0015
Sex, male	176 (59.9)	163 (57.0)	.483
Smoking	110 (40.6)	106 (38.4)	.601
Alcohol	31 (10.7)	33 (11.6)	.746
BMI (± SD)	27.6 ± 6.2	26.4 ± 5.6	.420
Obese subjects	59 (19.7)	50 (17.5)	.480
Overweight subjects	100 (34.0)	92 (32.2)	.630
Diabetes	69 (23.9)	80 (28.5)	.212
Hypercholesterolemia*	64 (22.2)	35 (12.5)	.002
Hypertension*	187 (65.2)	136 (48.6)	<.001
History of MI	40 (13.9)	41 (14.6)	.824
Atrial fibrillation*	74 (25.6)	43 (15.4)	.003
Peripheral arteriopathy	11 (3.9)	14 (5.0)	.516

BMI = body mass index; IQR = interquartile range; MI = myocardial infarction.
* Variables for which a significant difference was observed between cases and controls. Percentages are given in parentheses.

Table 2. Genotype frequencies of the β2-AR gene in cases and controls

Genotype	Frequency (%)	
	Cases	Controls
Arg16Gly		
Arg16Arg	45 (15.3)	39 (13.6)
Arg16Gly	113 (38.4)	120 (42.0)
Gly16Gly	136 (46.3)	127 (44.4)
HWE *P* value		.217
Gln27Glu		
Gln27Gln	82 (27.9)	97 (33.9)
Gln27Glu	89 (30.3)	103 (36.0)
Glu27Glu	123 (41.8)	86 (30.1)
HWE *P* value		<.001

with the three possible genotypes as two categories against a baseline wild type). Due to the selection of cases and controls with high cardiovascular risk, a multivariable logistic model was performed that included the genetic predictor and potential confounders. In particular, the multivariate model included variables that were significant ($P < .2$) in the univariate analysis or any potential confounder that changed the unadjusted odds ratio (OR) for genotype by >5% after adjustment.[23] Based on these criteria, age, atrial fibrillation, hypercholesterolemia, and hypertension were included in the model. Likelihood ratio tests were used to assess the significance of the model. In addition, the unadjusted OR and the variance were corrected to account for departures from HWE, as previously suggested.[24,25]

Haplotype frequencies were calculated using log-linear modeling and an expectation-maximization algorithm. Both crude and adjusted logistic regression analyses were carried out with haplotypes coded as categorical variables in the allele-based dataset.

Confidence intervals for ORs were calculated by using a profile likelihood approach. All calculations were considered significant at $P < .05$, with no adjustment for multiple testing. Analyses were carried out using Stata 9 (Stata Crop LD, College Station, TX).

Results

Risk factors for stroke in patients and controls are given in Table 1. Because the study recruited subjects from a neurological clinic, the age distributions in cases and controls were skewed toward higher ages.

Table 2 shows the genotype frequencies of β2-AR gene polymorphisms in cases and controls. Genotype frequencies in HWE were among controls at the Arg16Gly locus, but not at the Gln27Glu locus because of excess homozygosity.

The two single nucleotide polymorphisms (SNPs) were in linkage disequilibrium (D′, 0.54; $P < .001$; R^2, 0.14). Crude and adjusted analyses on the genotype and allele levels suggested that only the Gln27Glu locus had an effect on the odds of ischemic stroke when assuming either a recessive or an additive model of inheritance (Table 3). In particular, there was a significantly higher risk of ischemic stroke associated with the Glu27Glu genotype compared with the Gln27Gln genotype (crude OR, 1.69; 95% CI, 1.13–2.53; adjusted OR, 1.67; 95% CI, 1.09–2.55). After adjustment for deviations from HWE, the unadjusted odds of ischemic stroke when assuming a recessive model of inheritance were 2.40 (95% CI, 1.65–3.50).

To investigate this further, haplotypes were constructed. A logistic analysis showed that both haplotypes with a Glu27 allele conferred a significantly higher risk for ischemic stroke than did the Gly16Gln27 haplotype (OR, 1.36; 95% CI, 1.00–1.85; and OR, 1.67; 95% CI, 1.07–2.62, respectively; Table 4). However, after adjusting for hypercholesterolemia, hypertension, atrial fibrillation, and age, statistical significance was not attained (Table 4).

Finally, the analysis was repeated on cases of arterial origin only and their controls (215 cases and 236 controls). In this subgroup, crude and adjusted analyses showed that only the Gln27Glu locus had an effect on the risk of ischemic stroke when assuming either a recessive (adjusted OR, 1.71; 95% CI, 1.14–2.57; $P = .009$) or an additive (adjusted OR, 1.33; 95% CI, 1.05–1.69; $P = .02$) model of inheritance. Thus, subjects carrying the Glu27 allele had the highest risk.

Table 3. Risk of ischemic stroke in cases and in controls

	Unadjusted		Adjusted*	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
β2AR-16				
Recessive	1.08 (0.77–1.49)	.654	1.03 (0.73–1.46)	.860
Additive	1.00 (0.80–1.26)	.975	0.99 (0.78–1.26)	.943
Dominant	0.87 (0.55–1.39)	.568	0.91 (0.56–1.48)	.695
β2AR-27				
Recessive	1.67 (1.19–2.36)	.003	1.68 (1.17–2.41)	.005
Additive	1.31 (1.07–1.60)	.009	1.30 (1.05–1.61)	.016
Dominant	1.33 (0.93–1.89)	.117	1.29 (0.89–1.88)	.182

* Adjusted for hypercholesterolemia, hypertension, atrial fibrillation, and age.

Table 4. Haplotype frequencies of the β2-AR gene in cases and controls, and crude and adjusted* odds ratios

	Frequency (%)		Odds ratios (95% CI)	
Haplotype	**Cases**	**Controls**	**Crude**	**Adjusted***
Arg16Gln27	118 (20.0)	139 (24.3)	1.00 (reference)	1.00 (reference)
Gly16Gln27	135 (23.0)	158 (27.6)	1.00 (0.70–1.42)	0.95 (0.65–1.40)
Arg16Glu27	85 (14.5)	59 (10.3)	1.67 (1.07–2.62)	1.56 (0.96–2.53)
Gly16Glu27	250 (42.5)	216 (37.8)	1.36 (1.00–1.85)	1.25 (0.89–1.74)

* Adjusted for hypercholesterolemia, hypertension, atrial fibrillation, and age.

Discussion

The results of our study, after adjusting for possible confounders, suggest that subjects harboring the Glu27 allele of the β2-AR gene have an increased risk of ischemic stroke. The same significant results were obtained when only strokes of arterial origin were considered. β2-AR plays a fundamental role in the control of vascular tone and in the process of vascular remodeling.[7–9] Molecular variants of the β2-AR gene were previously identified, and their functional significance was investigated.[10,11] In particular, these in vitro studies revealed decreased receptor downregulation in the presence of the Glu27 allele. Moreover, enhanced vasodilation in response to isoproterenol in healthy subjects homozygous for the Glu27 allele was reported.[26]

Based on these findings, a potential contribution of the Glu27 allele in determining a predisposition to developing cardiovascular diseases could be hypothesized. In this regard, Large et al found that the Gln27Glu polymorphism is a genetic marker for human obesity.[12] However, other studies failed to confirm this evidence.[14,15] Moreover, contrasting evidence exists with regard to the role of the Glu27 allele on the risk of myocardial infarction, because a lower risk of coronary events was described in the elderly,[20] whereas an increased risk of coronary atherosclerosis was reported by others in a large prospective study.[19]

In regard to ischemic stroke, the only study that has been reported so far found no association of the β2-AR genotype and the occurrence of cerebrovascular events.[20] Therefore, to date, our findings represent the first demonstration that the Glu27 allele is significantly associated with an increased risk of ischemic stroke after adjustment for other stroke risk factors, including hypertension, present in our Sardinian sample. Because BMI did not represent a predisposing risk factor for stroke in our sample, it is unlikely that the effect of the β2AR/Glu27 allele could be mediated by obesity.

The strength of our study lies, first of all, in the characteristics of our human sample. In fact, it derives from a geographical area with a well-known elevated degree of genetic homogeneity,[27,28] and it was shown to be a highly conservative sample.[4,5] Moreover, the degree of association was unchanged when only strokes of arterial origin were considered, despite the decrease in sample size. Furthermore, the haplotype analysis showed that only the presence of the Glu27 allele, but not of the Gly16 allele, was responsible for an increased risk of stroke. However, statistical significance was not attained after adjusting for possible confounders.

In the latter regard, our study adds interesting information about the controversial issue of which allele (Gly16 or Glu27) dominates the phenotype. In fact, based on discrepancies between in vitro and ex vivo findings, it was suggested that Gly16 is the dominant allele. We confirmed a significant linkage disequilibrium between codon 16 and codon 27 in Sardinians, with allele frequencies similar to those reported for other white populations.[20] Furthermore, among Sardinians, we identified a percentage of individuals harboring the Arg16/Glu27 β2-AR haplotype higher than that reported for other ethnic groups. In fact, these individuals had a risk for stroke above 1 (1.67 in the crude analysis and 1.56 after multivariate analysis). Therefore, the Glu27 allele seems to be the most relevant determinant of the risk of stroke, at least among Sardinians.

In the attempt to find a pathophysiological link with the increased risk of stroke in carriers of the Glu27 allele, several observations can be taken into account. It was shown that β2-AR stimulates cellular proliferation in several tissues, including the endothelium, by activation of extracellular signal regulated kinase (ERK) and the release of vascular endothelial growth factor (VEGF).[8,9] Furthermore, β-blockers are known to exert a significant protective effect on the incidence of cardiovascular events and mortality,[29] and they were shown to inhibit coronary atherosclerosis in monkeys fed an atherogenic diet,[30] and to reduce the rate of progression of carotid atherosclerosis in clinically healthy and symptom-free subjects with carotid plaque.[31] Therefore, the reduced downregulation of β2-AR in subjects carrying the Glu27 allele may mediate, through an enhanced adrenergic stimulation of vascular tissue, a major effect on the progression of atherosclerosis and a determination of its clinical sequelae.

A potential limitation of our study is the recruitment of a hospital-based control cohort with an incidence of cardiovascular disease and related risk factors higher than expected in a cohort of healthy subjects. Thus, the domain of our study appears to be restricted mainly to patients

with vascular disease, ie, patients with cerebrovascular disease and those with other forms of atherosclerotic disease or risk factors for it.

Furthermore, we observed a deviation from HWE in controls for the Gln27Glu variant. The HWE may be violated because of genotyping error, chance, inbreeding, nonrandom mating, differential survivorship of marker carriers, genetic drift, population stratification, or a combination of these factors. However, there is no evidence in other studies on this cohort[4,5] of any deviation from HWE, which militates against this observation arising from random mating. It is likely that selective pressure acts to maintain Hardy-Weinberg disequilibrium at this locus because it may have a functional role.

Finally, we are aware of the issue of multiple testing performed thus far in our sample. However, multilocus testing is considered a rational approach when investigating the genetic basis of complex diseases such as stroke, particularly when it is well motivated by a priori hypotheses.[32]

In conclusion, we provide evidence of a direct, contributory role of the Gln/Glu27 β2-AR gene variant in the development of ischemic stroke. Identification of the molecular mechanisms underlying the relationship between the β2-AR gene and ischemic stroke may help to improve the prevention and treatment of this common disease.

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