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α-2 Genetic variant of α2β1 integrin and Risk of Stroke in Tunisian Population

Akram Jalel[1], Fatma Midani[2] ,Rim Mannai [3],Maher Kharrat [4], Fayçal Hentati [5], Mahjoubi Halima[6], Hayet Soualmia[7]

[1,6]Laboratory of Biophysics, Higher Institute of Medical Technologies of Tunis -TUNISIA,
[2] Neurology Service, National Institute of Neurology -TUNISIA,
[3]Genetic Department, University of Tunis Elmanar -TUNISIA,
[4]Université Tunis El Manar, Faculty of de Medicine of Tunis-TUNISIA,
[5]Neurology Service, National Institute of Neurology -TUNISIA,
[2,7] Biochemistry Department, and Laboratory of biochemistry La Rabta -TUNISIA,

AUTHOR to whom correspondence should be sent:
Dr. Akrem Jalel ✉
9 street Zouheir Essafi.1006
TUNISIA
Email: akrem.jalel@ist.tn
Or:
2852 University Street
Québec G2V 0A9
CANADA
Email: jalel.akrem.1@ulaval.ca

ABSTRACT: BACKGROUND: Integrins are membrane adhesion proteins belonging to the family of transmembrane receptors, and hetero-dimeric glycoprotein consisting of two subunits α and β . This protein is encoded by ITGA2 gene. it is essentially involved in cell-cell interaction, cell-pathogen agent and cell-extracellular matrix. It plays a major role in platelet adhesion to the sub endothelial matrix in thrombogenesis. In this study, we investigated the relationship between the Bgl II polymorphism of the α2β1 integrin and stroke in the Tunisian population. **METHODS**: Case-controlled study included 34 patients with stroke and 35 healthy controls. The Bgl II polymorphism of α2β1 integrins was determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) analysis method. **RESULTS**: In the whole population, the genotypic frequencies of the Bgl II polymorphism of the α2β1 integrin in stroke and control groups (+/+, +/-, -/-) were 16.7%, 40 %, 43.3% and 2.9%, 42.9%, 54.3% , respectively ($\chi 2 = 9.13$, $p< 0.01$). The genotype was not associated with stroke, OR=0.16 , 95% CI [0.01-1.56], p<0.11 for heterozygous and OR=0.13, 95% CI [0.01-1.31], p<0.08 for homozygous. All the other comparisons failed to show any significant result. Furthermore, a relationship was found between clinical characteristics and Bgl II genotypes. **CONCLUSION**: The present study does not support any association between Bgl II polymorphism of the α2β1 integrin and stroke. Then, our results suggest that this variant can't be considered as a genetic risk factor for stroke in the Tunisian population.

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Key words: Stroke, α2β1 integrin, genetic, α-2 variant, ITGA2 gene, thrombogenesis

INTRODUCTION

Stroke is a leading cause of morbidity and mortality in Tunisia and all around the world. This disease is a multifactorial disorder influenced by both genetic and environmental factors. The common point between the different forms of stroke onset is the existence of an inflammatory response interesting cerebral vascular bed. Following the genetic track, we mention the intergrin which is encoded by ITGA2 gene. This protein is essentially involved in cell-cell interaction, cell-pathogen agent and cell-extracellular matrix. It plays a major role in platelet adhesion to the sub endothelial matrix in thrombogenesis[1]. Several isoforms of the integrin have been isolated, among

them the α2β1 integrin which plays a major role in platelet adhesion to the sub endothelial matrix in thrombogenesis[2, 3, 4, 5] Patients deficient in the unit α2 of α2β1 suffer from prolonged bleeding time and the presence of chronic mucocutaneous bleeding[6, 7.] The patients deficient in α2 unit of suffer from prolonged bleeding time and the presence of chronic mucocutaneous bleeding[8,9,10]. In addition, serum antibodies against the α2 subunit have been found in patients with autoimmune platelet disease[11, 12, 13]. These antibodies block in vitro platelet adhesion to collagen as well as their aggregation[14, 15]**.** The two subunits forming the activated dimers are encoded by two different genes, located on two different chromosomes. The gene which encodes the α2 subunit is known under the name ITGA2 located on the short arm of chromosome 5 at the q11-2 locus spans approximately 110 kbp and contains 30 exons[16]. Several studies have shown an association between the α2β1 integrins and stroke and diabetic retinopathy[17, 18]. Other ones didn't found any association[19, 20]. However, so far the results are not consistent. The present study was undertaken to determine the possible association between Bgl II polymorphism of α2β1 integrins gene and stroke in Tunisian patients. To examine a hypothesis that a genetic variation on α2β1 integrin is associated with stroke, we analyzed the association between the Bgl II polymorphism and Bgl II polymorphism of α2β1 integrins gene and stroke in Tunisian patients. We also investigated genotype distributions of the ITGA2 gene.

SUBJECTS AND METHODS

Study population

A total of 79 unrelated subjects were included in the study. They consisted of 34 patients with stroke (195 women and 85 men) and 35 healthy controls. Patients and controls were homogeneous Tunisian subjects. Stroke patients were ambulatory subjects attending the consultation of the neurology Department of Rabta University Hospital of Tunis. Controls were unrelated healthy volunteers, randomly collected. They were without antihypertensive treatment, and their SBP and DBP were less than 140 and 90 mm Hg, respectively[22]. Informed written consent was obtained from all participants and the design of the study was approved by the local ethics committee. Weight and height were measured on the subjects barefooted and lightly clothed. Body mass index (BMI; kg/m2) was calculated and obesity was defined as BMI > 30 kg/m2 as recommended by (WHO, 1995). Diabetes mellitus was defined as hyperglycemia, requiring antidiabetic drugs or fasting blood sugar over 7.0 mmol/L. Dyslipidemia was defined as a total cholesterol (TC) level > 6.47 mmol/L and/or triglyceride (TG) level > 2.26 mmol/L. Cigarette smoking was quantified based on daily consumption and duration of smoking [21].Association between the -2518G/A polymorphism in the monocyte chemoattractant protein-1 (MCP-1) gene and hypertension in Tunisian patients.

Laboratory analysis

Blood samples were obtained after an overnight fast. Serum glucose, creatinine concentrations, uric acid, TC and TG were measured by standardized enzymatic methods using commercial kits (Roche Diagnostics, Mannheim, Germany) on a Hitachi 912 analyzer. LDL-cholesterol (LDL-C) was calculated according to Friedwald's formula[23].

DNA analysis

Genomic DNA was prepared from white blood cells by phenol extraction[23]. The genotypes were determined by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique[25, 26,27]. PCR products were subjected to enzymatic digestion with Bgl II enzyme. Then, the digested products were separated on 2 % agarose gels (figure1).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS 17.0 for Windows; SPSS Inc., Chicago, IL, USA). The Student *t*-test was used for continuous variables, and the χ^2 test was used for categorical variables to test for statistical significance.

We calculated unadjusted and multi-adjusted odds ratio (OR) together with their 95% approximate confidence intervals (95% CI) as estimators of the relative risk of stroke for α-2 variant of the α-2β-1 integrin genotypes. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

The clinical and demographic characteristics of our studied population are presented in Table 1. There were significant differences for PAS, PAD, BMI ($p<0.001$) between Hypertensive patients and control groups. The mean values of plasma total cholesterol, triglyceride, low-density lipoprotein cholesterol, creatinine and uric acid levels were significantly different ($p<0.005$). The frequencies of dyslipidemia, diabetes, obesity, and smoking were significantly higher in cases than in controls ($p <0.001$).

Genotype distribution was consistent with that predicted by Hardy–Weinberg equilibrium in patients and controls ($p>0.05$). The distribution of genotypes and alleles in patients with stroke in the whole population doesn't show a significant difference from that in the control subjects (Table 3). Patients with stroke had a frequency of 16.7%, for +/+genotype, 40% for the +/- genotype and 43.3% for the -/- genotype.

Controls had a frequency of 2.9%, for the +/+ genotype, 42.9%, for the +/- genotype and 54.3% for the -/- genotype ($\chi 2 = 9.13$, $p< 0.01$).

The risk of developing stroke was evaluated by OR. No risk of stroke was observed for +/- (OR, 0.16; 95% CI [0.01-1.56] p=0.11) and -/- genotypes (OR, 0.13;95% CI [0.01-1.31] p=0.08) compared to +/+. Two models are used, the dominant model and recessive one to analyze the association between stroke and Bgl II genotype. The comparison between the control group and stroke group did not show a significant difference for both models (0.38 and 0.08 respectively). Finally, our findings show relationship between clinical parameters and genotype distributions.

Table 1. Baseline characteristics of the study population.

Variables	controls (n=35)	Stroke patients (n=34)	p
Age (years)	41,83 ±1,67	61,79±1,42	<0,001
BMI (Kg/m2)	24,92±0.04	22,26±0,86	<0,01
SBP (mmHg)	122,86±1,12	136,50±4,46	<0,002
DBP (mmHg)	69,67±1,8	76,9±0,80	<0,005
Na+(mEq/L)	138.29 ± 0.22	142.85 ± 0.77	0.001
K+(mEq/L)	3.96 ± 0.05	3.95 ± 0.07	0.1
Cl-(mEq/L)	104.32 ± 0.36	106.14 ± 0.6	< 0.01
Na+(mEq/L)	138.29 ± 0.22	142.85 ± 0.77	< 0.001
Smoking (%)	17.8	34.9	< 0.001
TC (mmol/L)	1.90± 0.04	1.78± 0.084	0.03
TG (mmol/L)	1.05 ± 0.08	1.48 ± 0.28	0.001
HDL-C (mmol/L)	0.47 ± 0.02	0.52± 0.02	0.02
LDL-C (mmol/L)	1.14 ± 0.32	1.23 ± 0.34	0.001
Fasting glucose mmol /L)	0.96 ± 0.33	1.19 ± 0.55	<0.001
Creatinine (µmol/L)	7.74 ± 0.25	9.87 ± 0.62	<0.001
Uric Acid (mg/L)	43.75 ± 2.32	44.00± 1.00	0.1

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein-cholesterol; LDL-C:low-density lipoprotein-cholesterol. Variables are presented as means ± SD or % of patients and were compared by t-test. Significance was considered as $p<0.05$.

The values of hemostasis in stroke patients are determinate in the aim to evaluate the thrombogenesis background (Table2). Our results showed a significant increase in hemostatic parameters ($p <0.001$). Similarly, we observed a statistically significant increase in blood lipids: TG ($p <0.001$) and so that for natraemia (Na +) ($p <0.001$).

Table 2: Average values of hemostasis in stroke patients

Variables	Controls	Stroke (N = 3)	P
CRP (mg/L)	5	12.32± 2.77	<0.001
P T(%)	85%	79.29± 2.43	<0.001
Platelets/ µl	275000	192328 ± 17157.22	0.07
Ht	45 %	40.44 ± 1.5	0.01
ESR	15	29.14±6.96	<0.001
WBCs/ µl	7000	9252.14±565.7	<0.001
ASAT (U/L)	20	23.26±3.75	0.12
ALAT (U/L)	22	17.33±1.79	0.09
LDH (U/L)	300	232.33±24.67	<0.001
Total Bilirubin	6	14.06±1.87	<0.001

Table 3. Genotype and allele distributions of Bgl II polymorphism in controls and stroke patients.

	Genotype frequencies			Allelic frequencies	
	+/+	+/-	-/-	+	-
T (n=35)	1(2.9 %)	15(42.9%)	19(54.3%)	24.35%	75.65%
Stroke (n=34)	5(16.7 %)	12(40 %)	13(43.3%)	36.7%	63.3%
		p=0.152			

T: controls, Stroke: stroke patients

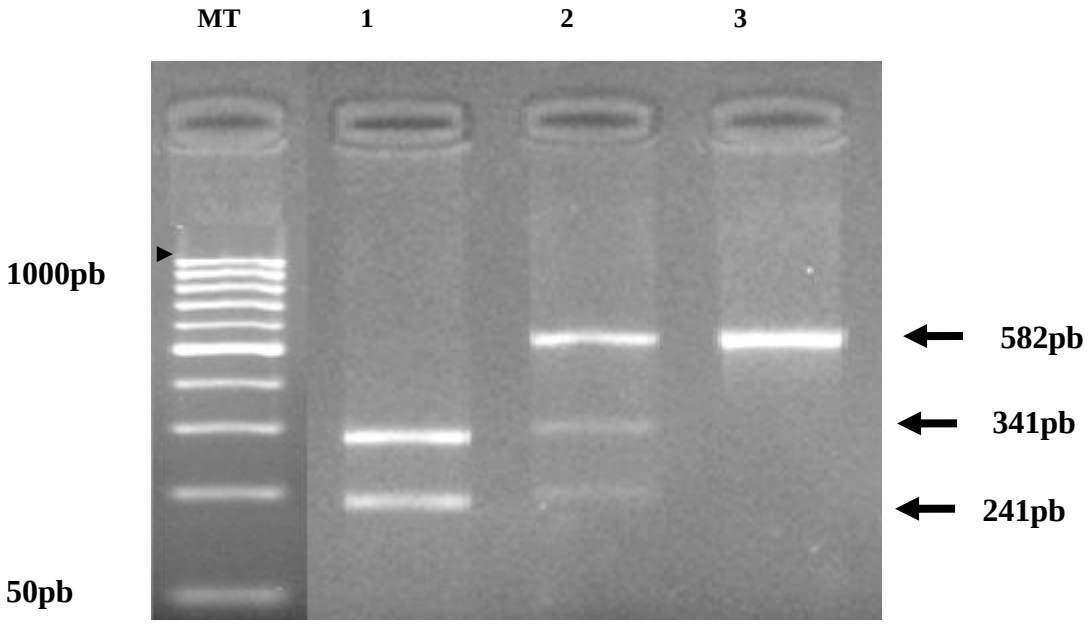

Figure 1. Agarose gel electrophoresis showing the genotypes polymorphism. M:marker; line 1 represents homozygous +/+ genotype; line 2 represents heterozygous +/- genotype; line 3 represents homozygous -/- genotype of Bgl II polymorphism of alpha integrin gene.
The elecrtophoretic profile shows:
• Two bands corresponding respectively to fragments and 341pb 241pb: the homozygous wild ++.
• Three bands corresponding respectively to fragments de582pb, and 341pb
241pb: the heterozygous + -.
• Only one band 582pb: the heterozygous mutated - -.

DISCUSSION

In this study, we investigated the association between the Bgl II polymorphism of α2β1 integrins and stroke in the Tunisian population. Our findings showed that this variant is not associated with stroke. Likewise, in the current study, we found that the Bgl II polymorphism of α2β1 integrins and stroke both before and after adjusting for confounding factors. These results are in agreement with some studies which failed to detect a significant association between this variant and stroke [28] .In contrast, our findings were different from other studies reporting positive associations[29,30,31]. We could explain these inconsistent results by the difference in the genetic background of the ethnic groups concerning the Bgl II polymorphism of α2β1 integrins. Additionally, environmental factors such as differences in diet, the lifestyle and stress could explain the contradictory results which may affect the expression of the Bgl II polymorphism of α2β1 integrins and stroke. In the other side, the possibility that hereditary variation in platelet Integrin α2β1 density is associated with silent polymorphisms in the α2 gene coding sequence[31] .Nevertheless, we should pay attention to the potential impact of variation in

α2β1 numbers on platelets considering that fibrillar collagens are major components of the subendothelial matrix that can trigger thrombus formation in flowing blood. The Bgl II polymorphism of α2β1 integrins induces the alteration of the Platelet adhesion to fibrillar collagen via the membrane glycoprotein (GP) Ia/IIa (α2β1), is a crucial event in the pathogenesis of arterial occlusive disorders[33,34]. Platelet adhesion to fibrillar collagen via the membrane glycoprotein (GP) Ia/IIa (α2β1), is a key event in the pathogenesis of arterial occlusive disorders. The polymorphism of the α2 integrin, (ITGA2) gene has been shown to correlate with the platelet GPIa/IIa density. Therefore, individuals who express the highest receptor density should present an augmented potential of platelet adhesion[35]. For this reason an increased risk of eventual stroke. Though, studies remain undecided and controversial. These findings on a possible association of these genetic susceptibility markers with hemorrhagic stroke are more restricted, and their implication in ischemic one stroke has not been beforehand detailed. Finally, because stroke is influenced by the interaction of various genes and environmental factors[36], we could not exclude other functional variant in α2β1 integrins or genes around to influence the expression of Bgl II polymorphism and its association with stroke. Besides, the several analyses may amplify the possibility of a spurious association (α1 or type I error).

CONCLUSION

In summary, the present study shows no association between Bgl II polymorphism of α2β1 integrins and stroke. In consequence, despite its implication in thrombogenesis dysfunction, we can't conclude that this variant gene may be considered as genetic risk factor for stroke in Tunisian population. We suggest additional studies, especially in young adults, to confirm these preliminary findings.

ABBREVIATIONS

BMI : Body Mass Index
BP: Blood Pressure
DBP: Diastolic Blood Pressure
HDL-C: High Density Lipoprotein Cholesterol
LDL-C: Low Density Lipoprotein Cholesterol
OR: Odds Ratio
PCR-RFLP: Polymerase Chain Reaction and Restriction Fragment Length Polymorphism
SBP: Systolic Blood Pressure
TC: Total Cholesterol
TG: Triglyceride

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