RESEARCH NOTE

First study of *C2491T FV* mutation with ischaemic stroke risk in Morocco

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[Diakite B., Hamzi K., Hmimech W., Nadifi S. and GMRAVC 2015 First study of *C2491T FV* mutation with ischaemic stroke risk in Morocco. *J. Genet*. **94**, 313–315]

This study evaluated the association of *C2491T FV* mutation with the risk of ischaemic stroke (IS). Carriers of *T* mutated allele were associated with a high risk of IS (OR: 3.77, 95% CI = 2.70–5.25; P <0.0001). But this risk was 8.95 fold when the subject carried *TT* genotype (P<0.0001) and 4.08 fold with *CT* genotype. Thus, we can suggest that *C2491T FV* mutation could be a genetic risk factor for IS in Moroccan population.

Stroke is a major public health problem causing a neurological deficit of sudden onset (Flemming *et al.* 2004). According to an epidemiological investigation, IS affects 4.1% of Moroccan population (International Symposium on Stroke, Rabat, Morocco). Hence, it is a multifactorial disease involving direct or indirect participation or synergy of several factors including known risk factors for IS, environmental and genetic factors (Domingues-Montanari *et al.* 2008). Indeed, in the past decade, many studies have highlighted the close relationship between genes involved in the coagulation mechanism such as *Factor V Leiden* (*FVL*) and cerebrovascular diseases, but the results remain controversial (Kim and Becker 2003; Mannucci *et al.* 2010). However, several previous studies have revealed that the distribution of *FVL* mutation remains low or almost absent in some populations (African population) compared to others (Mathonnet *et al.* 2002; They-They *et al.* 2010). Considering the heterogeneity of this mutation, van Wijk *et al.* (2001) identified five new mutations in *FV* gene of which one is specific to Moroccan population. It is *C2491T FV* mutation or FV (Q773Term) mutation which is a nonsense mutation in exon 13, in which cytosine is replaced by thymine at nucleotide 2491. This change modifies the sequence of amino acids by the appearance of a stop codon at residue 773 instead of the wild-type glutamine (van Wijk *et al.* 2001). Discovery of this new mutation has made us to explore the advantage of it in IS. Recently, in prelude of this work, a study from our team has confirmed the presence of *C2491T FV* mutation in general population (Hamzi *et al.* 2013). Given the existence of this mutation in our general population, in this study, we hypothesized that the *C2491T FV* mutation could have an important impact on IS. Thus, the aim of this work was to determine the effect of *C2491T FV* mutation in IS subjects and controls.

This is a case–control study, focussed on a series of 211 healthy subjects with no history of stroke and cognitive impairment, recruited in Moroccan population. And a series of 170 IS subjects recruited in neurology, University Hospital Center of Rabat and Casablanca. Patients' information on risk factors, age, sex, hypertension, diabetes, smoking and cholesterol were collected from medical records. According to the results of magnetic resonance imaging (MRI) or scanner, subjects were divided into IS subtypes following: atherosclerosis, cardio embolism, lacunar and other causes. This classification is known as TOAST. Informed consent was obtained from each patient and control.

Extraction of genomic DNA was performed from blood samples using the standard method with salt. The genotypes for *C2491T FV* mutation were detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described by van Wijk *et al.* (2001). The PCR product was performed in 25 μL. After amplification and digestion, PCR products of *C2491T FV* change have highlighted the following sizes, 605 bp with a restriction site for *Hph*I, 458 and 147 bp whose mutation abolished, 605, 458 and 147 bp for heterozygous *CT*. Three genotypic profiles *CC*, *CT* and *TT* of *FV* nonsense mutation genotyped by PCR-RFLP were also checked by high resonance melting (HRM) using 7500 fast real-time PCR system (AB Applied Biosystems, Foster City, USA) as described previously by Graham *et al.* (2005) and Price *et al.* (2007). The software 7500 Fast System SDS ver. 2.0.1 was used for the analysis of melting curves. The different genotypic profiles were interpreted from the temperature curves referring to the controls' curves.

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Keywords. C2941T FV; nonsense mutation; ischaemic stroke.

Table 1. Distribution of *C2491T FV* mutation according to the risk factors and IS subtype.

IS risk factor	% Patient ($n = 170$)	*C2491T FV* mutation %CC	%CT	%TT	*P* value
Age					0.33
≤ 55 years	73 (42.9)	22 (30.1)	31 (42.5)	20 (27.4)	
>55 years	97 (57.1)	35 (36.1)	48 (49.5)	14 (14.4)	
Sex					0.90
Male	96 (56.5)	33 (34.4)	47 (49.0)	16 (16.7)	
Female	74 (43.5)	24 (32.4)	32 (43.2)	18 (24.3)	
Smoking					0.29
Yes	58 (34.1)	23 (39.7)	24 (41.4)	11 (19.0)	
No	112 (65.9)	34 (30.4)	55 (49.1)	23 (20.5)	
HTA					<0.001*
Yes	86 (50.6)	5 (5.8)	48 (55.8)	33 (38.4)	
No	84 (49.4)	52 (61.9)	31 (36.9)	1 (1.2)	
Diabetic					0.14
Yes	39 (22.9)	8 (20.5)	24 (61.5)	7 (17.9)	
No	131 (77.1)	49 (37.4)	55 (42.0)	27 (20.6)	
Cholesterol					0.41
Yes	16 (9.4)	2 (12.5)	11 (68.8)	3 (18.8)	
No	154 (90.6)	55 (35.7)	68 (44.2)	31 (20.1)	
IS subtype					0.71
Atherosclerosis	67 (39.4)	26 (38.8)	29 (43.3)	12 (17.9)	
Cardio embolism	46 (27.1)	14 (30.4)	19 (41.3)	13 (28.3)	
Lacunar	11 (6.5)	4 (36.4)	5 (45.5)	2 (18.2)	
Others	46 (27.1)	13 (28.3)	26 (56.5)	7 (15.2)	

n, number; CC, wild genotypes; CT, heterozygous; TT, homozygous muted; *significant.

Data were analysed using SPSS 19.0 software. Chi-square test was used to evaluate the Hardy–Weinberg equilibrium. The correlation of the *C2491T FV* mutation with clinical parameters (IS risk factors and subtypes) were evaluated via an adjusted linear regression. The OR and 95% CI were used to assess the impact of *C2491T FV* mutation in stroke subjects and controls. For all tests, $P < 0.05$ was considered statistically significant.

We studied 170 cases and 211 controls with respective average age 57±3 and 54±2. By comparing PCR-RFLP technique and HRM analysis for genotyping of *C2491T FV* mutation in the present study, the concordance was 95.3%. The remaining percentages were DNA samples that could not be amplified. Table 1 shows the distribution of *C2491T FV* mutations according to IS risk factors and classification TOAST. In the cases, aged patients (age <50 years) versus young subjects (age ≤ 50 years) were overrepresented in our study sample (57.1% versus 42.9%). The proportion of cerebral ischaemia in male (56.5%) was slightly higher than in females (43.5%). In statistical terms, a negative correlation was observed between *C2491T FV* mutation and age, sex, smoking, diabetes and cholesterol, but except hypertension, where a significant inverse correlation ($P<0.004$) was noted with this nonsense mutation in multivariate linear regression model. Concerning the classification TOAST, the forms of atherosclerosis (39.4%) were more common than other forms. No correlation was observed between *C2491T FV* and IS subtypes.

Table 2. Genotypic and allelic frequencies of *C2491T FV* mutation in IS subjects and controls.

	Case (%) $n = 170$	Control (%) $n = 211$	OR (95% CI)	*P* value
Genotype				
CC	57 (33.5)	150 (71.1)	1	
CT	79 (46.5)	51 (24.2)	4.08 (2.55–6.49)	<0.0001*
TT	34 (20.0)	10 (4.7)	8.95 (4.15–19.29)	<0.0001*
Allele	$n = 340$	$n = 422$		
C	193 (56.8)	351 (83.2)	1	
T	147 (43.2)	71 (16.8)	3.77 (2.70–5.25)	<0.0001*
HWE: *P* value	<0.001	0.15		

*Significant; HWE, Hardy–Weinberg equilibrium.

Genotypes and *C2491T FV* allele frequencies of the two groups are shown in table 2. Genotype frequencies for *C2491T FV* were in agreement with the HWE in controls but not in cases. Therefore, this mutation was quite common in stroke subjects compared to controls. The proportion of allelic and genotypic frequencies in cases were 56.8% *C*, 43.2% *T* and 33.5% *CC*, 46.5% *CT*, 20.0% *TT*. For controls, the distribution of allele and genotype frequencies was 83.2% *C*, 16.8% *T* and 71.1% *CC*, 24.2% *CT*, 4.7% *TT*. However, *C2491T* nonsense mutation was significantly associated with an increased risk of IS with *CT* (OR: 4.08, 95% CI = 2.55–6.49; $P < 0.0001$), *TT* (OR = 8.95, 95% CI = 4.15–19.29; $P < 0.0001$) and *T* (3.77, 95% CI = 2.70–5.25; $P < 0.0001$).

In the present study, the low sampling may call into question the credibility of our results, which could lead to inconsistencies in the precision of variables. This can lead to a limitation of the associations between specific estimates. In other words, the specificity of *C2491T FV* mutation, which was detected first in a Moroccan patient with deep vein thrombosis, could play a key role in the pathogenesis of several thromboembolic diseases. Thus, we suggested that *C2491T FV* mutation could be considered as a potential genetic marker for IS in Moroccan population.

Acknowledgements

This work was financed by Academy Hassan II of Sciences and Technology and Laboratory of Genetics and Molecular Pathology (LGPM), Faculty of Medicine and Pharmacy, University Hassan II Ain Chock in Casablanca, Morocco. Many thanks to Dr van Wijk for the help provided. The members of the GRAVCM study group are Pr EL Alaoui Moulay Mustapha Faris, Pr Yahyaoui Mohammed and Pr Aidi Saadia (Neurological Departments of CHU Souissi Rabat), Pr Slassi Ilham and El Moutaouakil Bouchra (Neurological Departments of CHU Ibn Rochd, Casablanca), Pr Habbal Rachida (Cardiology Department of CHU Ibn Rochd); Adlouni Ahmed (Faculty of Sciences Ben Msik, Casablanca), Pr El Messal Mariame (Faculty of Sciences Ain Chock, Casablanca) and Genetics and Molecular Pathology department and Genetic and Molecular department.

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Received 8 September 2014, in revised form 3 December 2014; accepted 29 December 2014
Unedited version published online: 6 January 2015
Final version published online: 3 June 2015