LETTER TO THE EDITOR
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Evaluation of Novel Platelet Polymorphisms in Stroke. Dichotomic Effect of rs5443 in *GNB3*

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Dear Editor,

Our group recently characterized two single-nucleotide polymorphisms (SNPs), rs4366150 and rs1787566, on the genes encoding lysophosphatidic acid receptor-1 (*LPAR1*) and myosin VB (*MYO5B*), respectively, which are associated with platelet reactivity, in a cohort of 286 healthy children.[1] Furthermore, the recently identified SNPs rs5443 (on the gene encoding guanine nucleotide binding protein 3, *GNB3*) and rs3737224 (on the gene encoding platelet endothelial aggregation receptor 1, *PEAR1*)[2,3] may also be considered as potential new genetic factors implicated in platelet function. However, the role of these SNPs in thrombosis or hemorrhage disorders has either not been addressed, or is still controversial.

Given the functional effect of the four aforementioned SNPs, we aimed to determine their potential role in 1) the development of thrombosis in patients with ischemic stroke (IS) or 2) bleeding in patients with intracranial hemorrhage [subarachnoid hemorrhage (SAH) and intracerebral hemorrhage (ICH)].

For this purpose, the presence of the four SNPs was determined in consecutive patients who survived an IS and in patients who suffered from an intracranial hemorrhage. The cohort with IS according to the Trial of ORG 10172 in Acute Stroke Treatment criteria were patients enrolled in the Unit of Neurology in Reina Sofia Hospital (Murcia, Spain). We also recruited 611 healthy controls from the general population from blood donors (n=377) and traumatology and ophthalmology patients undergoing minor outpatient surgery (n=234). Cohorts with SAH and ICH were enrolled over a 3-year period and are described in a previous report from our group.[4] All subjects were Caucasians and gave their informed consent to enter the study, which was approved by the local Ethics Committee and was performed according to the Declaration of Helsinki.

Genomic DNA was isolated from whole blood samples according to standard procedures, and DNA was amplified using Taqman probes from Life Technologies (Madrid, Spain). Genotyping of the different cohorts for the different SNPs showed that they were all in Hardy-Weinberg equilibrium (not shown).

The general characteristics of patients and controls are given in Table 1. The univariate analyses revealed no association (p>0.05) between the presence of rs4366150 (*LPAR1*), rs1787566 (*MYO5B*), and rs3737224 (*PEAR1*), and the development of IS, SAH, or ICH (Table 1). Nevertheless, there was a trend toward significance for the rs5443 (T allele) SNP in *GNB3* as a risk factor in the development of IS (p=0.087) and as a protective factor in SAH (p=0.071). No association between rs5443 (T allele) and development of ICH was found (p=0.781). Multivariate analysis taking into account risk factors (age, sex, and hypertension) confirmed that the T allele of rs5443 was an independent protective factor against the development of SAH [odds ratio (OR)=0.608, 95% confidence interval (CI)=0.383−0.964, p=0.034], but excluded an effect on the risk of developing IS (p=0.152) (Table 1). We further refined our study by segregating by gender. The results showed that while in women the T allele of rs5443 was not associated with IS (p=0.713), there was a statistically significant, almost threefold increase in the risk of developing IS among men without

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Table 1. Clinical features and prevalence of selected risk factors in case-control study

	Controls (*n*=611)	IS (*n*=118)	*p**	OR (95% CI)	SAH (*n*=102)	*p**	OR (95% CI)	ICH (*n*=164)	*p**	OR (95% CI)
Age (year)										
Range	18–87	32–97	-	-	19–90	-	-	25–99	-	-
Mean±SD	48.6±19.6	73.3±12.3	<0.001	-	59.6±12.6	<0.001	-	69.6±4.7	<0.001	-
Male sex (%)	54	46	0.126	-	39	0.0062	-	66	0.0059	-
Risk factors (%)										
Current/former smoker	31	20	0.025	-	32	0.476	-	26	0.18	-
Hypertension	21	68	<0.001	-	42	<0.001	-	67	<0.001	-
Dyslipidemia	12	37	<0.001	-	N/A	-	-	N/A	-	-
Diabetes mellitus	9.5	45.7	<0.001	-	-	-	-	-	-	-
PEAR1 rs3737224, *n* (%)‡	138 (22.7)	28 (23.7)	0.814	-	27 (26.5)	0.409	-	39 (23.8)	0.778	-
LPAR1 rs4366150, *n* (%)†	387 (64.5)	77 (65.3)	0.876	-	74 (72.5)	0.115	-	104 (63.4)	0.797	-
MYO5B rs1787566, *n* (%)†	194 (32.1)	31 (26.3)	0.215	-	28 (27.7)	0.385	-	49 (29.9)	0.593	-
GNB3 rs5443, *n* (%)‡										
Total subjects	342 (60.0)	77 (65.3)	0.087	-	48 (47.1)	0.071	-	95 (57.9)	0.781	-
			0.152§	-	-	0.034§	0.608 (0.383–0.964)	-	-	-
Men	178 (53.9)	37 (68.5)	0.035‖	2.07 (1.051–4.097)	16 (40)	0.076‖	-	59 (54.6)	0.540	-
	178 (53.9)	37 (68.5)	0.069¶	-	-	-	-	-	-	-
Men without DM	159 (53.9)	22 (80.0)	0.028‖	2.717 (1.116–6.612)	-	-	-	-	-	-
Men with DM	19 (59.4)	15 (65.2)	0.908‖	-	-	-	-	-	-	-
Women	164 (59.4)	40 (62.5)	0.713‖	-	32 (51.6)	0.189‖	-	36 (64.3)	0.639	-

Data are mean±SD values or the percentage of individuals. Current/former smoker: the subject had ever smoked >10 cigarettes per day. Hypertension: blood pressure ≥140 mm Hg systolic or 90 mm Hg diastolic on repeated observations over 3 months, or if the subject was receiving chronic antihypertensive therapy. Dyslipidemia: total serum cholesterol level of >5.72 mmol/L (220 mg/dL).
*Statistical analysis was performed vs. controls, *t*-test or χ^2 were used to evaluate statistical differences between groups. Significance was accepted when $p<0.05$ (two-sided), †AG+GG genotypes, ‡CT+TT genotypes, §Multivariate analysis (hypertension, age, and sex included), ‖Multivariate analysis (hypertension and age included), ¶Multivariate analysis (diabetes mellitus, hypertension, and age included).
DM: diabetes mellitus, ICH: intracerebral hemorrhage, IS: ischemic stroke, N/A: not available, SAH: subarachnoid hemorrhage.

diabetes mellitus (OR=2.72, 95% CI=1.116−6.612, p=0.028). Indeed, the issue of sexual dimorphism in the occurrence of cardiovascular disease is well known, and the role of hormones in IS has also been addressed in several studies.[5]

Interestingly, our finding indicating that the T allele rs5443 SNP has an impact on both IS and SAH sheds light on the potential duality of SNPs as simultaneously being both risk and protective factors for different diseases. This concept of dichotomy has already been proposed by our group for other SNPs, such as factor V Leiden thrombophilia or FVII-323 Del/Ins, in the presence of which a phenotype of thrombosis or of bleeding may appear according to the presence of specific conditions and other risk factors.[4]

Future studies in larger cohorts are necessary to clarify the exact role of the rs5443 SNP in *GNB3* as a factor capable of modulating the individual thrombotic risk.

Conflicts of Interest

The authors have no financial conflicts of interest.

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