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Genetic Variant on Chromosome 12p13 Does Not Show Association to Ischemic Stroke in 3 Swedish Case-Control Studies

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Background and Purpose—In a genome-wide association study and subsequent case-control studies, the single-nucleotide polymorphism rs12425791 on chromosome 12p13 was reported to be associated with ischemic stroke, but this could not be validated in a recent well-powered study. We therefore investigated whether an association between ischemic stroke and rs12425791 could be detected in 3 different case-control studies from the southwest of Sweden.

Methods—We examined 3606 patients with ischemic stroke and 2528 controls from 3 independent case-controls studies.

Results—No significant association between ischemic stroke and the single-nucleotide polymorphism rs12425791 was detected in any of the 3 case-control samples or in the samples combined. The odds ratio for ischemic stroke for the minor allele in the combined sample was 1.02 (95% CI, 0.93 to 1.13).

Conclusions—The single-nucleotide polymorphism rs12425791 does not confer a substantial risk for ischemic stroke in our population. Our results support a recent large study including other European populations. **(*Stroke*. 2011;42:214-216.)**

Key Words: stroke ■ single-nucleotide polymorphism ■ genetic association studies

The Cohorts for Heart and Aging Research Consortium in Genetic Epidemiology performed a genome-wide association study of incident ischemic stroke (IS) and reported an association between IS and 2 single-nucleotide polymorphisms (SNPs) on chromosome 12p13, rs12425791 and rs11833579, located in close proximity to the ninjurin2 gene (*NINJ2*).[1] The association with rs12425791, but not with rs11833579, was replicated in a black and a white case-control sample.[1] In contrast, a recent well-powered meta-analysis of a combined case-control sample of IS of European ancestry, as well as of 1 population-based genome-wide cohort study conducted by the International Stroke Genetics Consortium (ISGC) and the Wellcome Trust Case-Control Consortium 2 (WTCCC2), did not replicate this association.[2] Furthermore, in an Italian case-control study, no association with IS was observed for the 2 SNPs on chromosome 12p13.[3] However, a large case-control study in a Japanese population detected a significant association between atherothrombotic stroke and rs12425791.[4] The aim of the present study was therefore to investigate whether an association between IS and the SNP rs12425791 could be detected in 3 different IS case-control samples from the southwest of Sweden.

Materials and Methods

All 3 study populations, the Lund Stroke Register, the Malmö Diet and Cancer study, and the Sahlgrenska Academy Study on Ischemic Stroke, were from the southwest of Sweden. Sample characteristics, data collection, and clinical definitions including those for risk factors have been described elsewhere.[5–7]

In brief, the Lund Stroke Register is a prospective, epidemiologic study that consecutively includes patients with first-ever stroke from the local area of Lund. Controls without stroke are randomly selected from the same geographic region.[6] The Malmö Diet and Cancer study is a prospective, population-based cohort study that includes 28 449 randomly selected men (born between 1923 and 1945) and women (born between 1923 and 1950) with baseline examinations between 1991 and 1996.[5] The Sahlgrenska Academy Study on Ischemic Stroke comprises patients who presented with first-ever or recurrent acute IS before reaching the age of 70 years and who were recruited consecutively between 1998 and 2008 at 4 stroke units in western Sweden.[7] Controls without cardiovascular disease were randomly selected from the same geographic region as the patients.[7]

In all 3 studies, the diagnosis of IS was ascertained in accordance with World Health Organization criteria and verified by computed tomography or autopsy. The study was approved by the ethics committee of the University of Gothenburg or by the ethics committee of Lund University. All participants provided informed consent before enrolment. For participants who were unable to communicate, consent was obtained from their next of kin.

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Table. Baseline Characteristics for the 3 Case-Control Samples as Well as Genotype Frequencies and Odds Ratios for the SNP rs12425791

	LSR		MDC		SAHLSIS		Combined	
	Control n=960	IS n=1865	Control n=900	IS n=897	Control n=668	IS n=844	Control n=2528	IS n=3606
Age, y (SD)	74 (12)	74 (12)	63 (7)	63 (7)	56 (10)	56 (11)	65 (12)	67 (13)
Male sex, n (%)	546 (57)	993 (53)	485 (54)	496 (55)	392 (59)	554 (66)	1423 (56)	2043 (57)
Hypertension, n (%)	452 (47)	1202 (66)	520 (59)	661 (74)	230 (34)	487 (58)	1202 (48)	2350 (66)
Diabetes, n (%)	70 (7)	454 (25)	25 (3)	87 (10)	33 (5)	153 (18)	128 (5)	694 (20)
Smoking, n (%)	95 (10)	352 (19)	191 (21)	293 (33)	131 (20)	324 (38)	417 (17)	969 (27)
Genotype frequency								
GG, n (%)	647 (67)	1294 (70)	611 (70)	596 (68)	450 (69)	547 (66)	1708 (69)	2437 (69)
AG, n (%)	267 (30)	499 (27)	237 (27)	250 (29)	183 (28)	255 (31)	687 (28)	1004 (28)
AA, n (%)	26 (3)	50 (3)	24 (3)	26 (3)	23 (4)	29 (3)	73 (3)	105 (3)
Minor allele frequency	0.17	0.16	0.16	0.17	0.17	0.19	0.17	0.17
Odds ratio for the *A* allele	Ref	0.95	Ref	1.07	Ref	1.08	Ref	1.02
95% CI		0.81–1.10		0.90–1.28		0.90–1.30		0.93–1.13
P value		*P*=0.46		*P*=0.46		*P*=0.42		*P*=0.64

LSR indicates Lund Stroke Register; MDC, the Malmö Diet and Cancer study; SAHLSIS, the Sahlgrenska Academy Study on Ischemic Stroke; and Ref, reference. Odds ratios were calculated with an additive model adjusted for age and sex.

Genotyping was performed, blinded to case-control status, by matrix-assisted, laser desorption ionization time-of-flight mass spectrometry on a MassARRAY platform with iPLEX genotyping technology (Sequenom, San Diego, Calif). Associations between the SNP rs12425791 and case-control status were investigated with an additive model in binary logistic regression, adjusted for age and sex. We also assessed the association after adjustment for hypertension, diabetes mellitus, and current smoking. We calculated that the samples combined would give 80% power at the 5% level to detect an odds ratio of 1.14 for an association between the analyzed SNP (minor allele frequency 0.175) and IS, assuming a multiplicative genetic model.

Results

Baseline characteristics and the genotype frequencies for the SNP rs12425791 are presented in the Table. Genotype distributions did not differ significantly from those predicted by Hardy-Weinberg equilibrium, and the genotyping success rate was 98%.

No association between rs12425791 and IS was detected in any of the 3 case-control samples separately or in the combined sample (Table). The odds ratio for IS for the minor allele in the combined sample was 1.02 (95% CI, 0.93 to 1.13) after adjustment for age and sex. After also adjusting for hypertension, diabetes mellitus, and current smoking, this odds ratio was 1.04 (95% CI, 0.94 to 1.15). Because stroke subtyping has not been performed in all samples, we did not investigate the etiologic subtypes separately.

Discussion

The present study did not detect an association between the SNP rs12425791 and IS in any of the 3 case-control samples or in the combined sample of 3606 patients with IS and 2528 controls. Thus, the association reported by Ikram et al[1] and Matsushita et al[4] could not be replicated. On the contrary, results from the present study support the results from the large meta-analysis performed by ISGC and WTCCC2 including 8915 patients with IS and 30 510 controls of European ancestry.[2] In the latter study, no association between either rs12425791 or rs11833579 and IS could be detected.[2] This was also true when analyzing atherothrombotic stroke. Furthermore, in the same study, no association between rs12425791 and IS was reported in cases and controls of black ancestry or in samples from Chinese and Pakistani subjects.[2] In addition, the lack of association between IS, as well as atherothrombotic stroke, and genetic variants on chromosome 12p13 was recently reported in a small Italian case-control sample with 419 young patients (<65 years).[3]

The present study had 98% power to detect an association with an effect size of 1.21, the lower limit of the 95% CI in the original report.[1] However, even though an association was not detected in the present study, an association with a lower odds ratio cannot be completely excluded. Also, the study by Ikram et al[1] was based on prospective cohorts, and one might argue that an association of the SNP with fatal IS could explain the lack of association in our case-control study.[1] However, we find this unlikely, as both the present study and the study by ISGC and WTCCC2 contained prospective cohorts.[2] Furthermore, 1 of the samples in the present study consisted of relatively young patients, who have a low case fatality.[7] In addition, the minor allele frequency detected in our study (0.17) is similar to that reported in the genome-wide association study performed by the Cohorts for Heart and Aging Research Consortium in Genetic Epidemiology (0.19).[1]

In conclusion, no association between the SNP rs12425791 on chromosome 12p13 and IS could be detected in 3 large, independent samples from the southwest of Sweden, which supports findings from the recent meta-analysis by ISGC and WTCC2.[2] Our results further underscore the importance of independent replication of novel genetic findings.

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Disclosures

None.

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