A genetic variant on chromosome 12p13 does not show association to ischemic stroke in three Swedish case-control studies

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Published in Stroke. 2011;42:214-6.
http://stroke.ahajournals.org/cgi/pmidlookup?view=long&pmid=21148441

Abstract

Background and purpose *In a genome-wide association study, and subsequent case-control studies the SNP 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 if an association between ischemic stroke and rs12425791 could be detected in three different case-control studies from the Southwest of Sweden.*

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

Results *No significant association between ischemic stroke and the SNP rs12425791 was detected in any of the three case-control samples or in the samples combined. Odds ratio for ischemic stroke for the minor allele in the combined sample was 1.02 (95% confidence interval 0.93-1.13).*

Conclusions

The SNP rs12425791 does not confer a substantial risk for ischemic stroke in our population. Our results support a recent large study including other European populations.

Background

The Cohorts for Heart and Aging Research Consortium in Genetic Epidemiology (CHARGE) consortium performed a genome-wide association study (GWAS) of incident ischemic stroke (IS) and reported an association between IS and two 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 both in an African-American and in 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 one population-based genome-wide cohort 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 two 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 if an association between IS and the SNP rs12425791 could be detected in three different IS case-control samples from the Southwest of Sweden.

Material and Methods

All three study populations, the Lund Stroke Register (LSR), the Malmö Diet and Cancer study (MDC), and the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) were from the Southwest of Sweden. Sample characteristics, data collection, and clinical definitions including risk factor definitions have been described elsewere. [5-7]

Briefly, LSR is a prospective, epidemiologic study which consecutively includes patients with first-ever stroke from the local area of Lund. Controls without stroke are randomly selected from the same geographical region.[6]

MDC is a prospective, population-based cohort study which 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]

SAHLSIS, 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 four stroke units in Western Sweden. Controls without cardiovascular disease were randomly

selected from the same geographical region as the patients.[7]

In all three studies, the diagnosis of IS was ascertained in accordance with WHO criteria, and verified by computed tomography (CT) 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 prior to enrolment. For participants who were unable to communicate, consent was obtained from their next-of-kin.

Genotyping was performed, blinded to case-control status, using matrix-assisted laser desorption ionisation time of flight mass spectrometry on the MassARRAY platform using the iPLEX genotyping technology (Sequenom, San Diego, CA, USA).

Associations between the SNP rs12425791 and case/control status were investigated using 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 (OR) of 1.14 for an association between the analyzed SNP (minor allele frequency (MAF) 0.175) and IS, assuming a multiplicative genetic model.

Results

Baseline characteristics and the genotype frequencies for the SNP rs12425791 are presented in Table 1. Genotype distributions did not differ significantly from those predicted by the Hardy-Weinberg equilibrium, and the genotyping success rate was 98%.

No association between rs12425791 and IS was detected in any of the three case-control samples separately, or in the combined sample (Table 1). The OR for IS for the minor allele in the combined sample was 1.02 (95% confidence interval (CI) 0.93-1.13) after adjustment for age and sex. After adjusting also for hypertension, diabetes mellitus, and current smoking this OR was 1.04 (95% CI 0.94-1.15). Because stroke subtyping has not been performed in all samples, we did not investigate the etiological subtypes separately.

Discussion

The present study did not detect an association between the SNP rs12425791 and IS in any of the three 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 8 915 patients with IS and 30 510 controls with 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 African-American ancestry or in samples from Chinese and Pakistani subjects.[2] In addition, 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% confidence interval in the original report.[1] However, even if an association was not detected in the present study an association with lower OR can not be completely excluded. Also, the study by Ikram et al is based on prospective cohorts, and one might thus

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 contain prospective cohorts.[2] Furthermore, one of the samples in the present study consists of relatively young patients, who have a low case fatality.[7] In addition, the MAF detected in our study (0.17) is similar to that reported in the GWAS performed by the CHARGE consortium (0.19).[1]

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

Acknowledgements
DNA isolation and biobank services were performed at Region Skåne Competence Centre (RSKC Malmö), Skåne University Hospital, Malmö, Sweden.

Sources of Funding
Supported by grants from the Swedish Research Council (K2008-65X-14605-06-03, K2007-61X-20378-01-3, K2010-61X-20378-04-3), the Swedish state (ALFBGB-11206), the Swedish Heart and Lung Foundation (20070404), the Yngve Land Foundation for Neurological Research, the Crafoord Foundation, Region Skåne, the Freemasons lodge of instruction EOS in Lund, Lund University, the Department of Neurology, Lund, the Swedish Stroke Association, the Tore Nilsson Foundation, and the Emelle Foundation.

Disclosure
None

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Table 1. Baseline characteristics for the three case-control samples as well as genotype frequencies and odds ratios for the SNP rs12425791.

	LSR		MDC		SAHLSIS		Combined	
	Control	IS	Control	IS	Control	IS	Control	IS
	n=960	n=1865	n=900	n=897	n=668	n=844	n=2528	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)
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)
MAF	0.17	0.16	0.16	0.17	0.17	0.19	0.17	0.17
OR 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; SD, standard deviation; MAF, minor allele frequency. Odds ratios (OR) were calculated using an additive model adjusted for age and sex