Genetic variation on chromosome 9p21 shows association with the ischemic stroke subtype large-vessel disease in a Swedish sample aged ≤70 years

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Abstract

Background*: The aim of this study was to investigate whether we could replicate a recent finding of an association between genetic variants on chromosome 9p21 and the ischemic stroke (IS) subtype large-vessel disease (LVD).*

Methods: *The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) comprises 844 IS patients, who suffered IS before reaching the age of 70 years, and 668 healthy controls. IS subtype was defined according to the TOAST criteria, and 111 patients were categorized as LVD. Seven SNPs on 9p21 were analyzed. Functional outcome was assessed three months after IS using the modified Rankin Scale.*

Results: *The SNP rs7857345 showed a significant association with the subtype LVD independent of traditional vascular risk factors. In addition, an association between rs7857345 and functional outcome after LVD was observed.*

Conclusion: *In this relatively young sample of patients with IS, genetic variation on 9p21 is associated with LVD.*

Introduction

Genome-wide association studies have identified a locus for risk of coronary artery disease on chromosome 9p21 [1]. Recent studies have also analyzed the association between 9p21 and overall ischemic stroke (IS), with diverse outcomes, although this association was evident in larger cohorts [2, 3]. Stroke is a heterogeneous disease and there are different etiologic subtypes of IS. A recent study by Gschwendtner *et al.* [2] revealed an association between 9p21 and IS that was confined to the subtype large-vessel disease (LVD), also denoted arteriosclerotic stroke. The aim of the present study was to investigate if we could replicate this finding in a Swedish sample of relatively young (≤70 years of age) individuals. A second aim was to investigate whether variants on 9p21 are associated with functional outcome after IS.

Methods

The study population comprises patients (*n*=844) and healthy controls (*n*=668) from the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), the design of which has been reported [4]. Briefly, patients with first-ever or recurrent acute IS before reaching the age of 70 years were consecutively recruited. The upper age limit was chosen based on studies that have

indicated that the genetic contribution is greater in patients with stroke at a younger age [5]. The patients were classified into etiologic IS subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [4]. Information on the subjects' vascular risk factors was collected as described [4]. Functional outcome 3 months after IS was assessed according to the modified Rankin Scale (mRS) for the first 600 consecutive patients (LVD, *n*=73). Written informed consent from participants, and approval by the Ethics Committee of the University of Gothenburg were obtained.

Using genotype data from the HapMap project (release 23a), a 44-kb region on 9p21, between positions 22071397 and 22115503, was tagged (r^2=0.8 and MAF>0.1). This resulted in six tagSNPs (Table 2). As rs1537378 was the lead SNP in the study by Gschwendtner *et al.* [2], this SNP was also analyzed. Assuming a multiplicative genetic model, the odds ratios (ORs) that can be detected with 80% power at the 5% level are in the range of 1.22 to 1.31, depending on the frequency (MAF 0.5-0.15) of the high risk allele for overall IS in the present sample. For LVD, the corresponding ORs are 1.50-1.63 for the same frequencies. (See details in supporting information about the study population, and genetic analyses.)

Associations between single SNPs and case/control status or functional outcome (mRS 0-2 versus mRS 3-6) were investigated using an additive model in binary logistic regression, primarily adjusted for age and sex (model 1). In a second model, hypertension, diabetes mellitus, and smoking were also included as covariates (model 2). Associations to outcome in the LVD group were investigated using exact binary logistic regression, adjusted for age and sex. Haplotype analysis was performed with THESIAS.

Results

Baseline characteristics are shown in Table 1. The distribution of the four major etiologic IS subtypes was as follows: LVD, *n*=111; small-vessel disease, *n*=165; cardioembolic stroke, *n*=151; and cryptogenic stroke, *n*=206.

The A allele of SNP rs7857345 showed a significant association with decreased risk of LVD after adjustment for age and sex (Table 2). This association remained after adjustment also for traditional risk factors, and in this analysis an association with LVD was also detected for rs10965227 (Table 2). In contrast, no significant associations were found for the other three IS subtypes (data not shown) or overall IS (Table 2). Haplotype analysis confirmed the single locus results without adding further information (data not shown).

Among LVD patients with mRS data at 3 month, 20 were dead or dependent, whereas 48 had a favorable outcome. The minor alleles of rs7857345 and rs1537378 were associated with a decreased risk of death or dependency after adjustment for age and sex (OR 0.25, 95% CI 0.06-0.78, *p*=0.01 and OR 0.29, 95% CI 0.09-0.74, *p*=0.005 respectively). For overall IS, no association between genetic variants and functional outcome was detected.

Discussion

The results from the present study with relatively young participants are consistent with a prior study by Gschwendtner *et al.* where an exclusive association between the IS subtype LVD and 9p21 was reported [2], although the significant SNPs differ. The lead SNP in the study by Gschwendtner *et al.*, rs1537378, and the SNP showing association with LVD in the present study, rs7857345, are located in different LD blocks, as defined in the HapMap CEU population using the method solid spine of LD. However, in the study by Gschwendtner et al, the SNP rs7857345 was not included, but two SNPs located in the same LD block as rs7857345, i.e.

rs2383207 and rs10757278, were found to be associated with LVD [2]. This implies that the association noted between the SNPs on 9p21 and LVD in the two different studies may represent either the same signal or different signals that give similar effects. The specific SNP within the region with the strongest association may reflect some intrinsic difference between the studied samples.

In a study, with somewhat older participants, by Lemmens *et al.* [6], one SNP on 9p21 was investigated (rs10757278). No significant association was found between this SNP and cerebrovascular disease (IS or TIA, *n*=648) or LVD (*n*=179). These results are in accordance with those of the present study (Table 2).

To our knowledge, this is the first study investigating whether genetic variation on 9p21 is associated with functional outcome after IS. The A allele of rs7857345 that showed a decreased risk for LVD, also showed association to a decreased risk of death or dependency. This result needs to be verified in additional studies.

The present results, and those of Gschwendtner *et al.*, suggest that genetic variation on 9p21 affects the atherosclerotic process. However, since there are studies showing association between 9p21 and intracranial aneurysms [*e.g.* 7], and there are studies reporting lack of association between 9p21 and carotid intima-media thickness [*e.g.* 8], 9p21 genetic variants may exert more general effects on arterial wall function.

In conclusion, the present study of a relatively young Swedish population confirms the association between genetic variation on chromosome 9p21 and the IS subtype of LVD. These findings emphasize the need to investigate the etiologic subtypes of IS in genetic studies.

Disclosure of conflict of interest

No conflict of interest.

Disclosure of sources of funding

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Table 1. Baseline characteristics of the control, large-vessel disease, and overall ischemic stroke groups.

	Control (*n*=668)	Large-vessel disease (*n*=111)	Ischemic stroke[a] (*n*=844)
Mean age, years (SD)	56 (10)	60 (7)[c]	56 (11)
Male, *n* (%)	392 (59)	84 (76)[c]	554 (66)[b]
Hypertension, *n* (%)	230 (34)	72 (65)[c]	487 (58)[c]
Diabetes mellitus, *n* (%)	33 (5)	38 (34)[c]	153 (18)[c]
Current smoking, *n* (%)	131 (20)	58 (53)[c]	324 (39)[c]

[a]Overall ischemic stroke includes the subtype of large-vessel disease; SD, standard deviation; *n*, numbers. [b]$p<0.01$ and [c]$p<0.001$, compared with the control group. Differences between the patients and the controls were examined with the χ^2 test for proportions, and with the Mann-Whitney U test for continuous variables.

Table 2. Genotype frequencies in the control, large-vessel disease, and overall ischemic stroke groups, with the odds ratios for the associations between the genetic variants and large-vessel disease and overall ischemic stroke.

Genotype and OR	Control (*n*=668)	Large-Vessel disease (*n*=111)	Ischemic stroke[a] (*n*=844)
rs10965227			
AA, *n* (%)	420 (63)	63 (58)	538 (64)
AG, *n* (%)	221 (33)	34 (31)	258 (31)
GG, *n* (%)	25 (4)	12 (11)	43 (5)
OR (95% CI)[b]	1.00	1.39 (1.00-1.94)	1.00 (0.84-1.20)
OR (95% CI)[c]	1.00	1.57 (1.06-2.34)[d]	1.04 (0.86-1.25)
rs1547705			
AA, *n* (%)	538 (83)	88 (82)	679 (83)
AC, *n* (%)	107 (16)	19 (18)	131 (16)
CC, *n* (%)	6 (1)	0 (0)	6 (1)
OR (95% CI)[b]	1.00	0.99 (0.59-1.65)	0.97 (0.75-1.25)
OR (95% CI)[c]	1.00	1.02 (0.54-1.93)	0.92 (0.69-1.21)
rs1333040			
AA, *n* (%)	177 (28)	33 (32)	248 (31)
AG, *n* (%)	314 (49)	49 (48)	392 (49)
GG, *n* (%)	150 (23)	21 (20)	163 (20)
OR (95% CI)[b]	1.00	0.86 (0.64-1.16)	0.88 (0.76-1.02)
OR (95% CI)[c]	1.00	0.82 (0.58-1.16)	0.88 (0.75-1.04)
rs7857345			
GG, *n* (%)	285 (43)	63 (58)	403 (48)
AG, *n* (%)	304 (46)	38 (35)	351 (42)
AA, *n* (%)	77 (12)	8 (7)	86 (10)
OR (95% CI)[b]	1.00	0.61 (0.44-0.86)[e]	0.86 (0.74-1.00)
OR (95% CI)[c]	1.00	0.58 (0.39-0.86)[e]	0.88 (0.74-1.04)
rs1333045			
GG, *n* (%)	159 (24)	33 (30)	236 (28)
AG, *n* (%)	349 (52)	54 (50)	419 (50)
AA, *n* (%)	158 (24)	22 (20)	183 (22)
OR (95% CI)[b]	1.00	0.80 (0.60-1.08)	0.88 (0.76-1.02)
OR (95% CI)[c]	1.00	0.81 (0.57-1.16)	0.91 (0.78-1.07)
rs10757278			
AA, *n* (%)	191 (29)	31 (28)	222 (27)
AG, *n* (%)	342 (51)	52 (48)	415 (50)
GG, *n* (%)	132 (20)	26 (24)	197 (24)
OR (95% CI)[b]	1.00	1.12 (0.83-1.50)	1.13 (0.98-1.31)
OR (95% CI)[c]	1.00	1.15 (0.82-1.63)	1.10 (0.94-1.28)
rs1537378			
GG, *n* (%)	202 (30)	43 (39)	274 (33)
AG, *n* (%)	336 (50)	47 (43)	426 (51)
AA, *n* (%)	128 (19)	19 (17)	139 (17)
OR (95% CI)[b]	1.00	0.82 (0.61-1.11)	0.90 (0.78-1.04)
OR (95% CI)[c]	1.00	0.80 (0.57-1.13)	0.91 (0.77-1.07)

OR, Odds ratio; CI, confidence interval. Binary logistic regression using an additive model. [a]Overall ischemic stroke includes the subtype large-vessel disese; [b]model 1 (adjusted for age and sex); [c]model 2 (adjusted for age, sex, hypertension, diabetes mellitus, and smoking); [d]$p<0.05$; [e]$p<0.01$

Supporting information:

Genetic variation on chromosome 9p21 shows association with the ischaemic stroke subtype large-vessel disease in a Swedish sample aged ≤70 years

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Methods

Study population

The study population comprised Caucasian patients (*n*=844) who participated in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) [1]. Patients who presented with first-ever or recurrent acute IS before reaching the age of 70 years were recruited consecutively between 1998 and 2008 at four stroke units in Western Sweden. Healthy Caucasian controls (*n*=668), from the same geographical region as the patients, were randomly selected from participants in a population-based health survey or the Swedish Population Registry. All patients underwent neuroimaging with CT and/or MRI. The patients were classified into IS etiologic subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into the etiologic categories large-vessel disease, small-vessel disease, cardioembolic stroke, other determined cause of stroke, cryptogenic stroke, and undetermined stroke. Cryptogenic stroke was defined when no cause was identified despite an extensive evaluation. Undetermined stroke included cases for which more than one cause was identified or when the evaluation was cursory. Stroke of other determined cause (*n*=92) and undetermined stroke (*n*=119) was not included in the subtype analysis in the present study. Adjudication of subtypes was performed by two neurologists (K.J., C.B.). Hypertension was defined by pharmacological treatment for hypertension, systolic blood pressure ≥160 mm Hg, and/or diastolic blood pressure ≥90mm Hg. Diabetes mellitus was defined by diet or pharmacological treatment, fasting plasma glucose ≥7.0 mmol/L, or fasting blood glucose ≥6.1 mmol/L. Smoking history was coded as current versus never or former (smoking cessation at least one year before inclusion in the study). Functional outcome 3 months after IS was assessed according to the modified Rankin Scale (mRS) for the first 600 consecutive patients (missing scores for 31 IS patients), including 73 patients with LVD (missing scores for 5 LVD patients). As previously described [1], the mRS score was dichotomized for death or dependency (mRS 3-6) versus a favourable outcome (mRS 0-2). This study was approved by the Ethics Committee of the University of Gothenburg. All participants gave their written informed consent. Next-of-kin consented for those participants who were unable to communicate.

Genetic analysis

Genotyping was performed as a part of the analysis of a larger panel of SNPs using the Golden Gate assay (Illumina Inc., San Diego, CA, USA). The SNP assay for rs10757278 failed, so this SNP was genotyped using the TaqMan SNP Genotyping Assay (assay C_11841860_10, Applied Biosystems, Foster City, CA, USA). Genotyping was performed by the SNP Technology Platform at the Uppsala University and the Uppsala University Hospital (www.genotyping.se) and at the Genomics Core Facility platform at the Sahlgrenska Academy, University of Gothenburg (www.cf.gu.se/Genomics). The genotyping success rates were between 95.5% and 99.6%. All SNPs genotyped were in Hardy–Weinberg equilibrium ($p>0.05$).

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