Genetic variation in complement component C3 shows association with ischemic stroke

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Abstract
Background*: The aim of this study was to investigate whether genetic variation at the third complement component (C3) locus is associated with ischemic stroke (IS).*
Methods: *The Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) comprises 844 patients with IS, and 668 healthy controls. Sixteen SNPs were analyzed.*
Results: *Two SNPs, rs2277984 and rs3745565, showed a significant association with overall IS. The SNP rs2277984 also showed association with the IS subtype cryptogenic stroke. These associations were independent of hypertension, diabetes, and smoking. The independent association between rs3745565 and overall IS withstand correction for multiple testing.*
Conclusion: *In this sample of patients with IS, genetic variation in C3 is associated with IS.*

Background

Evidence indicates that inflammation plays a role in the pathophysiology of stroke [1], and activation of the complement system after ischemic stroke (IS) has been reported. Components of the complement cascade (C1q, C3c and C4d) and terminal complexes (C5b-9) have been detected in cerebral infarcts [2, 3], and inhibition of complement activation has been observed to increase infarct volume and decrease neurogenesis after transient cerebral ischemia in mice [4]. C3 is the most abundant complement protein and plays a central role in the activation of the complement system [5], but little is known about its specific role in stroke. The aim of the present study was to investigate whether variation at the *C3* locus is associated with IS.

Methods

Study population
The study population comprised patients (n=844) who participated in the Sahlgrenska Academy Study on Ischemic

Stroke (SAHLSIS), the design of which has been reported [6, 7]. Briefly, patients who presented with first-ever or recurrent acute IS before reaching the age of 70 years were recruited consecutively at Stroke Units in western Sweden between 1998 and 2008. The upper age limit of the participants was chosen based on studies that have indicated that the genetic contribution is greater in patients who experience a stroke at a younger age [8]. Healthy community-based controls (n=668) were randomly selected as described [6]. The patients were classified into IS etiologic subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [6]. Written informed consent from participants, and approval by the Ethics Committee of the University of Gothenburg were obtained.

In the C3 gene, 16 SNPs were analyzed, including rs344555, which has previously been shown to associate with C3 plasma levels [9]. Assuming a multiplicative genetic model, the odds ratios (ORs) for overall IS that can be detected with 80% power at the 5% level are in the range of 1.22 to 1.34, depending on the minor allele frequency (MAF 0.48-0.12) in the present sample.

Associations between single SNPs and case/control status were investigated using an additive model in binary logistic regression, primarily adjusted for age and sex. Haplotype analysis was performed with HelixTree 6.3 (Golden Helix, Inc.). The presented *P*-values are crude uncorrected *P*-values. (See details in Supporting information about the study population, genetic analysis, and LD structure of the analyzed region.)

Results

Baseline characteristics of the present study population have been described previously [6, 7], and are summarized in the supporting information. All genotype distributions were compatible with the Hardy-Weinberg equilibrium ($p>0.05$), and the genotyping success rates were 98-100%.

ORs for the associations between the SNPs and IS are presented in Table 1. Genotype frequencies are presented in the supporting information. The SNPs rs2277984 and rs3745565 showed significant associations with IS. These associations remained significant after adjustment also for hypertension, diabetes, and smoking (OR 1.20, 95% CI 1.02-1.41, *P*=0.02 and OR 0.73, 95% CI 0.59-0.92, *P*=0.006, respectively). Haplotype analysis did not add any further information (data not shown). In our sample, the 16 SNPs are located in five LD blocks (See the Supporting information). After correcting for multiple testing by multiplying the crude *P*-values by the number of LD blocks, the multivariate association between rs3745565 and overall IS remained significant (*P*=0.03).

The SNPs that showed significant associations to overall IS were tested in each of the four main etiologic IS subtypes (Table 2). In this analysis, an association between rs2277984 and the subtype of cryptogenic stroke was observed. This association was also independent of hypertension, diabetes, and smoking (OR 1.37, 95% CI 1.07-1.74, *P*=0.01). However, this association does not remain after adjusting for multiple testing as described above.

Discussion

This case-control study is the first to present evidence of an association between genetic variation at the *C3* locus and IS.

No previous study has thoroughly investigated the effect of variations in the C3 gene on stroke. In the current study, the 16 SNPs analyzed tag 85% of the tagSNPs reported in HapMap (Rel 24) in the *C3* locus. Interestingly, rs2277984 and

rs3745565, showed associations with IS independent of vascular risk factors. However, only the multivariate association with rs3745565 remained significant after adjustment for multiple testing. Both rs2277984 and rs3745565 are intronic variations and are not linked to any known functional SNP(s). In contrast, no association was detected for rs2230199, a non-synonymous SNP in exon three. This is in line with previous studies, in which only rs2230199 was analyzed [10, 11]. Lack of association between cerebrovascular disease and the electrophoretic variations C3S and C3F (corresponding to rs2230199) has also been reported in an European study [12].

Because IS is a heterogenic disease we also investigated rs2277984 and rs3745565 in the different etiologic IS subtypes. We found a weak association between rs2277984 and cryptogenic stroke, which did not remain after adjustments for multiple tasting. If this is a subtype-specific effect of C3 gene variation or a lack of statistical power in the smaller subgroups remains to be determined.

In conclusion, the present study shows an association between genetic variation in *C3* and IS. As this is the first study showing such an association replication is necessary.

Disclosure of conflict of interest

No conflict of interest.

Disclosure of sources of funding

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Table 1. Odds ratios for the associations between the genetic variants at the *C3* locus and overall ischemic stroke.

SNP	MAF Controls	MAF Cases	OR (95% CI)	*P*-value
rs344555	0.19	0.20	1.03 (0.86-1.24)	0.73
rs2277984	0.45	0.49	1.18 (1.02-1.37)	**0.024**
rs344550	0.31	0.33	1.10 (0.94-1.29)	0.24
rs2241393	0.42	0.42	0.98 (0.85-1.14)	0.80
rs344548	0.17	0.18	1.10 (0.91-1.34)	0.33
rs2241392	0.39	0.39	0.99 (0.86-1.15)	0.93
rs237554	0.13	0.14	1.16 (0.94-1.43)	0.18
rs344540	0.38	0.41	1.11 (0.96-1.29)	0.16
rs3745568	0.13	0.14	1.10 (0.89-1.36)	0.40
rs3745565	0.16	0.14	0.81 (0.66-1.00)	**0.045**
rs423490	0.26	0.28	1.11 (0.93-1.31)	0.25
rs408290	0.42	0.42	1.00 (0.86-1.16)	1.00
rs11569450	0.11	0.11	0.99 (0.78-1.24)	0.90
rs11672613	0.38	0.37	0.98 (0.84-1.13)	0.75
rs2230205	0.15	0.15	1.02 (0.83-1.25)	0.85
rs2230199	0.19	0.19	1.00 (0.84-1.21)	0.96

OR, odds ratio; CI, confidence interval; MAF, minor allele frequency. Binary logistic regression using an additive model adjusted for age and sex. The *P*-values presented are the crude *P*-values.

Table 2. Odds ratios for the associations between the *C3* SNPs rs2227798 and rs3745565 and the four main etiologic subtypes of ischemic stroke.

SNP	Crypt. n=206	LVD n=111	SVD n=165	CE n=151
	OR (95% CI), *P*-value	OR (95% CI), *P*-value	OR (95% CI), *P*-value	OR (95% CI), *P*-value
rs2277984	1.37 (1.09-1.72), **0.007**	1.27 (0.95-1.70), 0.11	1.01 (0.79-1.30), 0.93	1.21 (0.94-1.56), 0.14
rs3745565	0.74 (0.53-1.04), 0.08	0.91 (0.60-1.37), 0.65	0.72 (0.49-1.04), 0.08	0.83 (0.57-1.20), 0.31

OR, odds ratio; CI, confidence interval; Crypt., cryptogenic stroke; LVD, large-vessel disease; SVD, small-vessel disease; CE, cardioembolic stroke. Binary logistic regression using an additive model adjusted for age and sex. The *P*-values presented are the crude *P*-values.

Supporting information:

Genetic variation in complement component C3 shows association with ischemic stroke

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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, 2]. 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. Additional diagnostic work-up was performed when clinically indicated, as described previously [1]. Baseline characteristics are shown in Table 1S. The patients were classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into the IS etiologic categories large-vessel disease (n=111), small-vessel disease (n=165), cardioembolic stroke (n=151), other determined cause of stroke (*n*=92), cryptogenic stroke (n=206), and undetermined stroke (*n*=119). Undetermined stroke included cases for which more than one cause was identified or when the evaluation was cursory. Stroke of other determined cause and undetermined stroke were not included in the subtype analysis in the present study. 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). All participants gave their written informed consent. Next-of-kin consented for those participants who were unable to communicate.

Genetic analysis

In *C3* locus 16 SNPs including rs344555, which has previously been shown to associate with C3 plasma levels [3], were selected (Table 2S). Together they cover 85% of all the HapMap tag SNPs in the gene (dbSNP b126). The SNPs were selected to cover a large amount of the variations in the gene using as few SNPs as possible. 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 rs3745565 failed, so this SNP was genotyped using the TaqMan SNP Genotyping Assay (assay C_27510821_10, Applied Biosystems, Foster City, CA, USA). The genotyping was performed blinded to case/control status. 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 presented *P*-values are crude uncorrected *P*-values. Correction for multiple testing was done by multiplying the *P*-values by the number of LD blocks. We think that Bonferroni correction would be too conservative as several of the SNPs are situated in the same LD blocks (Figure 1S).

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Table S1. Baseline characteristics of the control, and overall ischemic stroke groups.

	Control (*n*=668)	Ischemic stroke (*n*=844)	
Mean age, years (SD)	56 (10)	56 (11)	
Male, n (%)	392 (59)	554 (66)[a]	
Hypertension, n (%)	230 (34)	487 (58)[b]	
Diabetes mellitus, n (%)		33 (5)	153 (18)[b]
Current smoking, n (%)		131 (20)	324 (39)[b]

SD, standard deviation; n, numbers. [a]$p<0.01$ and [b]$p<0.001$, compared with the control group. Differences between patients and controls were examined with the χ^2 test for proportions, and with the Mann-Whitney U test for continuous variables

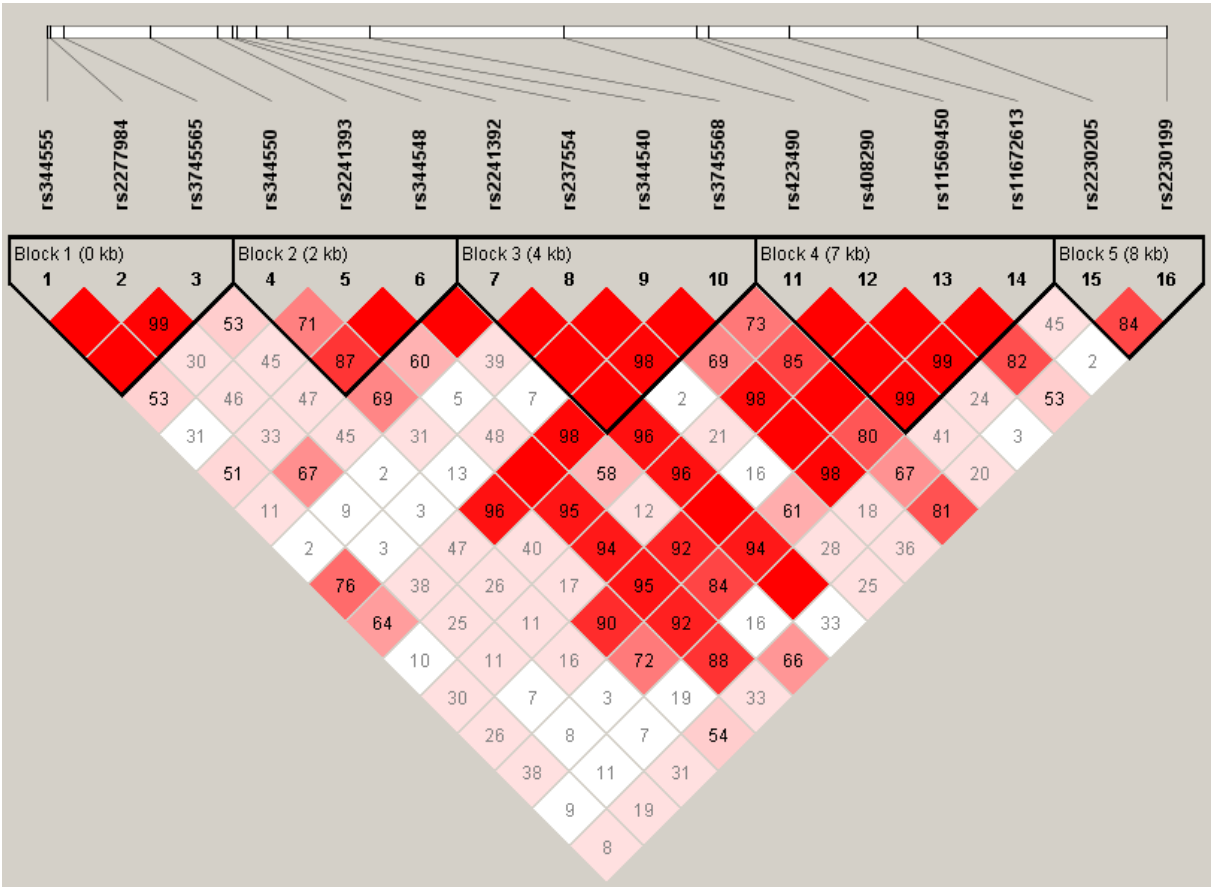

Figure 1S. Graphic representation of the linkage diseq uilibrium (LD) structure in the C3 gene for the analyzed SNPs. The LD blocks were defined using Haploview 4.1 and the method solid spine of LD.

Table S2. Genotype frequencies in the control and overall ischemic stroke groups.

SNP	Genotype	Control (*n*=668)	Ischemic stroke (*n*=844)
rs344555	GG, *n* (%)	438 (66)	540 (64)
	AG, *n* (%)	203 (30)	271 (32)
	AA, *n* (%)	25 (4)	29 (3)
rs2277984	AA, *n* (%)	198 (30)	208 (25)
	AG, *n* (%)	335 (50)	436 (52)
	GG, *n* (%)	133 (20)	196 (23)
rs344550	GG, *n* (%)	311 (47)	376 (45)
	CG, *n* (%)	299 (45)	374 (45)
	CC, *n* (%)	55 (8)	89 (11)
rs2241393	CC, *n* (%)	217 (33)	285 (34)
	CG, *n* (%)	336 (50)	406 (48)
	GG, *n* (%)	113 (17)	148 (18)
rs344548	CC, *n* (%)	456 (68)	563 (67)
	CG, *n* (%)	198 (30)	250 (30)
	GG, *n* (%)	12 (2)	27 (3)
rs2241392	CC, *n* (%)	246 (37)	309 (37)
	CG, *n* (%)	314 (47)	399 (47
	GG, *n* (%)	106 (16)	132 (16)
rs237554	GG, *n* (%)	502 (75)	615 (73)
	AG, *n* (%)	155 (23)	205 (24)
	AA, *n* (%)	8 (1)	19 (2)
rs344540	GG, *n* (%)	249 (37)	303 (36)
	AG, *n* (%)	327 (49)	389 (46)
	AA, *n* (%)	90 (14)	148 (18)
rs3745568	AA, *n* (%)	507 (76)	627 (75)
	AC, *n* (%)	150 (23)	194 (23)
	CC, *n* (%)	9 (1)	18 (2)
rs3745565	GG, *n* (%)	462 (70)	613 (75)
	CG, *n* (%)	189 (29)	178 (22)
	CC, *n* (%)	12 (2)	21 (3)
rs423490	GG, *n* (%)	355 (53)	441 (52)
	AG, *n* (%)	280 (42)	335 (40)
	AA, *n* (%)	31 (5)	64 (8)
rs408290	CC, *n* (%)	225 (34)	277 (33)
	CG, *n* (%)	326 (49)	425 (51)

	GG, *n* (%)	115 (17)	138 (16)
rs11569450	CC, *n* (%)	530 (80)	665 (79)
	CG, *n* (%)	127 (19)	168 (20)
	GG, *n* (%)	9 (1)	7 (1)
rs11672613	AA, *n* (%)	260 (39)	326 (39)
	AG, *n* (%)	309 (46)	404 (48)
	GG, *n* (%)	97 (15)	110 (13)
rs2230205	GG, *n* (%)	488 (73)	600 (71)
	AG, *n* (%)	159 (24)	226 (27)
	AA, *n* (%)	19 (3)	14 (2)
rs2230199	GG, *n* (%)	434 (65)	541 (64)
	CG, *n* (%)	203 (30)	268 (32)
	CC, *n* (%)	28 (4)	29 (3)