Lack of association between genetic variations in the *KALRN* region and ischemic stroke

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Abstract
Objectives: *To investigate whether KALRN gene variation is associated with ischemic stroke (IS).*
Design and methods*: Associations to overall IS and IS subtypes were investigated in SAHLSIS, which comprises 844 patients with IS and 668 controls.*
Results: *Associations between KALRN SNPs and overall IS and cardioembolic stroke were detected. Associations for overall IS were investigated in two additional Swedish samples, but could not be replicated.*
Conclusion: *KALRN gene variation is not associated with overall IS.*

Introduction

A susceptibility locus for coronary artery disease (CAD) has been mapped to chromosome 3q13 [1] and a fine mapping of this region identified the kalirin gene (*KALRN*) as a candidate gene [2]. Recently, an association between genetic variations in the *KALRN* region and ischemic stroke (IS) was reported [3]. Kalirin is a brain specific Rho guanine nucleotide exchange factor (RhoGEF).

The aim of the present study was to investigate if genetic variations in the KALRN region are associated with IS and/or IS subtypes.

Methods

The primary study was conducted on patients (*n*=844) and healthy controls (*n*=668) who participated in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), the design of which has been reported [4]. Briefly, patients who presented with first-ever or recurrent acute IS before reaching the age of 70 years were consecutively recruited. Information on the subjects' vascular risk factors was collected as described [4,5]. The patients in SAHLSIS were classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into IS etiologic subtypes. The Lund Stroke Register (LSR) and the Malmö Diet and Cancer study (MDC), were used as replication samples. Sample characteristics, data collection and clinical definitions have been described [5-7]. Briefly, LRS is a prospective, epidemiologic register initiated in 2001 which consecutively includes all patients with first ever stroke from the area of Lund University Hospital. Controls were selected from the same region. MDC is a prospective, population based cohort study which included 28 449 randomly selected persons at baseline examinations between 1991 and 1996 from which all incident cases of IS up to 2006 were included and matched for age and sex with stroke free control subjects. Smoking status was coded as current versus never or former. Written informed consent from participants, and approval by the Ethics Committee of the University of Gothenburg or by the Ethics Committee of Lund University were obtained.

Six SNPs in the *KALRN* region were selected for analysis based on their reported association to CAD or IS [2,3]. Genotyping of one SNP, rs7620580, failed. Genotyping was performed using the GoldenGate assay (Illumina Inc., San Diego, CA, USA) or TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Associations between single SNPs and case/control status 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). 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 1.23-1.34, depending on the minor allele frequency (MAF) (0.46-0.09) of the risk allele for overall IS in SAHLSIS. (See details in Supplementary material about the genetic analyses.)

Results

Baseline characteristics for all three studies are presented in the Supporting information, genotype frequencies in Table 1, and odds ratios in Table 2. In SAHLSIS two SNPs, rs9289231 and rs13075202, showed association to IS, after adjustment for age and sex. These associations remained after adjustments for vascular risk factors. After exclusion of patients with a history of CAD (n=138), the association for rs9289231, but not for rs13075202, remained (OR 1.33 (1.03-1.71), p=0.03, and OR 1.18 (0.98-1.42), p=0.08 respectively).

In order to investigate whether these findings could be replicated, rs9289231 and rs13075202 were genotyped in LSR (1864 patients and 960 controls) and MDC (898 patients and 900 controls). No association between the SNPs and IS was detected in LSR or MDC (Table 2). Excluding patients older than 70 years did not alter the results (IS in LSR and MDC, n=1053, data not shown).

As an interaction between SNPs in KALRN and smoking status was detected in a study on CAD [8], we conducted smoking–stratified analysis in SAHLSIS. No significant interaction between genotype and smoking status was detected (data not shown).

To further explore the associations detected for rs9289231 and rs13075202, subtype-specific analyses were conducted in SAHLSIS. Associations between both rs9289231 and rs13075202 and cardioembolic (CE) stroke were detected (OR 1.68 (1.15-2.46), p=0.008, and OR 1.37 (1.02-1.85), p=0.04, respectively). In addition, associations between rs9289231 and the subtypes large-vessel disease and small-vessel disease were observed (OR 1.69 (1.09-2.62), p=0.02, and OR 1.56 (1.07-2.27), p=0.02, respectively). However, after also including vascular risk factors in the model, only the associations for CE stroke remained significant (rs9289231, OR 1.67 (1.13-2.50), p=0.03, and rs13075202, OR 1.45 (1.06-1.98), p=0.02). Analyses of replication of the subtype-specific associations were not possible as information about IS subtypes is not available for LSR and MDC,

Discussion

In the present case-control study, the analyzed SNPs in the KALRN region were not major contributors to IS. The associations between IS and two SNPs in the KALRN region detected in SAHLSIS could not be replicated in two other Swedish samples.

The lack of association in the replication samples is probably not due to the older age in these samples as excluding patients older than 70 years did not alter the results. One could speculate that the association with genetic variations in the KALRN region is restricted to CE stroke, as independent associations were detected for rs9289231 and rs13075202 and this subtype in SAHLSIS. However, this is unlikely as LSR and MDC include older patients than SAHLSIS, and thus the proportion of patients with CE stroke is higher in the two replication samples.

In a recent study by Krug et al., five SNPs in *KALRN* showed association with overall IS (565 patients and 517 controls) [3]. However, two of these SNPs (rs17286604, and rs11712039) did not show association with overall IS in the present study. Contradictory to previous findings on CAD [2], the SNPs rs7613868, rs9289231 and rs13075202 did not show independent association to overall IS in the aforementioned study by Krug et al. [3]. In the present study rs9289231 and rs13075202, but not rs7613868, showed associations with overall IS in SAHLSIS.

An interaction between SNPs in *KALRN* and smoking status was detected in a study on CAD [8], where an association between SNPs in *KALRN* and CAD was only detected among young patients who smoked. This interaction

could not be detected in the present study on IS.

In conclusion, the present study indicates that genetic variations in the *KALRN* region are not associated to IS. This is to our knowledge the largest association study on IS investigating several genetic variations at the *KALRN* locus, and the first analysing the specific subtypes of IS. As subtype-specific analyses were only conducted in one of the samples, further studies on specific IS subtypes are warranted.

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Conflict of interest statement

The authors declare that they have no conflict of interests.

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Table 1. Genotype frequencies

	SAHLSIS		LRS		MDC	
Genotype	Control (*n*=668)	Ischemic Stroke (*n*=844)	Control (*n*=960)	Ischemic stroke (*n*=1864)	Control (*n*=900)	Ischemic stroke (*n*=898)
rs7613868						
GG, *n* (%)	359 (54)	405 (48)	-	-	-	-
AG, *n* (%)	259 (39)	381 (45)	-	-	-	-
AA, *n* (%)	48 (7)	53 (6)	-	-	-	-
rs9289231						
AA, *n* (%)	556 (83)	656 (78)	791 (83)	1504 (81)	707 (81)	718 (83)
AC, *n* (%)	104 (16)	172 (21)	154 (16)	331 (18)	154 (18)	145 (17)
CC, *n* (%)	6 (1)	11(1)	8 (1)	16(1)	9 (1)	7 (1)
rs13075202						
AA, *n* (%)	392 (60)	444 (53)	557 (59)	1093 (59)	506 (59)	504 (58)
AG, *n* (%)	232 (36)	348 (42)	350 (37)	652 (35)	306 (35)	313 (36)
GG, *n* (%)	29 (4)	39 (5)	44 (5)	96 (5)	51 (6)	51 (6)
rs17286604						
GG, *n* (%)	222 (33)	270 (32)	-	-	-	-
AG, *n* (%)	319 (48)	401 (48)	-	-	-	-
AA, *n* (%)	124 (19)	162 (19)	-	-	-	-
rs11712039						
AA, *n* (%)	241 (37)	323 (39)	-	-	-	-
AG, *n* (%)	314 (48)	389 (47)	-	-	-	-
GG, *n* (%)	105 (16)	115 (14)	-	-	-	-

Abbreviations: SAHLSIS, the Sahlgrenska Academy Study on Ischemic Stroke; LSR, the Lund Stroke Register; MDC, Malmö Diet and Cancer study.

Table 2. Odds ratios for the association between genetic variations and overall ischemic stroke in SAHLSIS and two replication samples, LRS and MDC

Genotype	SAHLSIS	LRS	MDC
	668/844 [a]	960/1864	900/898
rs7613868			
OR (95% CI) [b]	1.14 (0.96-1.35)	-	-
rs9289231			
OR (95% CI)[b]	1.38 (1.08-1.75) [d]	1.10 (0.91-1.34)	0.92 (0.73-1.15)
OR (95% CI)[c]	1.32 (1.02-1.71)[d]	-	-
rs13075202			
OR (95% CI)[b]	1.23 (1.03-1.46) [d]	0.99 (0.87-1.14)	1.01 (0.87-1.19)
OR (95% CI)[c]	1.27 (1.05-1.53)[d]	-	-
rs17286604			
OR (95% CI) [b]	1.02 (0.87-1.18)	-	
rs11712039			
OR (95% CI) [b]	0.90 (0.78-1.05)	-	

SAHLSIS, the Sahlgrenska Academy Study on Ischemic Stroke; LSR, the Lund Stroke Register; MDC, Malmö Diet and Cancer study; OR, odds ratio; CI, confidence interval

[a]Number of controls/cases.

[b]Binary logistic regression using additive model adjusted for age and sex. OR for the minor alleles are shown.

[c]binary logistic regression using additive model adjusted for age, sex, hypertension, diabetes mellitus and smoking. OR for the minor alleles are shown.

[d]$p<0.05$