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Sequence Variants on Chromosome 9p21.3 Confer Risk of Atherosclerotic Stroke

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Abstract

Objective—Recent studies have identified a major locus for risk of coronary artery disease (CAD) and myocardial infarction (MI) on chromosome 9p21.3. Stroke, in particular ischemic stroke (IS) due to atherosclerotic disease, shares common mechanisms with MI. We investigated whether the 9p21 region contributes to IS risk.

Methods—In an initial screen, 15 single nucleotide polymorphisms (SNPs) covering the critical genetic interval on 9p21 were genotyped in samples from Southern Germany (1090 cases, 1244 controls) and the United Kingdom (758 cases, 872 controls, 3 SNPs). SNPs significantly associated with IS or individual stroke subtypes in either of the screening samples were subsequently genotyped in 2528 additional cases and 2189 additional controls from Europe and North America.

Results—Genotyping of the screening samples revealed associations between seven SNPs and atherosclerotic stroke (all $p < 0.05$). Analysis of the full sample confirmed associations between six SNPs and atherosclerotic stroke in multivariate analyses controlling for demographic variables, CAD, MI, and vascular risk factors (all $p < 0.05$). The odds ratios (OR) for the lead SNP

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(rs1537378-C) were similar in the various subsamples with a pooled OR of 1.21 (95%CI=1.07-1.37) under both fixed and random effects models ($p=0.002$). The point estimate for the population attributable risk is 20.1% for atherosclerotic stroke.

Interpretation—The chromosome 9p21.3 region represents a major risk locus for atherosclerotic stroke. The effect of this locus on stroke seems to be independent of its relationship to CAD and other stroke risk factors. Our findings support a broad role of the 9p21 region in arterial disease.

INTRODUCTION

Stroke is the second most frequent cause of death and a major cause of disability worldwide 1·2. Stroke is aetiologically heterogeneous. The majority of patients have ischemic stroke (IS) which can further be subdivided according to stroke mechanisms. Major categories include atherosclerotic (i.e. large artery) stroke, cardioembolic stroke and small vessel stroke. There is substantial evidence for a genetic contribution to IS risk 3. However, the responsible genetic variants are still largely unknown 4·5.

Recently, genomewide association studies have identified a major locus for risk of coronary artery disease (CAD) and myocardial infarction (MI) on chromosome 9p21.3 6·9. This locus was uniformly identified as the strongest genetic signal for CAD in four independent screens and was subsequently confirmed in additional cohorts 10·12. The consistency of this finding coupled with a high frequency of the risk allele has attracted great attention all the more since the risk contributed by 9p21 was found to be independent of conventional vascular risk factors.

A sequence variant (rs10757278-G) in the same chromosomal region was subsequently shown to be associated with both abdominal aortic aneurysms (AAA) and intracranial aneurysms (IA) suggesting an even broader role of the 9p21 region in arterial disease 13.

Stroke, in particular atherosclerotic stroke, shares common risk factors and pathophysiological mechanism with CAD and MI 14·15 thus rendering the 9p21 region a strong candidate for stroke risk. Only recently, several small studies have looked for an association between sequence variants on 9p21 and IS risk 13·16·17. These studies have been inconclusive due in part to limited sample size and because stroke subtypes such as atherosclerotic stroke were not considered separately.

Drawing on the resources of the International Stroke Genetics Consortium, we assembled one of the largest collections of IS patients to date to investigate whether genetic variation at 9p21.3 is associated with risk of IS and, in particular, the subtype of atherosclerotic stroke. Furthermore, as CAD and MI are themselves risk factors for stroke 18·19, we sought to confirm whether any relationship between the locus and risk of stroke was independent of CAD.

METHODS

STUDY POPULATION

We studied subjects collected by six different centers across Europe and North America (Dept. of Neurology, Klinikum Großhadern, Munich, Germany; Clinical Neurosciences, St. Georges, London, United Kingdom; Dept. of Neurology, University of Maryland at Baltimore, Maryland District, USA; Dept. of Neurology, Mayo Clinic, Jacksonville, Florida, USA; Massachusetts General Hospital, Boston, Massachusetts, USA; University of Aberdeen, Aberdeen, United Kingdom) (Table 1 and Supplementary Material). Events with deficits lasting less than 24 hours with corresponding evidence of an acute ischemic infarct on neuroimaging were included. Strokes occurring as an immediate consequence of trauma

or associated with subarachnoid hemorrhage and strokes due to cerebral venous thrombosis were excluded. Stroke subtypes were classified at each participating center using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification system 20. The Ethics Committees of each study site approved the study protocol and all participants gave written informed consent.

SELECTION OF SNPs

For the screening phase we selected 10 SNPs showing the strongest association with CAD or MI in earlier GWA studies 6·7·11·13·21 (Figure 1) as well as five additional SNPs covering the critical genetic interval on 9p21 11·22.

GENOTYPING

Genotyping was performed on four different platforms. After completing local quality checks, all data were transferred to the central site (Munich) for central quality control and data analysis. Genotyping in the Munich and Boston samples was done in Munich using the iPlex Gold chemistry on a MassARRAY platform (Sequenom, San, Diego, CA, USA). The SNP assays were designed with the Sequenom Assay Design 3.1 software. In the Baltimore-Washington Young Stroke Study (BWYSS) sample and Jacksonville sample SNP genotyping was performed on site with Taqman technology (Applied Biosystems, Foster City, CA). The reaction protocol was specified according to manufacturer's instructions included with each individual primer set. The London and Aberdeen samples were genotyped by KBiosciences (<http://www.kbioscience.co.uk>), using a combination of their patented competitive allele specific PCR (KASPar) assays designed with proprietary Primer Picker software, and TaqMan® technology. Genotyping was successful in all samples and for all SNPs except for rs496892, which failed in the London sample for technical reasons. The genotyping call rates ranged from 97,8 to 99,2%. All SNPs are named according to the forward/T strand orientation as in dbSNP.

STATISTICAL ANALYSIS

Testing for a deviation from Hardy-Weinberg-Equilibrium (HWE) was done using the exact test for HWE from the R package “genetics” while correcting for multiple comparisons. Analysis for an association of the SNPs studied with the phenotypes of interest (IS as a whole, stroke subtypes, CAD and MI) was done using logistic regression implemented in the GLM procedure in R 2.7.1. (<http://cran.r-project.org>). Genotypes were coded as numbers of the minor alleles thus implementing Armitage's test for trend. Odds ratios (OR) and confidence intervals were also obtained from logistic regression while including the following possible confounders in the model: age (age-at-onset for cases and age at recruitment for controls), gender, ethnicity, center, CAD and MI, vascular risk factors. Meta-analysis, including testing for heterogeneity, was done using the R package rmeta (<http://cran.r-project.org/web/packages/rmeta/>). To determine the most likely causative SNPs we performed logistic regression conditioning on the most significant SNP. Testing for genetic models was done using a likelihood ratio testing procedure based on likelihoods obtained from logistic regression 23. Haplotype analysis was performed using Haploview Version 4.1 in LD blocks delineated using the four gamete rule. The LD structure in the German control population is given in Figure 1 and Supplementary Figure 1. Multiple testing corrections were performed using Sidak's method based on a total of 15 SNPs, thus not limiting our analyses to the seven SNPs entering stage 2 and also allowing joint analysis of the total sample 24. Post-hoc power analysis was done using the Genetic Power Calculator (<http://pngu.mgh.harvard.edu/~purcell/gpc/>). For an allele frequency of the risk allele of 0.64 and an allelic odds ratio of 1.2 the power of the current study to find a significant effect at a type I error level of 0.00333 (= 0.05/15) was 70%.

ROLE OF THE FUNDING SOURCES

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RESULTS

The majority of our cases and controls were of European origin. As expected, the relative distribution of atherosclerotic stroke differed across sites, ranging in frequency from 9.8% to 31.3% (Table 1). In addition, the frequency with which stroke cases had a history of MI or CAD ranged from 4.8% and 9.8%, respectively to 17.8% and 39%.

As an initial step we genotyped 15 SNPs covering the critical genetic interval on 9p21.3 (Figure 1) in 1090 cases and 1244 controls from Southern Germany. Of the 15 SNPs six (rs7044859, rs496892, rs564398, rs7865618, rs1537378, rs2383207) were associated with atherosclerotic stroke (all $p < 0.05$) (Supplementary Table 1) whereas no associations were found with IS in general or other stroke subtypes (data not shown). Genotyping of rs1333040, rs2383207, and rs10757278 done in parallel in 758 IS cases and 872 controls from the United Kingdom (UK) revealed associations between two SNPs (rs2383207 and rs10757278) and risk of atherosclerotic stroke (all $p < 0.05$) (Supplementary Table 1) whereas again no associations were found with other stroke subtypes (data not shown).

The seven SNPs (rs7044859, rs496892, rs564398, rs7865618, rs1537378, rs2383207, and rs10757278) associated with risk of atherosclerotic stroke in either of the two screening samples were subsequently genotyped in four additional samples from North America and Scotland adding 2528 cases and 2189 controls (Table 1). Genotyping in the UK sample was completed for the remaining SNPs. There was no significant deviation from Hardy-Weinberg equilibrium in any of the study groups.

Because of the observed association with atherosclerotic stroke in the screening samples we focused on this stroke subtype in subsequent analyses. Joint analysis of the full sample comprising 4376 cases and 4305 controls revealed significant associations between six of the seven SNPs and atherosclerotic stroke in multivariate analyses including age, gender, ethnicity, and center as covariates (Table 2). Importantly, these associations remained significant when including CAD and MI as covariates. In addition, these associations remained significant when adding vascular risk factors as covariates (rs10757278 was no longer significant whereas rs7044859 became significant) (Table 2). None of the interaction terms between SNPs and CAD, MI, or vascular risk factors approached significance.

Further analyses identified rs1537378 as the lead SNP among the polymorphisms tested as none of the SNPs in Table 2 displayed effects independent of rs1537378. Importantly, odds ratios (OR) were similar in all subsamples defined by ethnicity with an overall estimate of 1.19 (95% confidence interval [CI] = 1.06–1.33; $p = 0.003$). A meta-analysis restricted to samples with at least 50 ethnically homogeneous individuals showed similar results (Figure 2, Supplementary Table 2) with a pooled OR of 1.21 (95% CI = 1.07–1.37) under both fixed and random effects models ($p = 0.002$). Interestingly the results did not differ significantly between individuals of European and African American descent, suggesting that the effect of the SNP on risk of atherosclerotic stroke was similar across ethnicities. We also examined whether effects might differ across centers. There was no evidence for heterogeneity, when assessed within the meta-analysis ($p = 0.987$). Furthermore, OR were similar across subgroups divided by gender, age, and vascular risk factors (Supplementary Figure 2). We further examined whether there might be interactions between the culprit SNPs and CAD/MI or vascular risk factors. None of the interaction terms between SNPs and CAD, MI, or vascular risk factors approached significance. Testing for different modes of inheritance in

the joint data set revealed that the best fitting model was a recessive model for the C-allele of rs1537378 although the recessive model was not significantly better than the allele dosage model.

The effect of rs1537378 appeared to be restricted entirely to the subset of atherosclerotic stroke. There were no significant associations between any of the seven SNPs and the risk of other stroke subtypes with the exception of a nominally significant association between rs1537378 and stroke of undetermined aetiology, which encompasses multiple competing aetiologies including atherosclerosis ($p = 0.039$) (Supplementary Table 3). In addition, there were associations between two SNPs and risk of overall IS (rs564398, $p = 0.0328$; rs1537378, $p = 0.0114$) although OR were lower than for atherosclerotic stroke. When atherosclerotic strokes were excluded from the analysis, these associations disappeared, consistent with the hypothesis that the risk for stroke contributed by this locus is restricted to the subtype of atherosclerotic stroke. The point estimate for the population attributable risk was 20.1% for atherosclerotic stroke and 4% for overall IS.

741 (16.3%) subjects with IS and 243 (5.6%) control subjects had a history of MI or CAD. In order to confirm the results of prior studies, we repeated our analysis after reclassifying the overall group of cases and controls according to MI and CAD status. As expected, several SNPs were significantly associated with risk of MI or CAD in the overall sample (Supplementary Table 4). The ORs were similar in magnitude and identical in direction to those previously reported 11. The strongest signal was obtained with variant rs10757278-G. This SNP is highly correlated with rs1333049 ($r^2 > 0.95$) and rs2383207 ($r^2 \geq 0.8$), the lead SNPs in prior studies of MI/CAD 6·9 (Figure 1)

Haplotype analysis confirmed the single locus results without adding further information, owing to very high r^2 values between the associated SNPs (Figure 1). The LD structure was found to differ markedly between different ethnicities (Supplementary Figure 3).

DISCUSSION

Cardiovascular disease including stroke is the most frequent cause of death and a major cause of disability worldwide 1. This study demonstrates that the recently identified locus for CAD on chromosome 9p21.3 is also implicated in risk of atherosclerotic stroke. This relationship between the locus and risk of stroke appears to be mediated through mechanisms that are not dependent on the presence of MI, CAD, or other vascular risk factors. The observed ORs are substantial and the population attributable risk (PAR) is high, suggesting that the 9p21.3 region is a major locus for atherosclerotic stroke. Our findings add to recent observations in patients with abdominal aortic aneurysms (AAA) and intracranial aneurysms (IA) 13. Together these findings reveal a key role of the chromosome 9p21.3 region in arterial disease.

The validity of our findings is supported by several observations. First, associations between SNPs on 9p21 and atherosclerotic stroke were detected in both screening samples and were subsequently confirmed in the meta-analysis of the overall sample totalling 4376 IS cases and 4305 controls. Second, the ORs for the lead SNP (rs1537378) were remarkably consistent across subgroups including various populations from different geographical regions and ethnic backgrounds (Figure 2 and Supplementary Figure 2). Third, associations in the overall sample were robust when we controlled for potential confounders as well as intermediate variables. Fourth, the signal was strictly confined to a single stroke subtype both in the screening samples and in the overall population suggesting that the signal is clearly delimited and not related to stroke in general.

Significant associations were found with several SNPs covering a genomic interval of more than 100kb. It is evident from the distribution of associated SNPs in this region that the causally responsible variant remains to be identified. The genetic interval overlaps with exons 18-24 of *ANRIL*, a newly annotated gene encoding a large antisense non-coding RNA 25 (Figure 1). *ANRIL* has recently been shown to be expressed in human atheromatous vessels including both AAA and carotid endarterectomy (CEA) samples 12. It was further found to be expressed in isolated vascular endothelial cells, monocyte-derived macrophages and coronary smooth muscle cells all of which have a role in atherosclerosis. As for most non-coding RNAs the cellular function of *ANRIL* is still unknown. However, it appears from the above findings that *ANRIL* represents a good candidate for atherosclerosis risk. The genetic interval on 9p21 further overlaps with exon 5 of a splicing variant of the methylthioadenosine phosphorylase (*MTAP*) gene and is relatively close to the coding sequences of genes for two cyclin-dependent kinase inhibitors *CDKN2a* (encoding p16INK4a) and *CDKN2B* (encoding p15INK4b). These genes play a key role in regulating cell proliferation, cell senescence, and apoptosis 26, and may be implicated in atherosclerosis through their role in transforming growth factor (TGF)- β -induced growth inhibition 27-28. Of note, there is evidence for a coordinated transcriptional regulation of *ANRIL*, *p16/CDKN2A* and *p15/CDKN2B* as well as other genes in the 9p21.3 region 25. Further work is needed to determine, whether the association between atherosclerotic stroke and the 9p21 region is mediated through these genes or other pathways possibly through long-range regulatory effects.

Apart from demonstrating an association with atherosclerotic stroke we also replicated the reported association between 9p21.3 and both MI and CAD. The associated alleles and the mode of inheritance (recessive model, data not shown) match well with those previously reported for this phenotype and the OR are close to those found in recent studies 6-13.

Stroke, most notably atherosclerotic stroke, shares common risk factors and mechanisms with MI and CAD 15. Further, there is substantial comorbidity between stroke, CAD, and MI. Between 20 and 40% of stroke patients have an abnormal cardiac stress response with the rate being highest in patients with atherosclerotic stroke 15-29. Carotid intima-media thickness (IMT), a quantitative marker, and intermediate phenotype for early atherosclerosis strongly correlates with both CAD and risk of MI 30-31. Thus, one might speculate, that the 9p21.3 region contributes to vascular disease by promoting atherosclerosis.

However, several observations suggest the mechanisms are more complex. First, in this study associations with atherosclerotic stroke and CAD/MI were found to be independent from each other (Table 2 and Supplementary Table 4) suggesting that the underlying processes do not run strictly in parallel. Second, the two lead SNPs for CAD (rs10757278-G) and atherosclerotic stroke (rs1537378) identified here are more than 70kb apart. Accumulating data suggests that these two lead SNPs are likely to represent the same signal, each serving as a marker for the same causal variant 11. Thus, the difference in associated SNPs may just be coincidental. Alternatively however, this observation might reflect differences in the genetic architecture of atherosclerotic stroke and CAD/MI with regard to the 9p21.3 region as previously documented for CAD and type 2 diabetes 13. Third, it was recently shown that the 9p21.3 region is also implicated in risk of AAA and IA which are pathologically distinct from atherosclerosis 13. In fact, atherosclerosis is not considered a risk factor for IA 32-33. Together, these findings raise the possibility that the chromosome 9p21 region has a broader role in large artery disease possibly by impacting on vascular remodelling or repair rather than atherosclerosis per se 34-36. This concept would agree with results from a recent study that found no association of the rs1333049 genotype with carotid IMT in 3572 population-based subjects from Finland 37. Further studies are needed to determine the mechanisms by which the chromosome 9p21.3 region affects vascular risk.

Importantly, the observed effects of 9p21 on atherosclerotic stroke were found to be independent from established risk factors for cardiovascular disease. This finding agrees with similar data for CAD and MI showing that the effects of 9p21 on MI and CAD are not mediated by known vascular risk factors 7·9.

The strongest associations were seen with rs1537378. This SNP was also significant in the overall group of IS patients and in patients with stroke of “undetermined” aetiology. Most likely these associations likewise reflect an association with atherosclerotic stroke as both categories include patients with atherosclerotic stroke and there was no significant signal for any of the other stroke subtypes including cardioembolic stroke and small vessel stroke. In accord with this, OR were in the same direction but lower than for atherosclerotic stroke, which would be expected if the results were driven by atherosclerotic stroke.

Our findings might resolve some of the inconsistencies among recent studies that have looked for an association between sequence variants on 9p21 and IS risk 13·16·17. These studies provided no 13·16 or marginally 17 significant evidence for an independent association between SNPs on 9p21.3 and IS as a whole. However, sample sizes were limited (between 249 and 705 IS cases) and stroke subtypes such as atherosclerotic stroke were not considered separately. The current study documents the need for large sample sizes particularly when the ORs are moderate and associations are limited to stroke subtypes.

This study also has potential limitations. First, genotyping involved different methods and platforms which in theory might have impacted on our findings. However, we consider this possibility very unlikely for the following reasons: i), cases and controls were numerically well balanced on all three platforms; ii), ORs were similar across all sites and platforms (Figure 2); iii), ORs were similar for six neighbouring SNPs, which can thus be considered technical replicates. Second, samples were ascertained through different protocols without central phenotyping. Conceivably this might have affected the results. Yet again, we found no evidence for a center effect suggesting that differences in case ascertainment and phenotyping are less relevant. Finally, we cannot exclude that CAD and MI remained underdiagnosed in our stroke patients which in turn could have contributed to the association with stroke. However, a major bias seems unlikely. For one, this study replicated the known association with CAD and MI, which suggests that the level of phenotyping was good. Second, there was no evidence for an interaction between any of the SNPs and CAD or MI in multivariate analyses when considering stroke as the dependent variable. Regardless of this relationship the association between SNPs on 9p21 region and atherosclerotic stroke remains considerable and important.

The consistency of associations across a broad range of subjects and vascular conditions in conjunction with the substantial increase in IS risk particularly in homozygous individuals suggests that genotyping of this locus might have considerable clinical utility in risk prediction provided that the current findings are supported in further large population-based studies. Future studies will determine whether genotyping of the 9p21 region adds to already established scores for cardiovascular risk such as the Framingham risk score 38. Identifying individuals at risk for cardiovascular events may impact on preventive strategies. In this regard, the current findings might have therapeutic implications.

In conclusion, this study reveals an unexpectedly broad role of the chromosome 9p21 region in arterial disease. Identification of the molecular pathways and biological mechanisms might offer new perspectives for therapeutic interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 2.

Table 1

Baseline Characteristics of the Study Population Stratified by Affection Status

Subject Characteristic	Munich Sample		London Sample		Baltimore-Washington Young Stroke Study		Subject Characteristic		Ischemic Stroke Genetics Study		Boston Sample		Aberdeen Sample	
	Cases	Controls	Cases	Controls	Cases	Controls			Cases	Controls	Cases	Controls	Cases	Controls
Individuals, n (%)	1090	1244	758	872	652	718	Individuals, n (%)		603	435	608	519	607	517
Male	672 (61.7)	773 (62.1)	444 (58.6)	498 (57.1)	351 (53.8)	346 (48.2)	Male		256 (42.5)	163 (37.5)	335 (55.0)	249 (48.0)	334 (55.0)	265
Female	418 (38.3)	471 (37.9)	314 (31.4)	374 (42.9)	301 (46.2)	373 (51.9)	Female		347 (57.5)	272 (62.5)	274 (45)	270 (52.0)	273 (45.0)	252
Ethnicity							Mean (SD) age, y		64.6 (13.8)	60.7 (14.9)	65.2 (15.7)	66.8 (9.3)	69.6 (12.2)	67.1 (9.0)
White	1090 (100)	1244 (100)	758 (100)	872 (100)	327 (50.2)	384 (53.5)	Ethnicity							
Black	-	-	-	-	275 (42.2)	271 (37.7)	White		445 (73.8)	314 (72.2)	549 (90.3)	498 (96.0)	607 (100)	517 (100)
Other	-	-	-	-	50 (7.6)	63 (8.8)	Black		139 (23.0)	106 (24.4)	22 (3.6)	8 (1.5)	-	-
Mean (SD) age, y	65.4 (13.5)	62.4 (10.9)	66.0 (13.2)	65.3 (8.8)	41.1 (7.3)	39 (7.1)	Other		19 (3.2)	15 (3.4)	37 (6.1)	13 (2.5)	-	-
Ancestry	Southern Germany		United Kingdom		Baltimore-Washington Area, USA		Ancestry		USA and Canada		Boston, USA		Aberdeen and Borders, Scotland	
Stroke subtype, n (%)							Stroke subtype, n (%)							
Atherosclerotic stroke	318 (29.2)	-	237 (31.3)	-	64 (9.8)	-	Atherosclerotic stroke		120 (19.9)	-	111 (18.3)	-	170 (28.0)	-
Cardioembolic stroke	283 (26.0)	-	126 (16.6)	-	125 (19.2)	-	Cardioembolic stroke		135 (22.4)	-	23(38.7)	-	113 (18.6)	-
Small vessel stroke	107 (9.8)	-	136 (17.9)	-	97 (14.9)	-	Small vessel stroke		124 (20.6)	-	63 (10.4)	-	226 (37.2)	-
Other determined aetiology	48 (4.4)	-	5 (0.7)	-	80 (12.3)	-	Other determined aetiology		24 (4.0)	-	55 (9.0)	-	-	-
Undetermined aetiology	334 (30.6)	-	254 (33.5)	-	286 (43.9)	-	Undetermined aetiology		200 (33.2)	-	144(23.7)	-	98 (16.1)	-
Coronary artery disease, n (%)	156 (14.3)	-	93 (12.3)	48 (5.5)	64 (9.8)	30 (4.2)	Coronary artery disease, n (%)		132 (21.9)	38 (8.7)	119 (19.6)	35 (6.7)	237 (39.0)	102 (19.7)
Myocardial infarction, n (%)	119 (10.9)	-	80 (10.6)	48 (5.5)	33 (5.0)	4 (0.6)	Myocardial infarction, n (%)		107/602 (17.8)	21/434 (4.8)	39 (6.4)	15 (2.9)	29 (4.8)	11/317 (3.5)
Cardiovascular risk factor, n (%)							Cardiovascular risk factor, n (%)							
Hypertension	673 (61.7)	758 (60.9)	567 (74.8)	510/869 (58.7)	274/648 (42.3)	128/716 (17.9)	Hypertension		434 (72)	170/433 (39.3)	363 (59.7)	243 (46.8)	351 (57.8)	229 (44.3)
Former or current smoker	301 (27.6)	671 (53.9)	552/757 (38.4)	505/870 (58.0)	290/651 (44.5)	191 /708 (27.0)	Former or current smoker		399 (66.2)	218 (50.1)	373/593 (62.9)	253/517 (48.9)	259 (42.7)	299 (57.8)
Diabetes mellitus	248 (22.8)	79 (6.4)	119/754 (15.8)	38/867 (4.4)	109/650 (16.8)	35/716 (5.0)	Diabetes mellitus		157 (26)	59 (13.6)	116 (19.1)	34 (6.6)	89 (14.7)	22 (4.3)
Hypercholesterolemia	415 (38.1)	712 (57.2)	534/756 (70.6)	339/519 (65.3)	171/644 (26.6)	169/712 (23.7)	Hypercholesterolemia		297/600 (49.5)	138/430 (32.1)	222 (36.5)	225 (43.4)	350/603 (58)	312 (60.3)

Table 2

Association to Atherosclerotic Stroke on 9p21 in the overall sample

SNP	Alleles	Risk Allele	Cases (N)	Controls (N)	Frequency of Risk Allele	OR (95% CI)	P-value
Cases							
Controls							
Atherosclerotic stroke, corrected for age, gender, ethnicity, and center							
rs7044859	A/T	A	970	4256	0.4985	0.4899	1.10 (0.99-1.22)
rs496892	A/G	G	731	3360	0.5739	0.5531	1.15 (1.02-1.29)
rs564398	A/G	A	964	4190	0.6494	0.6227	1.19 (1.07-1.33)
rs7865618	A/G	A	962	4272	0.6398	0.6181	1.19 (1.07-1.32)
rs1537378	C/T	C	961	4202	0.6675	0.6423	1.21 (1.09-1.35)
rs2383207	A/G	G	962	4260	0.5889	0.5700	1.17 (1.06-1.30)
rs10757278	A/G	G	952	4262	0.5047	0.4693	1.12 (1.02-1.24)
Atherosclerotic stroke, corrected for age, gender, ethnicity, center, and CAD and MI							
rs7044859	A/T	A	970	4256	0.4985	0.4899	1.10 (0.99-1.21)
rs496892	A/G	G	731	3360	0.5739	0.5531	1.15 (1.02-1.29)
rs564398	A/G	A	964	4190	0.6494	0.6227	1.19 (1.07-1.32)
rs7865618	A/G	A	962	4272	0.6398	0.6181	1.18 (1.06-1.32)
rs1537378	C/T	C	961	4202	0.6675	0.6423	1.20 (1.07-1.34)
rs2383207	A/G	G	962	4260	0.5889	0.5700	1.17 (1.05-1.30)
rs10757278	A/G	G	952	4262	0.5047	0.4693	1.11 (1.01-1.23)
Atherosclerotic stroke, corrected for age, gender, ethnicity, center, CAD and MI, and vascular risk factors							
rs7044859	A/T	A	969	4240	0.4979	0.4896	1.12 (1.00-1.24)
rs496892	A/G	G	730	3346	0.5733	0.5528	1.16 (1.02-1.30)
rs564398	A/G	A	963	4175	0.6490	0.6225	1.18 (1.06-1.32)
rs7865618	A/G	A	961	4256	0.6394	0.6181	1.18 (1.06-1.32)
rs1537378	C/T	C	960	4187	0.6672	0.6424	1.19 (1.06-1.33)
rs2383207	A/G	G	961	4244	0.5890	0.5700	1.16 (1.04-1.29)
rs10757278	A/G	G	951	4246	0.5047	0.4694	1.11 (0.99-1.23)

Shown are all SNPs (single nucleotide polymorphisms) in the region on 9p21 that showed nominally significant association to atherosclerotic stroke in one of the two screening samples (see Supplementary Table 1); OR=odds ratio; CI=confidence interval; CAD=coronary artery disease