Published in final edited form as:
Stroke. 2014 July ; 45(7): 1920–1924. doi:10.1161/STROKEAHA.114.005208.

Effect of genetic variants associated with plasma homocysteine levels on stroke risk

Ioana Cotlarciuc, PhD[1], **Rainer Malik, PhD**[2], **Elizabeth G. Holliday, PhD**[3], **Kourosh R Ahmadi, PhD**[4], **Guillaume Pare, MD**[5], **Bruce M. Psaty, MD**[6,7,8,9], **Myriam Fornage, PhD**[10], **Nazeeha Hasan, PhD**[1], **Paul E. Rinne, MSc**[1], **M. Arfan Ikram, PhD**[11], **Hugh S Markus, DM**[12], **Jonathan Rosand, MD**[13,14,15], **Braxton D. Mitchell, PhD**[16,17], **Steven J. Kittner, MD**[16,17,18], **James F. Meschia, MD**[19], **Joyce BJ van Meurs, PhD**[20], **Andre G Uitterlinden, PhD**[20], **Bradford B. Worrall, MD**[21], **Martin Dichgans, MD**[2,23], **Pankaj Sharma, PhD, MD, FRCP**[1], and **METASTROKE and the International Stroke Genetics Consortium**

[1]Imperial College Cerebrovascular Research Unit (ICCRU), Department of Medicine, Imperial College London, UK [2]Institute for Stroke and Dementia Research (ISD), Medical Centre, Klinikum der Universität München, Ludwig-Maximilians-University, Munich, Germany [3]Center for Clinical Epidemiology and Biostatistics, School of Medicine and Public Health, University of Newcastle, New South Wales, Australia [4]Department of Nutritional Sciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, UK [5]Department of Pathology, McMaster University, Hamilton, Canada [6]Department of Epidemiology, University of Washington, Seattle, Washington, USA [7]Department of Medicine, University of Washington, Seattle, Washington, USA [8]Department of Health Services, University of Washington, Seattle, Washington, USA [9]Group Health Research Institute, Group Health, Seattle, WA, USA [10]University of Texas Health Science Center at Houston, Houston, TX, USA [11]Department of Epidemiology, Neurology, Radiology, Erasmus MC University Medical Center, Rotterdam, Netherlands [12]Clinical Neurosciences, University of Cambridge, Cambridge, UK [13]Department of Neurology, Massachusetts General Hospital, Boston, MA, USA [14]Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA [15]Program in Medical and Population Genetics, Broad Institute, Cambridge, MA, USA [16]Veterans Affairs Medical Center, Baltimore, Maryland, USA [17]Department of Medicine, University of Maryland School of Medicine, Maryland, USA [18]Department of Neurology, University of Maryland School of Medicine, Maryland, USA [19]Department of Neurology, Mayo Clinic, Jacksonville, FL, USA [20]Department of Internal Medicine, Erasmus MC University Medical Center, Rotterdam, Netherlands [21]Department of Neurology, University of Virginia, Charlottesville, VA, USA [2]Department of Public Health Science, University of Virginia, Charlottesville, VA, USA [23]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany

Abstract

Corresponding author's contact information: Ioana Cotlarciuc, PhD, Imperial College Cerebrovascular Research Unit (ICCRU), Department of Medicine, Imperial College London, Fulham Palace Rd, London, W6 8RF, UK. i.cotlarciuc@imperial.ac.uk Tel: +44(0)2033117284 Fax: +44(0)2033117284.

Disclosures
Worrall received support from US NINDS and NHGRI. Rosand received support from NIH and he is a consultant for Boehringer Ingelheim.

Background and Purpose—Elevated homocysteine (tHcy) levels are known to be associated with increased risk of ischemic stroke (IS). Given that both tHcy and IS are heritable traits, we investigated a potential genetic relationship between homocysteine levels and stroke risk by assessing 18 polymorphisms previously associated with tHcy levels for their association with IS and its subtypes.

Methods—Previous meta-analysis results from an international stroke collaborative network, METASTROKE, were utilized to assess association of the 18 tHcy associated SNPs in 12,389 IS cases and 62,004 controls. We also investigated the associations in regions located within 50kb from the 18 tHcy related SNPs, and the association of a genetic risk score including the 18 SNPs.

Results—One SNP located in the *RASIP1* gene and a cluster of three SNPs located at and near *SLC17A3* were significantly associated with IS (P<0.0003) after correcting for multiple testing. For stroke subtypes, the sentinel SNP located upstream of *MUT* was significantly associated with SVD (small vessel disease) (P=0.0022), while one SNP located in *MTHFR* was significantly associated with LVD (large vessel disease) (P=0.00019). A genetic risk score including the 18 SNPs did not show significant association with IS or its subtypes.

Conclusions—This study found several potential associations with IS and its subtypes: an association of an *MUT* variant with SVD, an *MTHFR* variant with LVD, and associations of *RASIP1* and *SLC17A3* variants with overall IS.

Keywords

homocysteine; ischemic stroke; genetic association; genotype risk score

Introduction

The relationship between plasma homocysteine (tHcy) levels and stroke risk has been investigated by numerous observational studies, which together provide compelling evidence that elevated homocysteine levels are associated with an increased risk of ischemic stroke (IS).[1] However, residual confounding and reverse causation impair causal inference from the results of observational studies.[2] Mendelian randomization studies investigating a potential causal relationship between tHcy and IS risk have yielded inconclusive results.[3]

Recent studies showed that lowering homocysteine levels through vitamin B (folic acid and vitamin B12) intervention reduced the risk of stroke in patients with normal renal function and with normal vitamin B12 metabolism.[4-6] These findings may explain the negative results obtained by earlier studies[7, 8] where renal function and vitamin B12 metabolism status were not taken into account. In addition, the folic acid fortification of grains in the US and some European countries may have also reduced the benefit of folic acid intervention trials.[9] Thus, the relationship between stroke and homocysteine is complex and careful consideration is required in designing future clinical trials.[10]

Recently, a genome-wide association study (GWAS) meta-analysis of plasma homocysteine levels in 44,147 individuals of European ancestry identified 13 associated genetic loci at genome-wide significance ($P<5\times10^{-8}$).[11] Within the 13 associated loci, this meta-analysis

identified 18 independent single-nucleotide polymorphisms (SNPs) explaining 6% of variation in tHcy levels.

Since both tHcy and IS have a genetic component, the reported association between tHcy and stroke risk may result from shared genetic risk factors. Thus, we investigated the 18 SNPs previously associated with homocysteine levels for their association with IS and its subtypes: large-vessel (LVD), small-vessel (SVD) and cardioembolic (CE) stroke. We also evaluated association of a genotype risk score (GRS) including the18 tHcy-associated SNPs for association with IS and its subtypes.

Materials and Methods

Study Population

The study population included 12,389 IS cases and 62,004 controls of European ancestry from 15 cohorts contributing to the METASTROKE collaboration[12]. Details of the study designs for each participating study are included in the Online Supplement (Please see http://stroke.ahajournals.org).

SNP selection

Plasma homocysteine associated SNPs reaching genome-wide significance ($P<5\times10^{-8}$) in a published meta-analysis of GWAS[11] were selected for inclusion. In total, 18 independent SNPs ($r^2<0.2$) were found to be significantly associated with tHcy at a $P<5\times10^{-8}$ and selected for assessment in and its subtypes (Supplementary Table I). As a secondary analysis, to account for potential population differences in linkage disequilibrium (LD) between functional and tag-SNPs, we included additional SNPs located ± 50kb around the 18 tHcy associated SNPs, and a total of 3160 SNPs were selected out of which 166 variants were independent ($r^2<0.2$).

Statistical analysis

Summary statistics for association of the 18 tHcy associated SNPs with IS and its subtypes were provided by METASTROKE consortium. Details on genotyping, imputation and quality control methods are provided in Supplemental Methods. Logistic regression was performed to test association of individual SNPs with IS and its subtypes assuming an additive model, and adjusting for study-specific covariates age, sex and ancestry principal components (Supplemental Methods).

Considering that stroke subtypes are independent of each other[12-16], we pre-specified a Bonferroni-adjusted significance threshold of $\alpha=0.0027$ (where $\alpha=0.05/18$ SNPs) to adjust for primary analyses testing 18 independent SNPs for association with IS and its subtypes.

For the secondary analysis of SNPs located within ±50kb of the 18 tHcy related SNPs we specified a Bonferroni-adjusted significance threshold of 0.0003 ($\alpha=0.05/166=0.0003$, for 166 independent SNPs tested).

We then tested the association between an additive genotype risk score (GRS) of the 18 homocysteine associated SNPs and increased risk of IS and its subtypes. The GRS was calculated using a previously described method[17] (Supplemental Methods).

Using the CATS genetic power calculator[18], our study had 80% power to detect odds ratios (OR) of 1.06–1.11 for IS, and subtype-specific OR of 1.13–1.23 for variants with allele frequency 10– 50% at a Bonferroni-corrected threshold of 0.0027 (α=0.05/18).

Results

Study population characteristics

The discovery meta-analysis of IS studies included 12,389 cases and 62,004 controls of European descent. Stroke subtypes CE, LVD and SVD accounted for 19% (n=2,365), 17.4% (n=2,167) and 15.2% (n=1,894) respectively, of all IS cases. Detailed characteristics of the participating studies have been summarised previously.[12]

Association of tHcy associated SNPs with Overall IS

Firstly, we assessed the association of each tHcy associated SNP with IS risk, and subsequently with its subtypes. Then, we tested the combined effect of the 18 sentinel tHcy associated SNPs on risk of IS and its subtypes.

For overall IS, the OR for the 18 tested SNPs ranged from 0.96–1.04 (Supplementary Table II). Of the 18 SNPs tested, two SNPs located at/near *FUT1* (rs838133, OR 1.04; 95%CI 1.00– 1.07; *P*=0.013) and *CPS1* (rs7422339, OR 0.96; 95%CI 0.92–0.99; *P*=0.045) were associated with IS at a nominal P-value (P<0.05), but did not pass Bonferroni corrected P-value of 0.0027 (P=0.05/18).

The combined GRS including the 18 independent tHcy SNPs did not show a significant association with IS (OR 1.02; 95%CI 0.91–1.15; *P*=0.63) (Supplementary Table III).

Association of tHcy associated SNPs with IS Subtypes

Next, we investigated the association of the tHcy related SNPs with IS subtypes. For SVD, two SNPs located near *MUT* (rs9369898, OR 1.12; 95%CI 1.04–1.21; *P*=0.0022) and *FUT1* (rs838133, OR 1.07; 95%CI 1.00–1.15; *P*=0.04) respectively, were nominally associated at a P-value <0.05 (Supplementary Table IV). The variant located near *MUT* (rs9369898) also passed Bonferroni corrected P-value of 0.0027. The major allele A of rs9369898 associated with higher tHcy levels was also associated with increased risk of SVD. There was no evidence of between study heterogeneity for rs9369898 (I^2=7.4%; P-het=0.37). The GRS including the 18 independent tHcy SNPs did not show an association with SVD risk (OR 1.1; 95%CI 0.85– 1.42; *P*=0.43) (Supplementary Table III).

For LVD, one SNP (rs838133, OR 1.08; 95%CI 1.01–1.16; *P*=0.018) located near *FUT1* gene was associated at a nominal P-value (P<0.05), but did not pass Bonferroni correction for multiple testing (Supplementary Table V). The GRS of the 18 independent tHcy SNPs did not show an association with LVD risk (OR 1.06; 95%CI 0.82–1.35; *P*=0.64) (Supplementary Table III). None of the tested SNPs were associated with CE risk even at a

nominal P-value (P<0.05) (Supplementary Table VI). In addition, the GRS of the 18 independent tHcy SNPs did not show an association with CE risk (OR 0.9; 95%CI 0.71–1.14; *P*=0.4) (Supplementary Table III).

Investigation of associations in regions located ±50kb around the 18 tHcy associated SNPs

As a secondary analysis, we assessed the associations with IS and its subtypes for SNPs located within ±50kb of the 18 tHcy associated SNPs.

For overall IS, three variants (rs9379800, rs17271121 and rs12664474) located within 50kb from the tHcy associated polymorphism, rs548987, were associated with IS at P-values lower than the Bonferroni corrected threshold (P=0.05/166=0.0003) (Table 1). Two SNPs (rs9379800 and rs12664474) located upstream of *SLC17A3* were highly correlated (r^2=0.766) with each other, and moderately correlated with the third SNP, rs17271121 located in an intron of *SLC17A3* (r^2[rs9379800, rs17271121]=0.306; r^2[rs17271121, rs12664474]=0.545). None of the three SNPs were in LD with the tHcy associated polymorphism, rs548987 (r^2<0.035).

In addition, another SNP rs2287921 located in an intron of *RASIP1* gene, within 50kb from the *FUT1* polymorphism, rs838133, was associated with IS at a P-value of 0.0002 (OR 0.94; 95%CI 0.91-0.97), lower than Bonferroni correction for multiple testing (P<0.0003). This SNP was in moderate LD (r^2=0.658) with the sentinel SNP rs838133, which may suggest that this could be a broader risk region spanning the two neighboring genes *RASIP1* and *FUT1*.

For LVD, one SNP (rs1801131), a missense variant located in *MTHFR* gene, near the two sentinel SNPs in this gene, rs1801133 and rs12134663, was associated with LVD with a P-value of 1.92×10^{-4} (OR 1.15; 95%CI 1.06–1.23) lower than the Bonferroni correction for multiple testing (P<0.0003). This missense SNP was in low LD with the two tHcy sentinel SNPs (r^2[rs1801131, rs1801133]=0.19; r^2[rs1801131, rs12134663]=0.268).

For SVD, one SNP (rs566295) located upstream *MUT*, 44kb from the tHcy associated polymorphism, rs9369898, was associated with SVD with a P-value of 2.2×10^{-4} (OR 0.87; 95%CI 0.80–0.93) passing Bonferroni correction for multiple testing. This SNP was in low LD with the *MUT* polymorphism, rs9369898 (r^2=0.264).

For CE, no significant associations were observed at a threshold exceeding Bonferroni correction for multiple testing (P<0.0003).

Discussion

This large study of 12,389 IS cases and 62,004 controls, has identified several potential novel associations with IS and its subtypes by testing previously reported associations with homocysteine levels in stroke. We found evidence of an association of *MUT* gene with SVD, an association of *MTHFR* gene with LVD, and associations of *RASIP1* and *SLC17A3* with overall IS.

Of the 18 tHcy polymorphisms investigated, one polymorphism located upstream of *MUT* gene was significantly associated with SVD, while none of the 18 tHcy related SNPs was significantly associated with LVD, CE or overall IS. The allele correlated with increased tHcy levels at *MUT* gene showed to be also associated with increased risk of SVD suggesting a potential small but significant effect on SVD risk.

On a closer inspection of this region, another SNP located 44kb from the sentinel SNP and in low LD with the sentinel SNP, was also associated with SVD. This polymorphism was also significantly associated with homocysteine levels at a genome-wide significance level (P=2.27E-09)[11], but conditional analysis has not been conducted to establish if these two polymorphisms were independently influencing homocysteine levels. These two polymorphisms may thus potentially be correlated with either a single, or multiple regulatory variants in this region that modulate both tHcy levels and SVD risk.

The *MUT* gene is known to encode the mitochondrial enzyme methylmalonyl Coenzyme A mutase, a vitamin B12-dependent enzyme. Considering that vitamin B12 is an important cofactor in homocysteine metabolism, a potential pleiotropic effect of *MUT* gene on both plasma homocysteine and vitamin B12 levels was suggested previously.[11] In addition, vitamin B12 deficiency is highly prevalent in SVD and may underlie blood-brain barrier damage, leading to small vessel dysfunction, especially periventricular white matter lesions[19], suggesting a potential role of *MUT* polymorphisms in SVD via mechanisms involving vitamin B12 deficiency.

Moreover, hyperhomocysteinaemia as an independent risk factor for SVD may act via endothelial dysfunction[20], suggesting that homocysteine lowering therapy may be particularly effective in this stroke subgroup. In support of this, the VITATOPS trial found that after a 3 year vitamin B supplementation period, the risk of stroke was reduced only in patients with symptomatic small vessel disease.[8] Therefore, considering that in our study the only homocysteine significant association was with SVD, brings more evidence to support the hypothesis that homocysteine may be a risk factor in particular to SVD.

Our study also found an association with LVD, a missense variant located in *MTHFR* (A1298C), 2kb from the well-studied homocysteine associated polymorphism *MTHFR* C677T.[2, 3] Previous studies showed that A1298C was associated with a decrease in *MTHFR* activity, but was not associated with increased homocysteine levels.[21] The functional differences between the two polymorphisms could be explained by their location: C677T is located within the N-terminal catalytic region, while A1298C is located within the C-terminal regulatory domain, being involved in enzyme regulation.[21]

Given that high folate levels have been associated with a reduced effect of *MTHFR* C677T on homocysteine levels and stroke[22], the non-association of *MTHFR* C677T with stroke could be explained by the inclusion in the METASTROKE study of individuals from countries where folic acid fortification has already been implemented.[12] The relationship between *MTHFR* A1298C and folate status has not been yet investigated and it is not known if this polymorphism is influenced by folate levels. However, if this SNP is not influenced or

influenced at a lesser extent by folate status compared to C677T, may explain why A1298C polymorphism, and not C677T, was found to be associated with LVD in our stroke cohort.

For overall IS, two highly correlated SNPs located upstream *SLC17A3* and one SNP located in an intron of *SLC17A3* were found to be associated with IS after correction for multiple testing. None of these SNPs were in LD with the sentinel polymorphism suggesting the presence of another independent potential risk locus in this region.

SLC17A3 encodes a voltage-driven transporter that excretes intracellular urate and organic anions from the blood into renal tubule cells.[23] A significant association between a polymorphism (rs1165205) located in intron 1 of *SLC17A3* and serum uric acid concentrations has been found by a GWAS study.[24] This SNP was located up to 80 kb from our investigated SNPs, but was in low LD (r^2<0.2) with all our *SLC17A3* SNPs suggesting. Therefore, the possibility exists that these polymorphisms are correlated with another variant that has a causal role on influencing urate levels. Alternatively, there may be several independent loci associated with urate levels in this region as it has been suggested previously[24], and our investigated SNPs may be correlated with another polymorphism influencing urate levels independent from the GWAS reported association. Epidemiological studies have shown that elevated serum uric acid is a strong independent risk factor for hypertension[25], and considering that hypertension is a risk factor for stroke, it has been suggested that increased uric acid levels may be involved in predicting stroke risk.[26]

Another association with IS was a variant located in *RASIP1* gene. *RASIP1* is required for the proper formation of vascular structures that develop via both vasculogenesis and angiogenesis.[27] As it is well known that insufficient vessel growth or maintenance can lead to stroke among other disorders[28], we provide a possible link between *RASIP1* and stroke risk. Further, this polymorphism has been reported to be significantly associated with retinal vascular caliber in a previous GWAS.[29] Furthermore, considering that changes in retinal vascular caliber are associated with cardiovascular diseases including IS[30], provides more evidence of a potential role of this gene in stroke.

So far, several GWAS have been conducted on ischemic stroke and its subtypes which have identified associations of common polymorphisms specific to each stroke subtype[12, 16, 31], endorsing the fact that different stroke subtypes have different risk factor profiles and pathophysiological mechanisms. Our study supported this hypothesis and identified a potential association of *MUT* with SVD, and of *MTHFR* with LVD. In addition, we also identified potential associations of *RASIP1* and *SLC17A3* with overall IS. The reason why we did not find significant associations of *RASIP1* and *SLC17A3* with any of the stroke subtypes could be due to the smaller sample sizes and, thus reduced power of the stroke subtype cohorts. However, it is important to highlight that our findings need to be validated by replication in independent cohorts to avoid considering spurious results.

In addition, the combined genetic score of the 18 independent tHcy SNPs did not show a significant association with overall IS or its subtypes. The low power of our study to detect small effects, together with the fact that stroke and homocysteine may only partly share their allelic architecture may explain the lack of association of the tHcy genotype risk score.

However, our results apply to European population and to countries with established policies of folic acid fortification of grains. A recent study has shown that in these countries, the evidence from genetic studies and from randomised trials with folic acid suggested no benefit from lowering homocysteine levels for stroke prevention.[22] Thus, to elucidate the controversial role of homocysteine in stroke, future studies should be conducted in regions with low folate levels where homocysteine-lowering interventions may have an important effect in reducing stroke risk.

To conclude, our study provides evidence of several potential associations with IS and its subtypes: an association of *MUT* gene with small vessel stroke, an association of *MTHFR* gene with large vessel stroke, and associations of *RASIP1* and *SLC17A3* with overall IS, highlighting possible roles of these genes in IS and its subtypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We gratefully acknowledge the METASTROKE Collaboration.[12]

Sources of Funding

This study was supported by the Department of Health (UK), Henry Smith Charity, British Council, United Kingdom-India Education and Research Institute (UKIERI), and the Qatar National Research Foundation.

Genotpying of the cohorts within METASTROKE was supported by the Wellcome Trust, UK Medical Research Council (MRC), Australian National and Medical Health Research Council, National Institutes of Health (NIH) including National Heart, Lung and Blood Institute (NHLBI), the National Institute on Aging (NIA), the National Human Genome Research Institute (NHGRI), the National Institute of Neurological Disorders and Stroke (NINDS), and the American Heart Association-Bugher Foundation Centers for Stroke Prevention.

References

1. Homocysteine Studies Collaboration. Homocysteine and Risk of Ischemic Heart Disease and Stroke: A Meta-analysis. JAMA. 2002; 288:2015–2022. [PubMed: 12387654]
2. Brattström L, Wilcken DE. Homocysteine and cardiovascular disease: cause or effect? The American Journal of Clinical Nutrition. 2000; 72:315–323. [PubMed: 10919920]
3. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. The Lancet. 2005; 365:224–232.
4. Huang T, Chen Y, Yang B, Yang J, Wahlqvist ML, Li D. Meta-analysis of B vitamin supplementation on plasma homocysteine, cardiovascular and all-cause mortality. Clinical Nutrition. 2012; 31:448–454. [PubMed: 22652362]
5. Saposnik G, Ray JG, Sheridan P, McQueen M, Lonn E, the HI. Homocysteine-Lowering Therapy and Stroke Risk, Severity, and Disability: Additional Findings From the HOPE 2 Trial. Stroke. 2009; 40:1365–1372. [PubMed: 19228852]
6. Hankey GJ, Eikelboom JW, Yi Q, Lees KR, Chen C, Xavier D, et al. Antiplatelet therapy and the effects of B vitamins in patients with previous stroke or transient ischaemic attack: a post-hoc subanalysis of VITATOPS, a randomised, placebo-controlled trial. The Lancet Neurology. 2012; 11:512–520.
7. Toole JF, Malinow MR, Chambless LE, Spence JD, Pettigrew LC, Howard VJ, et al. Lowering Homocysteine in Patients With Ischemic Stroke to Prevent Recurrent Stroke, Myocardial Infarction, and Death: The Vitamin Intervention for Stroke Prevention (VISP) Randomized Controlled Trial. JAMA. 2004; 291:565–575. [PubMed: 14762035]

8. The VITATOPS Trial Study Group. B vitamins in patients with recent transient ischaemic attack or stroke in the VITAmins TO Prevent Stroke (VITATOPS) trial: a randomised, double-blind, parallel, placebo-controlled trial. The Lancet Neurology. 2010; 9:855–865.
9. Baigent C, Clarke R. B Vitamins for the Prevention of Vascular Disease: Insufficient Evidence to Justify Treatment. JAMA. 2007; 298:1212–1214. [PubMed: 17848657]
10. Spence JD. B vitamin therapy for homocysteine: renal function and vitamin B12 determine cardiovascular outcomes. Clin Chem Lab Med. 2013; 51:633–637. [PubMed: 23449527]
11. van Meurs JBJ, Pare G, Schwartz SM, Hazra A, Tanaka T, Vermeulen SH, et al. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. Am J Clin Nutr. 2013; 98:668–676. [PubMed: 23824729]
12. Traylor M, Farrall M, Holliday EG, Sudlow C, Hopewell JC, Cheng Y-C, et al. Genetic risk factors for ischaemic stroke and its subtypes (the METASTROKE Collaboration): a meta-analysis of genome-wide association studies. The Lancet Neurology. 2012; 11:951–962.
13. Jerrard-Dunne P, Cloud G, Hassan A, Markus HS. Evaluating the Genetic Component of Ischemic Stroke Subtypes: A Family History Study. Stroke. 2003; 34:1364–1369. [PubMed: 12714707]
14. Grau AJ, Weimar C, Buggle F, Heinrich A, Goertler M, Neumaier S, et al. Risk Factors, Outcome, and Treatment in Subtypes of Ischemic Stroke: The German Stroke Data Bank. Stroke. 2001; 32:2559–2566. [PubMed: 11692017]
15. Kirshner HS. Differentiating ischemic stroke subtypes: Risk factors and secondary prevention. Journal of the Neurological Sciences. 2009; 279:1–8. [PubMed: 19185319]
16. Bellenguez C, Bevan S, Gschwendtner A, Spencer CCA, Burgess AI, Pirinen M, et al. Genome-wide association study identifies a variant in HDAC9 associated with large vessel ischemic stroke. Nat Genet. 2012; 44:328–333. [PubMed: 22306652]
17. The International Consortium for Blood Pressure Genome-Wide Association Studies. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature. 2011; 478:103–109. [PubMed: 21909115]
18. Skol AD, Scott LJ, Abecasis GR, Boehnke M. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat Genet. 2006; 38:209–213. [PubMed: 16415888]
19. Pieters B, Staals J, Knottnerus I, Rouhl R, Menheere P, Kessels A, et al. Periventricular White Matter Lucencies Relate to Low Vitamin B12 Levels in Patients With Small Vessel Stroke. Stroke. 2009; 40:1623–1626. [PubMed: 19286604]
20. Hassan A, Hunt BJ, O'Sullivan M, Bell R, D'Souza R, Jeffery S, et al. Homocysteine is a risk factor for cerebral small vessel disease, acting via endothelial dysfunction. Brain. 2004; 127:212–219. [PubMed: 14607791]
21. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R. A Second Genetic Polymorphism in Methylenetetrahydrofolate Reductase (MTHFR) Associated with Decreased Enzyme Activity. Molecular Genetics and Metabolism. 1998; 64:169–172. [PubMed: 9719624]
22. Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, et al. Effect modification by population dietary folate on the association between MTHFR genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. The Lancet. 2011; 378:584–594.
23. Jutabha P, Anzai N, Kitamura K, Taniguchi A, Kaneko S, Yan K, et al. Human Sodium Phosphate Transporter 4 (hNPT4/SLC17A3) as a Common Renal Secretory Pathway for Drugs and Urate. Journal of Biological Chemistry. 2010; 285:35123–35132. [PubMed: 20810651]
24. Dehghan A, Kottgen A, Yang Q, Hwang S-J, Kao WHL, Rivadeneira F, et al. Association of three genetic loci with uric acid concentration and risk of gout: a genome-wide association study. The Lancet. 2008; 372:1953–1961.
25. Shankar A, Klein R, Klein BEK, Nieto FJ. The association between serum uric acid level and long-term incidence of hypertension: population-based cohort study. J Hum Hypertens. 2006; 20:937–945. [PubMed: 17024135]
26. Bos MJ, Koudstaal PJ, Hofman A, Witteman JCM, Breteler MMB. Uric Acid Is a Risk Factor for Myocardial Infarction and Stroke: The Rotterdam Study. Stroke. 2006; 37:1503–1507. [PubMed: 16675740]

27. Mitin NY, Ramocki MB, Zullo AJ, Der CJ, Konieczny SF, Taparowsky EJ. Identification and Characterization of Rain, a Novel Ras-interacting Protein with a Unique Subcellular Localization. Journal of Biological Chemistry. 2004; 279:22353–22361. [PubMed: 15031288]

28. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011; 473:298–307. [PubMed: 21593862]

29. Ikram MK, Xueling S, Jensen RA, Cotch MF, Hewitt AW, Ikram MA, et al. Four Novel Loci (19q13, 6q24, 12q24, and 5q14) Influence the Microcirculation In Vivo. PLoS Genet. 2010; 6:e1001184. [PubMed: 21060863]

30. Ikram MK, De Jong FJ, Van Dijk EJ, Prins ND, Hofman A, Breteler MMB, et al. Retinal vessel diameters and cerebral small vessel disease: the Rotterdam Scan Study. Brain. 2006; 129:182–188. [PubMed: 16317022]

31. Holliday EG, Maguire JM, Evans T-J, Koblar SA, Jannes J, Sturm JW, et al. Common variants at 6p21.1 are associated with large artery atherosclerotic stroke. Nat Genet. 2012; 44:1147–1151. [PubMed: 22941190]

Table 1

Association with IS and its subtypes of SNPs located ±50kb from the 18 tHcy associated SNPs at a P<0.0003 obtained after adjustment for multiple testing.

SNP	Sentinel SNPs	Chr	Risk Locus	CA	Freq	Beta	SE	P-value	OR	OR (95L)	OR (95U)	P_het	I^2	Q
IS														
rs9379800	rs548987	6	SLC17A3	G	0.17	0.087	0.0233	$1.65x10^{-4}$	1.09	1.13	1.05	0.63	0	9.74
rs17271121	rs548987	6	SLC17A3	C	0.13	0.089	0.0246	$2.8\ x10^{-4}$	1.09	1.13	1.05	0.42	0	13.24
rs12664474	rs548987	6	SLC17A3	G	0.17	0.082	0.0226	$2.86\ x10^{-4}$	1.08	1.12	1.04	0.67	0	10.21
rs2287921	rs838133	19	FUT1	C	0.55	0.061	0.0166	$2.09\ x10^{-4}$	1.06	1.09	1.03	0.08	27.2	20.61
LVD														
rs1801131	rs1801133 rs12134663	1	MTHFR	T	0.68	0.141	0.0378	$1.92\ x10^{-4}$	1.15	1.06	1.23	0.79	0	6.20
SVD														
rs566295	rs9369898	6	MUT	C	0.54	0.138	0.0374	$2.2\ x10^{-4}$	1.13	1.2	1.07	0.84	0	4.88

Abbreviations: SNP=single-nucleotide polymorphism; Chr=chromosome; CA=coded allele; Freq=frequency of the coded allele; Beta=beta coefficient; SE=standard error; OR=odds ratio; 95L&95U=lower and upper 95 percentile; P_het=P-value for the Cochran's heterogeneity statistic; Q=Cochran's heterogeneity statistic; I^2=Higgins Heterogeneity index.