Rutten-Jacobs, L. C. A., Traylor, M., Adib-Samii, P., Thijs, V., Sudlow, C., Rothwell, P. M., ... Markus, H. S. (2016). Association of MTHFR C677T Genotype With Ischemic Stroke Is Confined to Cerebral Small Vessel Disease Subtype. *Stroke*, 47(3), 646—651. https://doi.org/10.1161/strokeaha.115.011545

Citing this paper
Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights
Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the Research Portal

Take down policy
If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Association of MTHFR C677T Genotype With Ischemic Stroke Is Confined to Cerebral Small Vessel Disease Subtype

Loes C.A. Rutten-Jacobs, PhD; Matthew Traylor, PhD; Poneh Adib-Samii, MBBS; Vincent Thijs, MD; Cathie Sudlow, FRCP; Peter M. Rothwell, FMedSci; Giorgio Boncoraglio, MD; Martin Dichgans, MD; James Meschia, MD; Jane Maguire, PhD; Christopher Levi, MD; Natalia S. Rost, MD; Jonathan Rosand, MD; Ahamad Hassan, FRCP; Steve Bevan, PhD; Hugh S. Markus, FRCP

Background and Purpose—Elevated plasma homocysteine levels are associated with stroke. However, this might be a reflection of bias or confounding because trials have failed to demonstrate an effect from homocysteine lowering in stroke patients, although a possible benefit has been suggested in lacunar stroke. Genetic studies could potentially overcome these issues because genetic variants are inherited randomly and are fixed at conception. Therefore, we tested the homocysteine levels-associated genetic variant MTHFR C677T for association with magnetic resonance imaging–confirmed lacunar stroke and compared this with associations with large artery and cardioembolic stroke subtypes.

Methods—We included 1359 magnetic resonance imaging–confirmed lacunar stroke cases, 1824 large artery stroke cases, 1970 cardioembolic stroke cases, and 14448 controls, all of European ancestry. Furthermore, we studied 3670 ischemic stroke patients in whom white matter hyperintensities volume was measured. We tested MTHFR C677T for association with stroke subtypes and white matter hyperintensities volume. Because of the established association of homocysteine with hypertension, we additionally stratified for hypertension status.

Results—MTHFR C677T was associated with lacunar stroke (P=0.0003) and white matter hyperintensity volume (P=0.04), but not with the other stroke subtypes. Stratifying the lacunar stroke cases for hypertension status confirmed this association in hypertensive individuals (P=0.0002), but not in normotensive individuals (P=0.30).

Conclusions—MTHFR C677T was associated with magnetic resonance imaging–confirmed lacunar stroke, but not large artery or cardioembolic stroke. The association may act through increased susceptibility to, or interaction with, high blood pressure. This heterogeneity of association might explain the lack of effect of lowering homocysteine in secondary prevention trials which included all strokes. (Stroke. 2016;47:646-651. DOI: 10.1161/STROKEAHA.115.011545.)

Key Words: cerebral small vessel disease • genetic association • homocysteine • hypertension • lacunar stroke • MTHFR

Elevated plasma homocysteine levels (tHcy) have been consistently associated with the risk of ischemic stroke in observational studies. Moreover, experimental studies suggest that increases in total homocysteine aggravates vascular disease. However, some clinical trials that investigated the benefit of lowering tHcy with B vitamins to reduce the risk of stroke have been negative. In contrast, a recent large primary prevention trial in China, the China Stroke Primary Prevention trials which included all strokes.

Received September 17, 2015; final revision received November 25, 2015; accepted December 31, 2015.

From the Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK (L.C.A.R.-J., M.T., H.S.M.); Department of Medical & Molecular Genetics, King’s College London, London, UK (M.T.); Stroke and Dementia Research Centre, Department of Clinical Neuroscience, St George’s University of London, London, UK (P.A.-S.); KULeuven Department of Experimental Neurology and Leuven Research Institute for Neuroscience and Disease, University of Leuven, and Laboratory of Neurobiology, Vesalius Research Center, VIB, Leuven, Belgium and Department of Neurology, Austin Health and Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia (V.T.); Division of Clinical Neurosciences, Neuroimaging Sciences and Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK (C.S.); Stroke Prevention Research Unit, Nuffield Department of Neurosurgery, University of Oxford, UK (P.M.R.); Department of Cerebrovascular Diseases, Fondazione IRCCS Istituto Neurologico “Carlo Besta”, Milano, Italy (G.B.); Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University Munich and Munich Cluster of Systems Neurology, SyNergy, Munich, Germany (M.D.); Department of Neurology, Mayo Clinic, Jacksonville (J.M.); School of Nursing and Midwifery (J.M.) and Hunter Medical Research Institute (J.M., C.L.), University of Newcastle, NSW, Australia; Center for Human Genetic Research and Department of Neurology, Massachusetts General Hospital, Boston (N.S.R., J.R.); Department of Neurology, Leeds General Infirmary, Leeds Teaching Hospitals NHS Trust, Leeds, UK (A.H.); and School of Life Science, University of Lincoln, Lincoln, UK (S.B.).

Guest Editor for this article was Louis Caplan, MD.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.115.011545/-/DC1.

Correspondence to Loes Rutten-Jacobs, PhD, Stroke Research Group, Department of Clinical Neurosciences, University of Cambridge, Cambridge, CB2 0QQ, UK. E-mail LR406@medschl.cam.ac.uk

© 2016 The Authors. Stroke is published on behalf of the American Heart Association, Inc., by Wolters Kluwer. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution, and reproduction in any medium, provided that the original work is properly cited.

Stroke is available at http://stroke.ahajournals.org DOI: 10.1161/STROKEAHA.115.011545
Prevention Trial (CSPPT), which recruited only hypertensive patients, demonstrated a beneficial effect in reducing risk of stroke.7 Possible reasons for this conflicting evidence include insufficient stroke phenotyping particularly if homocysteine is a predominant risk factor for one type of stroke, dietary folate fortification reducing tHcy in populations in which trials have been performed, interactions between treatment and risk factors, and insufficient treatment duration.

A subtype-specific effect, with elevated homocysteine primarily increasing risk for small vessel disease (SVD) stroke, has been suggested by both epidemiological data and secondary analysis of clinical trials. Case–control studies have suggested that elevated homocysteine is primarily a risk factor for lacunar stroke6,9 and that there may be heterogeneity even within this subtype, with strongest associations in those SVD cases with multiple lacunar infarcts and confluent leukoaraiosis on magnetic resonance imaging (MRI).10 Most previous clinical trials that investigated the benefit of lowering homocysteine studied stroke as a combined event lumping together all different etiologies (ie, hemorrhage and ischemic and ischemic stroke subtypes). A secondary analysis in the Vitamins to Prevent Stroke (VITATOPS) trial11 found a borderline treatment effect in patients with lacunar stroke (hazard ratio 0.80 (95% confidence interval [CI] 0.67–0.96)), whereas an MRI VITATOPS substudy found that elevated homocysteine is primarily a risk factor for lacunar infarct on MRI (subcortical infarct ≤15 mm in diameter). All MRI scans were centrally reviewed by one physician (H.S. Markus). Exclusion criteria were any other cause of white matter disease, including lacunar infarcts >15 mm in diameter, as these can be caused by embolic mechanisms (striatocapsular infarcts); any other specific cause of stroke (eg, lupus anticoagulant, cerebral vasculitis, dissection, monogenic forms of stroke, eg, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)). Large-artery and cardioembolic stroke cases were obtained from Genetic Risk Factors for Leukoaraiosis Study (GENESIS), LSS, MCLSC, and the Wellcome Trust Case Control Immunochip Consortium (WTCCC2-Immunochip), including cohorts from the UK, Germany, Belgium, Italy, Sweden, Poland, Austria, and Australia. Cases were classified into stroke subtypes according to the pathophysiological TOAST classification,16 using clinical assessment, as well as brain and vascular imaging where available. Hypertension was defined as prescription of antihypertensives before stroke or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg >1 week post stroke. Fourteen thousand four hundred and forty-eight ancestry-matched controls were obtained from the same geographical location as the cases in each group. A description and characteristics of all cohorts are given in Table I in the online-only Data Supplement.

Methods

Stroke Populations

We included 1359 MRI-defined lacunar stroke cases from the UK young lacunar stroke DNA study, the Leuven Stroke Study (LSS), and the MRI-confirmed lacunar stroke collaborative (MCLSC), including cohorts from the UK, Germany, Italy, and Australia (Table I in the online-only Data Supplement). Lacunar stroke was defined as a clinical lacunar syndrome17 with an anatomically corresponding lacunar infarct on MRI (subcortical infarct ≤15 mm in diameter). All MRI scans were centrally reviewed by one physician (H.S. Markus). Exclusion criteria were any other cause of white matter disease, including lacunar infarcts >15 mm in diameter, as these can be caused by embolic mechanisms (striatocapsular infarcts); any other specific cause of stroke (eg, lupus anticoagulant, cerebral vasculitis, dissection, monogenic forms of stroke, eg, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL)). Large-artery and cardioembolic stroke cases were obtained from Genetic Risk Factors for Leukoaraiosis Study (GENESIS), LSS, MCLSC, and the Wellcome Trust Case Control Immunochip Consortium (WTCCC2-Immunochip), including cohorts from the UK, Germany, Belgium, Italy, Sweden, Poland, Austria, and Australia. Cases were classified into stroke subtypes according to the pathophysiological TOAST classification,16 using clinical assessment, as well as brain and vascular imaging where available. Hypertension was defined as prescription of antihypertensives before stroke or systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg >1 week post stroke. Fourteen thousand four hundred and forty-eight ancestry-matched controls were obtained from the same geographical location as the cases in each group. A description and characteristics of all cohorts are given in Table I in the online-only Data Supplement.

White Matter Hyperintensity Volumes Population

The white matter hyperintensity (WMH) volume population (n=3670) was derived from the International Stroke Genetics Consortium (ISGC) WMH collaboration. This collaboration measured WMH volumes in patients with ischemic stroke from the UK, Italy, Belgium, Germany, Australia, and USA (Table II in the online-only Data Supplement). Inclusion criteria were >18 years of age, self-reported European ancestry, and a diagnosis of ischemic stroke. Exclusion criteria were any other cause of white matter disease, including CADASIL, vasculitis, and demyelinating and mitochondrial disorders. MRI scans were acquired as part of routine clinical practice for evaluation of ischemic stroke. Fluid-attenuated inversion recovery sequences were primarily used for leukoaraiosis analysis; however, in their absence, T2 sequences were used. All scans were quantitatively graded to obtain a WMH volume, which was normalized for intracranial volume. WMH volume was measured in the hemisphere contralateral to the infarct and doubled to obtain whole brain volumes. Patients with bilateral nonlacunar infarcts were excluded. All neuroimaging analyses have been previously described19.

Genotyping

Direct genotyping of rs1801133 was performed in all cohorts except WTCCC2-Immunochip using commercially available arrays from
Affymetrix or Illumina. In WTCCC2-Immunochip, rs1801133 was imputed from the 1000 Genomes integrated variant set (March 2012) using IMPUTE v2.21 The SNP was imputed with high accuracy (imputation info score = 0.91). The SNP passed genotyping frequency thresholds (>97%) and was in Hardy–Weinberg equilibrium in all groups. To control for population stratification, individuals were removed that did not segregate with Hapmap II European populations based on ancestry informative principal component analysis using EIGENSTRAT software or multidimensional scaling in PLINK software, and ancestry-informative covariates were included in all analyses.21,22

**Statistical Analysis**

In each cohort, logistic regression was performed to test for association of *MTHFR C677T* with MRI-defined lacunar stroke, cardioembolic stroke, and large artery stroke, assuming an additive model and adjusting for ancestry-informative principal components.

For each cohort, the association between WMH volume and *MTHFR C677T* was determined by performing linear regression of WMH volume on genotype dosages. Results across all cohorts were combined using a fixed-effects inverse variance weighted meta-analysis method. Subsequently, we repeated the analyses for MRI-defined lacunar stroke and WMH volume stratified by hypertension status.

We set our *P* value threshold for the main analyses to *P*<0.05 and then used a Bonferroni-corrected value (*P*=0.005) to assess significance in secondary analyses.

**Results**

**Stroke Analyses**

*MTHFR C677T* was significantly associated with lacunar stroke (odds ratio [OR] 1.20, 95% CI 1.09–1.33; *P*=0.0003), but not with large-artery stroke (OR 1.01, 95% CI 0.93–1.08; *P*=0.88) or cardioembolic stroke (OR 1.03, 95% CI 0.96–1.11; *P*=0.44; Figure 1).

In the lacunar stroke cases, the association was most pronounced in homozygotes (OR 1.48, 95% CI 1.20–1.84 for TT versus CC; *P*=0.0003 and OR 1.17, 95% CI 1.01–1.36 for CT versus CC; *P*=0.03).

The overall prevalence of hypertension in the lacunar stroke cases was 72.6%. There were no differences in the prevalence of hypertension according to *MTHFR* genotype (72.0% in CC, 73.4% in CT, and 71.2% in TT). Stratifying the lacunar stroke cases for hypertension status demonstrated the association of *MTHFR C677T* with lacunar stroke (rs9369898, *MUT*), but no association with WMH volume on genotype dosages. Results across all cohorts were combined using a fixed-effects inverse variance weighted meta-analysis method. Subsequently, we repeated the analyses for MRI-defined lacunar stroke and WMH volume stratified by hypertension status.

We set our *P* value threshold for the main analyses to *P*<0.05 and then used a Bonferroni-corrected value (*P*=0.005) to assess significance in secondary analyses.

**WMH Volumes Analyses**

*MTHFR C677T* was significantly associated with WMH volume (OR 1.06, 95% CI 1.01–1.11; *P*=0.04; Figure 2). In the secondary analyses in which we stratified by hypertension status, there was a borderline association for either hypertensive cases (OR 1.06, 95% CI 1.00–1.13; *P*=0.05), although this did not pass the Bonferroni-corrected threshold, whereas there was no association in normotensive cases (OR 1.02, 95% CI 0.95–1.11; *P*=0.57).

**Discussion**

In the present study, we showed that the tHcy-associated genetic variant *MTHFR C677T* was associated with lacunar stroke risk and cerebral SVD, but not for large artery or cardioembolic stroke, and that this association was restricted to patients with hypertension. Thereby, this study supports the hypothesis that homocysteine is a risk factor for specifically SVD and not for the other stroke subtypes.

Previous genetic association studies linking homocysteine to ischemic stroke have produced conflicting results.15 Such candidate studies may be influenced by publication bias, which is reduced in large multicentre GWAS studies. A recent analysis of 18 SNPs associated with tHcy reported equivocal results in 12 389 ischemic stroke cases (METASTROKE).23 There was no association between any SNPs associated with tHcy and all ischemic stroke or large artery or cardioembolic subtypes, consistent with this study. However, one SNP was associated with lacunar stroke (rs9369898, *MUT*), but no association was found with the *MTHFR* polymorphism.

Our study is the first large-scale study to include MRI-based phenotyping of lacunar stroke. The majority of stroke cases in previous studies have relied on CT brain imaging in combination of a diagnosis of a lacunar stroke syndrome. In ≤50% of cases, a clinical lacunar syndrome is caused by pathologies other than SVD.24
Table. Association Between MTHFR C677T Genotypes and MRI-Defined Lacunar Stroke

| MTHFR Genotype | OR (95% CI) | P Value |
|----------------|-------------|---------|
| All lacunar stroke |             |         |
| CT vs CC | 1.17 (0.90–1.13) | 0.10 |
| TT vs CC | 1.34 (1.17–1.53) | <0.0001 |
| Hypertensive lacunar stroke |             |         |
| CT vs CC | 1.19 (0.95–1.50) | 0.10 |
| TT vs CC | 1.35 (1.16–1.58) | <0.0001 |
| Normotensive lacunar stroke |             |         |
| CT vs CC | 1.07 (0.84–1.37) | 0.58 |
| TT vs CC | 1.23 (0.85–1.78) | 0.28 |

CI indicates confidence interval; MRI, magnetic resonance imaging; and OR, odds ratio.

Another possible reason for the conflicting results between different studies is dietary folic acid fortification in certain populations. This was present in some cohorts in METASTROKE and might have attenuated any association with MTHFR C677T in METASTROKE. In the present study, folic acid fortification was not used in any of the included stroke cohorts, but was used in some of the included WMH volume cohorts. The association shown between MTHFR C677T and WMH volume in the present study might have been attenuated by the inclusion of these folic acid–fortified cohorts.

The SVD-specific effect of MTHFR C677T in the present study might reflect the effects of increased tHcy in SVD patients with this genetic variant. Although we did not assess tHcy in the present study and, therefore, cannot draw conclusions on this association in the present study, previous studies support this hypothesis of possible SVD-specific effect of increased tHcy. Secondary analyses of the VITATOPS trial suggested that homocysteine-lowering therapy might be associated with improved outcome in SVD (both lacunar stroke and WMH) but not in other stroke subtypes. In the VITATOPS trial, in which lacunar stroke subtyping was largely based on a clinical stroke syndrome with computed tomography imaging, a borderline significant reduction in recurrent stroke occurred in patients with SVD; based on our results, one could hypothesise that this treatment effect might be stronger in MRI-confirmed lacunar stroke. Consistent with this specific effect in SVD are the results of an MRI sub-study in 359 individuals from VITATOPS; although no association was found in the group as a whole, in a subanalysis of patients with MRI evidence of severe SVD at baseline, B vitamin supplementation was associated with a significant reduction in WMH volume change. Two meta-analyses on previous genetic association studies linking MTHFR with WMH on MRI could not confirm an association between MTHFR and WMH. Individual studies that were included in these meta-analyses had only small number of patients, and it was suggested that much larger studies would be needed to detect an association. In the present study, we included twice as many subjects as the largest study in the previous meta-analyses that assessed WMH on a dichotomous scale and three times as many subjects as the study that assessed WMH volume.

We found that the association of MTHFR C677T with lacunar stroke was restricted to hypertensive individuals. One possible explanation for this finding exclusively in hypertensive individuals might be that the association acts through increased susceptibility to, or interaction with, high blood pressure. Interestingly, the recent large primary prevention CSPPT trial showed a benefit of homocysteine-lowering therapy in reducing stroke risk in hypertensive individuals from a population in which folic acid fortification was not occurring.

The major strength of our study is the confirmation of all lacunar strokes by MRI and the relatively large sample size. Furthermore, the approach of using a genetic proxy for tHcy reduces the likelihood of reversed causality and residual confounding compared with previous observational studies. The relationship between tHcy and stroke in observational studies might be confounded by unmeasured or not adequately measured factors (eg, other dietary factors) that are causally associated with stroke. In contrast, genetic studies rely on the fact that genetic variants are fixed from conception and are not influenced by other traits.

Moreover, the stroke cohorts were derived from countries in which folic acid fortification was not implemented at the time of stroke, which maximized the chances of demonstrating an effect for MTHFR C677T. We also included an analysis of WMH volume in ischemic stroke patients. Stroke patients represent an enriched population in whom WMH are increased. It has been shown, however, that the genetic factors underlying

Figure 2. Forest plot for the association of MTHFR C677T with WMH. The size of the box is inversely proportional to the estimate variance of the effect estimator. ASGC indicates Australian Stroke Genetics Collaborative; GENESIS, Genetic Risk Factors for Leukoaraisis Study; ISGS, Ischemic Stroke Genetics Study; LSS, Leuven Stroke Study; MGH, Massachusetts General Hospital; SGUL, St George’s University of London; SWISS, Sibling with Ischemic Stroke Study; and WTCCC2, Wellcome Trust Case-Control Consortium 2.
WMH in ischemic stroke patients seem to be similar to those in population-based studies of WMH.\textsuperscript{15} A potential limitation of the present study is that we did not have independent replication cohorts available to validate our findings, and therefore, future studies are warranted to confirm these interesting findings. Furthermore, although \textit{MTHFR} C677T is strongly associated with tHcy in other studies,\textsuperscript{12,14} we could not also directly assess the association of tHcy with stroke subtype in the present study because tHcy measurements were not available in all of our cohorts.

In summary, we showed that \textit{MTHFR} C677T was associated with lacunar stroke in hypertensive individuals, supporting a possible causal role for homocysteine in the pathogenesis of cerebral SVD. Our results suggest that any future trials investigating the benefit of lowering homocysteine in stroke patients should focus on the SVD subtype and that they should incorporate MRI-based diagnosis.

Acknowledgments

We thank all study staff and participants for their important contributions. Study-specific collaborators are reported in the online-only Data Supplement.

Sources of Funding

Collection of the UK Young Lacunar Stroke DNA Study (DNA Lacunar) was primarily supported by the Wellcome Trust (WT072952) with additional support from the Stroke Association (TSA 2010/01). Genotyping of the DNA Lacunar samples and Dr Traylor were supported by a Stroke Association Grant (TSA 2013/01). Genotyping of WTCCC2 ischemic stroke study was funded by the Wellcome Trust. The Oxford Vascular Study has been funded by Wellcome Trust, Wolfson Foundation, UK Stroke Association, British Heart Foundation, Dunhills Medical Trust, National Institute of Health Research (NIHR), Medical Research Council, and the NIHR Oxford Biomedical Research Centre. Funding for the genotyping at Massachusetts General Hospital was provided by the Massachusetts General Hospital-Dean Institute for the Integrative Study of Atrial Fibrillation and Stroke and the National Institute of Neurological Disorders and Stroke (U01 NS069208). Dr Rutten-Jacobs was supported by a Stroke Association/British Heart Foundation programme grant (TSA BHF 2010/01). Dr Adib-Samii was supported by a Medical Research Council (United Kingdom) training fellowship. Drs Markus and Bevan are supported by the National Institute for Health Research Cambridge University Hospitals Comprehensive Biomedical Research Centre. Dr Markus is supported by a National Institute for Health Research Senior Investigator award. Dr Thijss is supported by a Clinical Investigator Grant from the scientific research fund, Fonds Wetenschappelijk Onderzoek Flanders. Dr Levi is supported by a National Health and Medical Research Council (NHMRC Australia) Practitioner Fellowship and the Australian Stroke Genetics Collaboration has received Project Grant support from the NHMRC (App 1010287). Dr Rost was supported by a National Institute of Neurological Disorders and Stroke grant (R01 NS082285-01). Professor Rothwell is in receipt of an NIHR Senior Investigator Award and a Wellcome Trust Senior Investigator Award. We also acknowledge the use of the facilities of the Acute Vascular Imaging Centre, Oxford, and the Cardiovascular Clinical Research Facility, Oxford. The sponsors of the study had no role in the study design, data collection, data analysis, interpretation, writing of the article, or the decision to submit the article for publication.

Disclosures

None.

References

1. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. \textit{JAMA.} 2002;288:2015–2022.
2. Faraci FM, Lentz SR. Hyperhomocysteinemia, oxidative stress, and cerebral vascular dysfunction. \textit{Stroke.} 2004;35:345–347. doi: 10.1161/01.STR.0000115161.10646.67.
3. 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. \textit{Lancet Neurol.} 2010;9:855–865.
4. Lonn E, Yusuf S, Arnold MJ, Sheridan P, Pogue J, Micks M, et al; Heart Outcomes Prevention Evaluation (HOPE) 2 Investigators. Homocysteine lowering with folic acid and B vitamins in vascular disease. \textit{N Engl J Med.} 2006;354:1567–1577. doi: 10.1056/NEJMoa060900.
5. Armitage JM, Bowman L, Clarke RJ, Wallendouskus K, Bulbulia R, Rahimi K, et al; Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) Collaborative Group. Effects of homocysteine-lowering with folic acid plus vitamin B12 vs placebo on mortality and major morbidity in myocardial infarction survivors: a randomized trial. \textit{JAMA.} 2010;303:2486–2494. doi: 10.1001/ jama.2010.840.
6. 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. \textit{JAMA.} 2004;291:565–575. doi: 10.1001/ jama.291.5.565.
7. Huo Y, Li J, Qin X, Huang Y, Wang X, Gottesman RF, et al; CSPPt Investigators. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPt randomized clinical trial. \textit{JAMA.} 2015;313:1325–1335. doi: 10.1001/ jama.2015.2274.
8. Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, et al. Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. \textit{Circulation.} 2004;109:2766–2772. doi: 10.1161/01. CIR.0000139412.76355.2D.
9. Kwan U, Crosseley C, Barry L, Rudd A, Wolfe CD, Collinson P, et al. Homocysteine and its relationship to stroke subtypes in a UK black population: the south London ethnicity and stroke study. \textit{Stroke.} 2008;39:2943–2949. doi: 10.1161/STROKEAHA.107.513416.
10. 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. \textit{Brain.} 2004;127(pt 1):212–219. doi: 10.1093/ brain/awh023.
11. Cavaliere M, Schmidt R, Chen C, Mok V, de Freitas GR, Song S, et al; VITATOPS Trial Study Group. B vitamins and magnetic resonance imaging-detected ischemic brain lesions in patients with recent transient ischemic attack or stroke: the VITAMins TO Prevent Stroke (VITATOPS) MRI-substudy. \textit{Stroke.} 2012;43:3266–3270. doi: 10.1161/ STROKEAHA.112.665703.
12. Casas JP, Bautista LA, Yip SF, Sharma P, Hingorani AD. Homocysteine and stroke: evidence on a causal link from mendelian randomisation. \textit{Lancet Neurol.} 2005;6:225–232. doi: 10.1016/S1474-4422(05)71742-3.
13. Dichgans M. Genetics of ischemic stroke. \textit{Lancet Neurol.} 2007;6:149–161. doi: 10.1016/S1474-4422(07)70028-5.
14. van Meurs JB, Pare G, Schwartz SM, Haara A, Tanaka T, Vermeulen SH, et al. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. \textit{Am J Clin Nutr.} 2013;98:666–676. doi: 10.3945/ajcn.112.045455.
15. Holmes MV, Newcombe P, Hubacek JA, Sofat R, Ricketts SL, Cooper J, et al; Effect modification by population dietary folate on the association between \textit{MTHFR} genotype, homocysteine, and stroke risk: a meta-analysis of genetic studies and randomised trials. \textit{Lancet.} 2011;377:584–594. doi: 10.1016/S0140-6736(11)60872-8.
16. Wardlaw JM, Smith EE, Bis Seattle, Cordomier C, Fazeekas F, Fryane R, et al; Standards for Reporting Vascular changes on \textit{Structured Reporting (STRIVE) V1}. Neuroimaging standards for research into small vessel disease and its contribution to ageing and neurodegeneration. \textit{Lancet Neurol.} 2012;11:822–838. doi: 10.1016/S1474-4422(12)70124-8.
17. Bamford J, Sandercock P, Dennis M, Burn J, Warlow C. Classification and natural history of clinically identifiable subtypes of cerebral infarction. \textit{Lancet.} 1991;337:1521–1526.
for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke*. 1993;24:35–41.

19. Adib-Samii P, Rost N, Traylor M, Devan W, Biffi A, Lanfranconi S, et al; Australian Stroke Genetics Collaborative; Wellcome Trust Case-Control Consortium-2 (WTCCC2); METASTROKE; International Stroke Genetics Consortium. 17q25 Locus is associated with white matter hyperintensity volume in ischemic stroke, but not with lacunar stroke status. *Stroke*. 2013;44:1609–1615. doi: 10.1161/STROKEAHA.113.679936.

20. Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, et al. An integrated map of genetic variation from 1,092 human genomes. *Nature*. 2012;491:56–65.

21. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet*. 2006;38:904–909. doi: 10.1038/ng1847.

22. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet*. 2007;81:559–575. doi: 10.1086/519795.

23. Cotlarciuc I, Malik R, Holliday EG, Ahmadi KR, Paré G, Psaty BM, et al; METASTROKE and the International Stroke Genetics Consortium. Effect of genetic variants associated with plasma homocysteine levels on stroke risk. *Stroke*. 2014;45:1920–1924. doi: 10.1161/STROKEAHA.114.005208.

24. Markus HS, Khan U, Birns J, Evans A, Kalra L, Rudd AG, et al. Differences in stroke subtypes between black and white patients with stroke: the South London Ethnicity and Stroke Study. *Circulation*. 2007;116:2157–2164. doi: 10.1161/CIRCULATIONAHA.107.699785.

25. Paternoster L, Chen W, Sudlow CL. Genetic determinants of white matter hyperintensities on brain scans: a systematic assessment of 19 candidate gene polymorphisms in 46 studies in 19,000 subjects. *Stroke*. 2009;40:2020–2026. doi: 10.1161/STROKEAHA.108.542050.

26. Tran T, Cotlarciuc I, Yadav S, Hasan N, Bentley P, Levi C, et al. Candidate-gene analysis of white matter hyperintensities on neuroimaging. [published online ahead of print April 2, 2015]. *J Neurol Neurosurg Psychiatry*. doi: 10.1136/jnnp-2014-309685. http://jnnp.bmj.com/content/early/2015/04/02/jnnp-2014–309685. Accessed November 23, 2015.
Association of MTHFR C677T Genotype With Ischemic Stroke Is Confined to Cerebral Small Vessel Disease Subtype

Loes C.A. Rutten-Jacobs, Matthew Traylor, Poneh Adib-Samii, Vincent Thijs, Cathie Sudlow, Peter M. Rothwell, Giorgio Boncoraglio, Martin Dichgans, James Meschia, Jane Maguire, Christopher Levi, Natalia S. Rost, Jonathan Rosand, Ahamad Hassan, Steve Bevan and Hugh S. Markus

Stroke. 2016;47:646-651; originally published online February 2, 2016; doi: 10.1161/STROKEAHA.115.011545

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/47/3/646
Free via Open Access

Data Supplement (unedited) at:
http://stroke.ahajournals.org/content/suppl/2016/02/02/STROKEAHA.115.011545.DC1.html

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

Association of MTHFR C677T genotype with ischemic stroke is confined to cerebral small vessel disease subtype

Loes CA Rutten-Jacobs, PhD^1^, Matthew Traylor, PhD^{1,2}, Poneh Adib-Samii, MBBS^{3}, Vincent Thijs, MD^{4}, Cathie Sudlow, FRCP^5^, Peter M Rothwell, FMedSci^{6}, Giorgio Boncoraglio, MD^{7}, Martin Dichgans, MD^{8}, James Meschia, MD^{9}, Jane Maguire, PhD^{10}, Christopher Levi, MD^{10}, Natalia S Rost, MD^{11}, Jonathan Rosand, MD^{11}, Ahamad Hassan, FRCP^{12}, Steve Bevan, PhD^{13}, Hugh S Markus, FRCP^{1}

Affiliations

^1^ University of Cambridge, Department of Clinical Neurosciences, Cambridge, UK
^2^ Department of Medical & Molecular Genetics, King’s College London, London, UK
^3^ Stroke and Dementia Research Centre, St George’s University of London, London, UK
^4^ KULeuven Department of Experimental Neurology and Leuven Research Institute for Neuroscience and Disease, University of Leuven, and Laboratory of Neurobiology, Vesalius Research Center, VIB, Leuven, Belgium and Department of Neurology, Austin Health and Florey Institute of Neuroscience and Mental Health, Heidelberg, Australia
^5^ Division of Clinical Neurosciences, Neuroimaging Sciences and Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh, UK
^6^ Stroke Prevention Research Unit, Nuffield Department of Neuroscience, University of Oxford, UK
^7^ Department of Cerebrovascular Diseases, Fondazione IRCCS Istituto Neurologico "Carlo Besta”, Milano, Italy
^8^ Institute for Stroke and Dementia Research, Klinikum der Universität München, Ludwig-Maximilians-University Munich and Munich Cluster of Systems Neurology, SyNergy, Munich, Germany
^9^ Department of Neurology, Mayo Clinic, Jacksonville, USA
^10^ Centre for Clinical Epidemiology and Biostatistics, Hunter Medical Research Institute and School of Medicine and Public Health, University of Newcastle, NSW, Australia
^11^ Center for Human Genetic Research and Department of Neurology, Massachusetts General Hospital, Boston, USA
^12^ Department of Neurology, Leeds General Infirmary, Leeds Teaching Hospitals NHS Trust, Leeds, UK
^13^ School of Life Science, University of Lincoln, UK

Corresponding Author:
Loes Rutten-Jacobs
Department of Clinical Neurosciences
University of Cambridge
Cambridge
CB2 0QQ
LR406@medschl.cam.ac.uk
Cohorts description

UK Young Lacunar Stroke DNA Study (DNA Lacunar)

DNA Lacunar is a multicentre cohort study, which constitutes a large DNA resource of young patients with well phenotyped lacunar stroke and stroke-free community controls.

Between 2005 and 2012, 1030 white patients of European ancestry with lacunar stroke, aged ≤ 70 years, were recruited from 72 specialist stroke centres throughout the UK. All patients underwent brain MRI, imaging of the carotid arteries and ECG. Echocardiography was performed when appropriate. All MRI’s and clinical histories were reviewed centrally by one experienced stroke physician.

970 Unrelated Caucasian controls, free of clinical cerebrovascular disease, were obtained by random sampling from general practice lists from the same geographical location as the patients. Sampling was stratified for age and sex.

MRI-confirmed ischemic stroke collaboration (MCISC)

Wellcome Trust Case-Control Consortium 2 (WTCCC2)

The WTCCC2 samples were genotyped as part of the WTCCC 2 ischemic stroke study. Stroke cases were recruited from three centres in the UK (St. George's University London, Oxford and Edinburgh) and one centre in Germany, University and Klinikum Großhadern, Ludwig-Maximilians-University, Munich.

WTCCC2-UK: The St George’s Stroke Study consecutively recruited ischemic stroke patients attending cerebrovascular services in London between 1995 and 2008. All patients had clinically relevant diagnostic workup performed, including brain imaging with computed tomography (CT) and/or magnetic resonance imaging (MRI) as well as ancillary diagnostic investigations including duplex ultrasonography of the carotid and vertebral arteries, echocardiography, Holter monitoring, magnetic resonance angiography (MRA), CT-angiography (CTA) and blood tests. The Oxford Vascular Study recruited patients with acute ischemic stroke or transient ischemic attack (TIA) with evidence of infarction on brain imaging between 2002 and 2008 as part of a population-based study. All cases were phenotyped by one experienced stroke neurologist with review of original imaging. The Edinburgh Stroke Study prospectively recruited consecutive stroke inpatients and outpatients between 2002 and 2005. An experienced stroke physician assessed each patient as soon as possible after the stroke, prospectively recording demographic and clinical details, including vascular risk factors and results of brain imaging and other investigations.

WTCCC2-Germany: The Munich study recruited consecutively between 2002 and 2008, from a single Stroke Unit with a high rate of MR imaging (>80%) (n=1383). All subjects were over 18 years of age, of self-reported European ancestry and with a diagnosis of ischemic stroke classified according to TOAST by an experienced neurologist or stroke physician. All patients had brain imaging as well as ancillary diagnostic investigations where clinically relevant.

Controls for the UK samples were drawn from shared WTCCC controls obtained from the 1958 Birth Cohort. This is a prospectively collected cohort of individuals born in 1958 (http://www.b58cgene.sgu.ac.uk/), and ascertained as part of the national child development study (http://www.cls.ioe.ac.uk/studies.asp?section=000100020003). Data from this cohort are available as a common control set for a number of genetic and epidemiological studies.

For the German samples controls were Caucasians of German origin participating into the population KORAgen study (www.gsf.de/kora/en/english.html). This survey represents a gender- and age stratified random sample of all German residents of the Augsburg area and
consists of individuals 25 to 74 years of age, with about 300 subjects for each 10-year increment. All controls were free of a history of stroke or transient ischemic attack.

**Besta Stroke Study (Milano)**
This study includes consecutive Italian patients referred to Besta Institute from 2000 to 2009 with stroke and included in the Besta Cerebrovascular Diseases Registry (CEDIR). Ischemic stroke cases, first ever or recurrent, confirmed on brain imaging, were selected for this study. An experienced stroke neurologist assessed all cases.

**Australian Stroke Genetics Collaborative (ASGC)**
Stroke cases comprised European-ancestry patients admitted to four clinical centres across Australia (The Neurosciences Department at Gosford Hospital, Gosford, New South Wales (NSW); the Neurology Department at John Hunter Hospital, Newcastle, NSW; The Queen Elizabeth Hospital, Adelaide; and the Royal Perth Hospital, Perth) between 2003 and 2008. Stroke was defined by WHO criteria as a sudden focal neurologic deficit of vascular origin, lasting more than 24 hours and confirmed by brain imaging. Other investigative tests such as electrocardiogram, carotid Doppler and trans-oesophageal echocardiogram were conducted to define stroke aetiology as clinically appropriate.

**Leuven Stroke Study**
Patients with cerebral ischemia, defined as a clinical stroke with imaging confirmation or a TIA with a new ischemic lesion on diffusion weighted MRI, who were admitted to the Stroke Unit of the University Hospitals in Leuven were enrolled. All patients underwent brain imaging and a standardized protocol including carotid ultrasound or CT angiography and cardiac examination (echocardiography and Holter monitoring) in all patients. Control individuals were selected from the same population and were either spouses of patients with multiple sclerosis, amyotrophic lateral sclerosis or stroke or healthy community dwelling subjects partially from the Leuven University Gerontology Database. Controls either confirmed they never had a stroke or TIA or responded negative to any item of the Verification of Stroke Free Status questionnaire.

**GENESIS**
This study recruited patients attending cerebrovascular services at St. George’s Hospital, London between 2011-2013. All patients had clinically relevant diagnostic workup performed, including brain imaging with magnetic resonance imaging (MRI) as well as ancillary diagnostic investigations including duplex ultrasonography of the carotid and vertebral arteries, echocardiography, Holter monitoring, magnetic resonance angiography (MRA), CT-angiography (CTA) and blood tests.

**WTCCC2-Immunochip**

Bio-Repository of DNA in Stroke (BRAINS): London
The Bio-Repository of DNA in Stroke (BRAINS) is an international study recruiting highly phenotyped patients with stroke. For the purposes of the current work all patients were Caucasians. Diagnosis of stroke was confirmed using positive imaging (MRI or CT) and ischemic stroke subtypes were assigned using TOAST criteria, based on clinical, imaging and risk factor data. The cohort has been described in detail elsewhere (Yadav S, Schanz R,
Maheshwari A, et al. Bio-Repository of DNA in stroke (BRAINS): a study protocol. BMC Med Genet 2011; 12: 34).

**Glasgow: Scotland**

Patients with ischemic stroke attending the cerebrovascular service of the Western Infirmary, Glasgow, were recruited between 1990 and 2004 as part of an ongoing study of genetic and circulating biomarkers in stroke. All patients underwent brain imaging and extracranial carotid ultrasound in accordance with a standard clinical protocol. The study was approved by the West Ethics Committee.

Controls for the UK samples were drawn from shared WTCCC controls obtained from the 1958 Birth Cohort. This is a prospectively collected cohort of individuals born in 1958 (http://www.b58cgene.sgu.ac.uk/), and ascertained as part of the national child development study (http://www.cls.ioe.ac.uk/studies.asp?section=000100020003). Data from this cohort are available as a common control set for a number of genetic and epidemiological studies.

**Lund Stroke Register, Sweden**

Lund Stroke Register (LSR) since 2001 continuously enrolls patients aged 18 and older with first-ever stroke, living in the primary uptake area of Skane University Hospital, Lund. The study is mainly hospital-based but has a good coverage of the whole geographical population. All included patients are examined with CT/MR or autopsy of the brain. When clinically indicated, the patients are examined with ultrasound imaging of carotid arteries, echocardiography, and angiography. In this study, first-ever ischemic stroke patients from LSR between 2001 and 2004 were included. All patients were assessed by a neurologically trained physician regarding stroke type. Informed consent was obtained from all individuals or when they were not able to respond from their next-of-kin. The study was approved by the Ethics Committee of Lund University. Biobank services were performed at Region Skane Competence Centre (RSK C Malmo), Malmo University Hospital, Malmo, Sweden.

**Swedish Control Samples**

Controls for the Lund cases were provided by the Swedish SLE network. Controls were healthy blood donors from the geographical areas of Uppsala, Stockholm and Lund. The studies were approved by the regional ethics boards and all subjects gave their informed consent to participate. Genotyping of the Swedish control samples was performed at the SNP&SEQ technology platform in Uppsala, Sweden (www.genotyping.se).

**Munich: Germany**

Cases were consecutive European Caucasians recruited from a single dedicated Stroke Unit at the Department of Neurology, Klinikum Großhadern, Ludwig-Maximilians-University, Munich. Ischemic stroke subtypes were determined according to TOAST criteria based on relevant clinical and imaging data.

**German control samples**

German healthy control individuals were obtained from the PopGen biobank [Krawczak et al., Community Genet 9:55-61, 2006]. Written, informed consent was obtained from all study participants and all protocols were approved by the institutional ethical review committee of the participating centre. The panel is a cross-sectional control cohort from the Kiel area in Northern Germany. More than 300 phenotypes were collected for the cohort and a 3-year follow has recently been completed. All data and biomaterials are accessible via the PopGen biobank. The Genotyping was part of the German National Genome Research Network.
Poland: Krakow

Patients were recruited in the stroke unit of the Jagiellonian University in Krakow, Poland (a single-center study). All stroke patients and controls were >18 years of age and were white. All patients had clinically relevant diagnostic workup performed, including brain imaging with computed tomography (CT) (100%) and/or magnetic resonance imaging (MRI) (8%) as well as ancillary diagnostic investigations including duplex ultrasonography of the carotid and vertebral arteries (85.2%), echocardiography (54.8%). MR-angiography, CT-angiography, Holter monitoring, transesophageal echocardiography and blood tests for hypercoagulability were performed were indicated. Patients were classified into etiologic subtypes according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST). The control group included unrelated subjects taken from the population of southern Poland. Control subjects had no apparent neurological disease based on the findings in a structured questionnaire and a neurological examination. The study was approved by local research ethics committees and informed consent was obtained from all participants.

White Matter Hypertensions collaboration International Stroke Genetics Consortium

St Georges University of London (SGUL)
This study recruited patients attending cerebrovascular services at St. George’s Hospital, London between 2007-2011. All patients had clinically relevant diagnostic workup performed, including brain imaging with magnetic resonance imaging (MRI) as well as ancillary diagnostic investigations including duplex ultrasonography of the carotid and vertebral arteries, Holter monitoring, magnetic resonance angiography (MRA), CT-angiography (CTA) and blood tests.

Massachusetts General Hospital (MGH)
Cases presenting with ischemic stroke and admitted to the Massachusetts General Hospital (MGH) Stroke Unit through the Emergency Department, or evaluated in the MGH Neurology outpatient clinics, as well as on the inpatient Medical and Vascular Surgical services from January 2003 to July 2008. Ischemic stroke was defined as either (1) a radiographically proven (head CT or MRI) infarct associated with the appropriate clinical stroke syndrome, or (2) a fixed neurological deficit persisting more than 24 hours, consistent with a vascular pattern of involvement and without radiographic evidence of demyelinating or other non-vascular disease. All subjects were evaluated by a neurologist upon presentation and clinical and laboratory data were collected during the admission for qualifying ischemic stroke event. All patients had acute brain imaging as well as ancillary diagnostic investigations: cervical and intracranial vessel imaging using CT or MR angiography (75%), cervical ultrasound (24%), echocardiography (86%), and Holter monitoring (16%).

Ischemic Stroke Genetics Study (ISGS)
Ischemic Stroke Genetics Study (ISGS) was a 5-center, prospective, case-control study of first-ever ischemic stroke cases in the United States. All affected individuals had WHO-defined stroke confirmed by a study neurologist to be ischemic on the basis of head CT or brain MRI. Peripheral blood DNA samples were collected between May 2003 and September 2008.
Sibling with Ischaemic Stroke Study (SWISS)

This is a prospective, multicentre study of sibling pairs with first-ever or recurrent ischemic stroke. Probands were recruited from 70 clinical centres across the US and Canada. Ischemic stroke affected and unaffected siblings were recruited primarily using proband-initiated contact. All affected individuals had WHO-defined stroke confirmed by a study neurologist to be ischemic on the basis of brain imaging. Peripheral blood DNA samples were collected between October 2000 and December 2009.
Appendix

UK Young Lacunar Stroke DNA Study collaborators

Study managers: Josie Monaghan; Alan Zanich, Samantha Febrey, Eithne Smith, Jenny Lennon, St George’s University of London

Participating centres (number of enrolled patients per centre; local investigators):
Aberdeen Royal Infirmary, Aberdeen (12; Mary Macleod). Addenbrooke’s Hospital, Cambridge (54; Jean-Claude Baron, Elizabeth Warburton, Diana J Day, Julie White). Airedale General Hospital, Steeton (4; Samantha Mawer). Barnsley Hospital, Barnsley (3; Mohammad Albazzaz, Pravin Torane, Keith Elliott, Kay Hawley). Bart’s and the London, London (2; Patrick Gompertz). Basingstoke and North Hampshire Hospital, Basingstoke (13; Elio Giallombardo, Deborah Dellafera). Blackpool Victoria Hospital, Blackpool (11; Mark O'Donnell). Bradford Royal Infirmary, Bradford (1; Chris Patterson). Bristol Royal Infirmary, Bristol (8; Sarah Caine). Charing Cross Hospital, London (12; Pankaj Sharma). Cheltenham General and Gloucester Royal Hospitals, Cheltenham and Gloucester (10; Dipankar Dutta). Chesterfield Royal Hospital, Chesterfield (4; Sunil Punnoose, Mahmud Sajid). Countess of Chester Hospital, Chester (22; Kausik Chatterjee). Derriford Hospital, Plymouth (4; Azlisham Mohd Nor). Dorset County Hospital NHS Foundation Trust, Dorchester (6; Rob Williams). East Kent Hospitals University NHS Foundation Trust, Kent (22; Hardeep Baht, Guna Gunathilagan). Eastbourne District General Hospital, Eastbourne (4; Conrad Athulathmudali). Frenchay Hospital, Bristol (1; Neil Baldwin). Frimley Park Hospital NHS Foundation Trust, Frimley (6; Brian Clarke). Guy’s and St Thomas’ Hospital, London (14; Tony Rudd). Institute of Neurology, London (25; Martin Brown). James Paget University Hospital, Great Yarmouth (1; Peter Harrison). King’s College Hospital, London (16; Lalit Kalra). Leeds Teaching Hospitals NHS Trust, London (125; Ahamad Hassan). Leicester General Hospital and Royal Infirmary, Leicester (9; Tom Robinson, Amit Mistri). Luton and Dunstable NHSFT University Hospital, Luton (16; Lakshman Sekaran, Sakthivel Sethuraman, Frances Justin). Maidstone and Tunbridge Wells NHS Trust (3; Peter Maskell). Mayday University Hospital, Croydon (14; Enas Lawrence). Medway Maritime Hospital, Gillingham (5; Sam Sanmuganathan). Milton Keynes Hospital, Milton Keynes (1; Yaw Duodu). Musgrove Park Hospital, Taunton (9; Malik Hussain). Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne (12; Gary Ford). Ninewells Hospital, Dundee (5; Ronald MacWalter). North Devon District Hospital, Barnstaple (8; Mervyn Dent). Nottingham University Hospitals, Nottingham (17; Philip Bath, Fiona Hammonds). Perth Royal Infirmary, Perth (2; Stuart Johnston). Peterborough City Hospital, Peterborough (1; Peter Owusu-Agyei). Queen Elizabeth Hospital, Gateshead (5; Tim Cassidy, Maria Bokhari). Radcliffe Infirmary, Oxford (5; Peter Rothwell). Rochdale Infirmary, Rochdale (4; Robert Namushi). Rotherham General Hospital, Rotherham (1; James Okwera). Royal Cornwall Hospitals NHS Trust, Truro (11; Frances Harrington, Gillian Courtauld). Royal Devon and Exeter Hospital, Exeter (22; Martin James). Royal Hallamshire Hospital, Sheffield (1; Graham Venables). Royal Liverpool University Hospital and Broadgreen Hospital, Liverpool (9; Aravind Manoj). Royal Preston Hospital, Preston (18; Shuja Punekar). Royal Surrey County Hospital, Guildford (23; Adrian Blight, Kath Pasco). Royal Sussex County Hospital, Brighton (14; Chakravarthi Rajkumar, Joanna Breeds). Royal United Hospital, Bath (6; Louise Shaw, Barbara Madigan). Salford Royal Hospital, Salford (16; Jane Molloy). Southampton General Hospital, Southampton (1; Giles Durward). Southend Hospital, Westcliff-on-Sea (26; Paul Guyler). Southern General Hospital, Glasgow (34; Keith Muir,
Wilma Smith). St George’s Hospital, London (108; Hugh Markus). St Helier Hospital, Carshalton (10; Val Jones). Stepping Hill Hospital, Stockport (4; Shivakumar Krishnamoorthy). Sunderland Royal Hospital, Sunderland (1; Nikhil Majumdar). The Royal Bournemouth Hospital, Bournemouth (15; Damian Jenkinson). The Walton Centre, Liverpool (15; Richard White). Torbay Hospital, Torquay (19; Debs Kelly). University Hospital Aintree, Liverpool (19; Ramesh Durairaj). University Hospital of North Staffordshire, Stoke-on-trent (16; David Wilcock). Wansbeck General Hospital and North Tyneside Hospital, Ashington and North Shields (6; Christopher Price). West Cumberland Hospital, Whitehaven (6; Olu Orugun, Rachel Glover). West Hertfordshire Hospital, Watford (20; David Collas). Western General Hospital, Edinburgh (12; Cathie Sudlow). Western Infirmary, Glasgow (33; Kennedy R. Lees, Jesse Dawson). Wycombe Hospital and Stoke Mandeville, High Wycombe (20; Dennis Briley and Matthew Burn). Yeovil District Hospital, Yeovil (46; Khalid Rashed). York Teaching Hospital, York (1; John Coyle).
Table I Stroke cohorts

| Cohort                          | N   | Mean age (sd) | % Male | % Hypertensive |
|---------------------------------|-----|---------------|--------|---------------|
| **DNA Lacunar & GENESIS**       |     |               |        |               |
| Lacunar stroke                  | 998 | 57.3 (9.6)    | 704 (71%) | 705 (71%)     |
| Large artery stroke             | 64  | 70.1 (11.6)   | 44 (69%) |               |
| Cardioembolic stroke            | 80  | 73.5 (14.6)   | 47 (59%) |               |
| Controls                        | 970 | 59.7 (4.3)    | 510 (53%) | NA            |
| **MCLSC**                       |     |               |        |               |
| Lacunar stroke                  | 319 | 68.5 (11.8)   | 190 (60%) | 262 (82%)     |
| Large artery stroke             | 1338| 68.9 (11.9)   | 904 (68%) | 906 (68%)     |
| Cardioembolic stroke            | 1094| 73.7 (13.8)   | 558 (51%) | 761 (70%)     |
| Controls                        | 7623| 62.5 (10.2)   | 4002 (52%) | NA            |
| **LSS**                         |     |               |        |               |
| Lacunar stroke                  | 42  | 65.5 (13.9)   | 29 (69%) | 26 (62%)      |
| Large artery stroke             | 70  | 63 (61%)      |        | 48 (69%)      |
| Cardioembolic stroke            | 157 | 56 (61%)      | 96 (61%) | 98 (62%)      |
| Controls                        | 455 | 55.7 (14.5)   | 212 (47%) | NA            |
| **WTCCC2-Immunochip**           |     |               |        |               |
| Large artery stroke             | 352 | 69.1 (12.2)   | 220 (62%) | 234 (67%)     |
| Cardioembolic stroke            | 639 | 75.7 (11.8)   | 339 (53%) | 417 (67%)     |
| Controls                        | 5401| NA            | 2304 (43%) | NA            |

Abbreviations: DNA Lacunar, UK Young Lacunar Stroke DNA Study; GENESIS, Genetic Risk factors for Leukoaraiosis study; MCLSC, MRI-confirmed lacunar stroke collaboration; LSS, Leuven Stroke Study, WTCCC2-Immunochip, Wellcome Trust Case-Control Consortium II Immunochip
| Centre                           | Country | N  | Mean age (sd) | % Male | % Hypertensive |
|---------------------------------|---------|----|---------------|--------|---------------|
| Milano                          | Italy   | 151| 57 (14)       | 60%    | 57%           |
| WTCCC2-Edinburgh               | UK      | 64 | 68 (13)       | 50%    | 72%           |
| WTCCC2-Munich FLAIR            | Germany | 447| 66 (12)       | 66%    | 72%           |
| WTCCC2-Munich T2               | Germany | 203| 67 (12)       | 55%    | 67%           |
| WTCCC2-Oxford Flair            | UK      | 65 | 65 (15)       | 54%    | 65%           |
| WTCCC2-Oxford T2               | UK      | 75 | 67 (13)       | 59%    | 68%           |
| WTCCC2-SGUL                    | UK      | 323| 70 (14)       | 63%    | 77%           |
| GENESIS 1                      | UK      | 121| 67 (14)       | 67%    | 62%           |
| GENESIS 2                      | UK      | 228| 69 (15)       | 58%    | 76%           |
| SGUL 1                         | UK      | 70 | 70 (13)       | 61%    | 61%           |
| SGUL 2                         | UK      | 57 | 68 (14)       | 58%    | 72%           |
| DNA Lacunar                     | UK      | 303| 57 (9)        | 72%    | 68%           |
| LSS                            | Belgium | 361| 66 (15)       | 58%    | 59%           |
| MGH-Affymetrix                 | US      | 476| 67 (14)       | 60%    | 64%           |
| MGH-Omni                       | US      | 84 | 64 (15)       | 63%    | 68%           |
| MGH-Illumina                   | US      | 228| 66 (15)       | 64%    | 61%           |
| ASGC                            | Australia | 96 | 65 (13)       | 57%    | 77%           |
| ISGS                            | US      | 207| 68 (14)       | 62%    | 61%           |
| SWISS                           | US      | 111| 66 (11)       | 48%    | 74%           |
| Overall                         |         | 3670 |             |      |               |

Abbreviations: Milano, Besta Stroke Register; WTCCC2, The Wellcome Trust Case-Control Consortium II; GENESIS, Genetic Risk factors for Leukoaraiosis study; SGUL, St Georges University of London; DNA Lacunar, UK Young Lacunar Stroke DNA Study; LSS, Leuven Stroke Study; MGH, Massachusetts General Hospital; ASGC, Australian Stroke Genetics Collaborative; ISGS, Ischemic Stroke Genetics Study; SWISS, Sibling with Ischaemic Stroke Study