Common NOTCH3 Variants and Cerebral Small-Vessel Disease

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Background and Purpose—The most common monogenic cause of cerebral small-vessel disease is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, caused by NOTCH3 gene mutations. It has been hypothesized that more common variants in NOTCH3 may also contribute to the risk of sporadic small-vessel disease. Previously, 4 common variants (rs10404382, rs1043994, rs10423702, and rs1043997) were found to be associated with the presence of white matter hyperintensity in hypertensive community-dwelling elderly.

Methods—We investigated the association of common single nucleotide polymorphisms (SNPs) in NOTCH3 in 1350 patients with MRI-confirmed lacunar stroke and 7397 controls, by meta-analysis of genome-wide association study data sets. In addition, we investigated the association of common SNPs in NOTCH3 with MRI white matter hyperintensity volumes in 3670 white patients with ischemic stroke. In each analysis, we considered all SNPs within the NOTCH3 gene, and within 50-kb upstream and downstream of the coding region. A total of 381 SNPs from the 1000 genome population with a mean allele frequency >0.01 were included in the analysis. A significance level of P<0.0015 was used, adjusted for the effective number of independent SNPs in the region using the Galway method.

Results—We found no association of any common variants in NOTCH3 (including rs10404382, rs1043994, rs10423702, and rs1043997) with lacunar stroke or white matter hyperintensity volume. We repeated our analysis stratified for hypertension but again found no association.

Conclusions—Our study does not support a role for common NOTCH3 variation in the risk of sporadic small-vessel disease. (Stroke. 2015;46:00-00. DOI: 10.1161/STROKEAHA.114.008540.)

Key Words: CADASIL | cerebral small vessel diseases | genetic association studies | stroke, lacunar

Cerebral small-vessel disease (SVD) accounts for nearly one quarter of all ischemic strokes and is an important cause of dementia. Lacunar infarction and white matter hyperintensities (WMH) on MRI are lesions commonly seen in SVD. Genetic factors have been suggested to play an important role in SVD.1-3 Several monogenic causes of SVD have been described, the most common of which is cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). CADASIL is caused by mutations in the NOTCH3 gene, and among the main features are recurrent ischemic strokes and white matter lesions on MRI.4 Besides CADASIL causing mutations, it has been suggested that more common variants in NOTCH3 may also contribute to the risk of sporadic SVD.5 This study in a community-dwelling elderly cohort, the Austrian Stroke Prevention Study, found 4 common single

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nucleotide polymorphism (SNP) polymorphisms at the 
*NOTCH3* gene (rs10404382, rs1043994, rs10423702, and 
rs1043997) to be associated with the presence of WMH. 
However, these associations seemed to be restricted to 
hypertensive subjects. In contrast, another study in 120 
patients with lacunar stroke found no association between 
2 common *NOTCH3* SNPs (rs3815188 and rs1043994) 
and the presence of WMH.6 One other study investigated 
the association between common *NOTCH3* variation and 
ischemic stroke in white patients.7 This study identified 
the SNP rs78501403 to be associated with ischemic stroke, 
but power was lacking to investigate this association in the 
SVD subtype. Lacunar infarcts are small and frequently 
not seen on computed tomography; therefore, MRI is important 
for accurate diagnosis.

To test the hypothesis that common *NOTCH3* variation 
is associated with SVD, we investigated the association of 
common variants in *NOTCH3* with both clinical and MRI-
confirmed lacunar stroke and with WMH lesion volume 
quantified on MRI.

**Methods**

**Lacunar Stroke Population**

Lacunar stroke cases were obtained from cohorts from the United 
Kingdom, Germany, and Belgium (n=1350; aged, 60 years [SD, 11]; 
68% men; Table I in the online-only Data Supplement). Lacunar 
stroke was defined as a clinical lacunar syndrome7 with a compatible 
lesion on MRI (subcortical infarct ≤15 mm in diameter). Exclusion 
criteria were as follows: stenosis >50% in the extra- or intracranial 
vascular bed; cardioembolic source of stroke, defined according to 
the Trial of Org 10172 in Acute Stroke Treatment (TOAST) 
criteria8 as high or moderate probability; subcortical infarct >15 mm 
in diameter, as these can be caused by embolic mechanisms (strieto 
atocapsular infarcts); any other specific cause of stroke (eg, lupus 
anticoagulant, cerebral vasculitis, and dissection). A description of 
all cohorts is given in the online-only Data Supplement. Controls 
(n=7397) for the United Kingdom and German analyses were de-
erived from population cohorts and were therefore not confirmed 
sto be stroke free. Belgian controls were ascertained from the local 
population.

SVD stroke subtype, classified using the TOAST criteria,9 and 
leukoaraiosis grading using the semiquantitative Fazekas scale was per-
formed with central review of all MRI scans by 1 physician (H.S.M.). 
The Fazekas scale has been shown to reflect pathological severity of 
SVD in a postmortem validation study.16 In addition, lacunar infarcts 
were determined as high signal recovery or low signal lesions on T1 sequences.

A preplanned secondary analysis was performed in those SVD 
cases with confluent leukoaraiosis (Fazekas grade ≥2) or multiple 
lacunar infarcts (n=717; 53%), as cases of CADASIL present with this phenotype, and compared with the controls (n=7397).

**WMH Volumes Population**

The WMH volumes population (n=3670) was derived from ischemic stroke cohorts from United Kingdom, Italy, Belgium, Germany, 
Australia, and United Stated (Table II in the online-only Data Supplement). Inclusion criteria were as follows: aged ≥18 years, self-reported European ancestry, and a diagnosis of ischemic stroke. Exclusion criteria were CADASIL, vasculitis, and demyelinating 
and mitochondrial disorders. For the present study, we included all available patients with ischemic stroke from each cohort who met the inclusion and exclusion criteria and had MRI and genome-wide association study data available. MRI scans were acquired as a part of routine clinical practice for evaluation of ischemic stroke. Fluid 
atenuated 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 infarcts and doubled 
to obtain whole brain volumes. All neuroimaging analyses have been 
previously described.13

**Genotyping**

Genotyping of all cohorts was performed on commercially available 
arrays from Affymetrix or Illumina. All cohorts performed extensive 
quality control steps before imputation, removing SNPs showing 
significant departure from Hardy-Weinberg equilibrium, high levels 
of missingness or low minor allele frequency. Individuals were re-
moved that did not segregate with the Hapmap II European population 
based on ancestry informative principal component (PC) analysis using 
eigenstrat software package or multidimensional scaling in 
plink software package.12,13 In addition, individuals with high levels 
of missingness or heterozygosity were excluded. All data sets were 
imputed to 1000 genomes integrated variant set (March 2012) using 
impute version 2.14

**Lacunar Stroke Analyses**

We analyzed binary case/control status for each lacunar stroke 
population using a score test, as implemented in SNPTTEST 
version 2.5. Imputed genotype probabilities were taken into account 
using a missing data likelihood score test or an expectation-maxi-
mization method for SNPs with low mean allele frequency or high 
uncertainty. The first 2 ancestry informative PCs, age and sex were 
included as covariates in the model where possible (sex and PC1, 
PC2 only in the UK-Wellcome Trust Case-Control Consortium-2 
and Germany-Wellcome Trust Case-Control Consortium-2 stud-
ies; Table I in the online-only Data Supplement). We meta-ana-
lyzed the 4 cohorts using a fixed-effects inverse variance-weighted 
method, as implemented in METAL.15 We first performed analyses 
using an additive model and then under dominant and recessive 
models.

**WMH Volumes Analysis**

The association between WMH volume and each autosomal SNP 
was determined by performing linear regression of WMH vol-
ume on genotype dosages using plink version 1.07.13 SNPs with 
PLINK INFO (information content metric) score >0.7 or mean al-
lele frequency <0.01 were removed from further analyses. We used 
genomic inflation to evaluate inflation of test statistics in each cen-
ter.16 Results across all centers were combined using a fixed-effects 
inverse variance-weighted method using METAL.15 Heterogeneity 
was assessed using Cochran q statistic. After the meta-analysis, we 
considered only SNPs present in ≥12 centers, and with heterogene-
ity ≥p>0.001, for analysis.

**NOTCH3 SNPs Analyzed and Assessment of Statistical Significance**

In each analysis, we considered all SNPs within the *NOTCH3* gene, 
and within 50-kb upstream and downstream of the coding region. 
A total of 381 SNPs from the 1000 genomes population with mean allele 
frequency >0.01 were included in the analysis. We used the Galwey 
method to estimate the effective number of independent SNPs in the 
region,17 based on the linkage disequilibrium patterns from European 
individuals in the 1000 genomes population.18 This method has 
been shown to give the best agreement with random permutations. 
Using the method, we estimated there to be 34 effective independent 
SNPs in the region. Therefore, we set our P value threshold for each 
analysis to P<0.0015. Power calculations were conducted using the 
Genetic Power Calculator for a case–control study of discrete traits 
under an additive disease risk model and a disease prevalence of 0.25% 
for lacunar stroke.18
Results

Lacunar Stroke Analyses

We first tested for an association of any NOTCH3 SNP with lacunar stroke under an additive model. No SNP met our criteria for statistical significance for association with lacunar stroke. Results for all SNPs in the region by chromosomal position are given in Figure 1A.

We then performed secondary analyses under recessive and dominant models. Again, none of these SNPs met our criteria for statistical significance (Figure 1A and 1B in the online-only Data Supplement). All SNPs had \( P > 0.005 \) in all analyses.

We next tested for association of any NOTCH3 SNP in those patients with lacunar stroke who also had confluent leukoaraiosis or multiple lacunar infarcts (n=717) under an additive model. No SNP met our criteria for statistical significance for association with lacunar stroke with confluent leukoaraiosis or multiple lacunar infarcts (Figure 1B). The associations for the SNPs that were identified in previous studies are given in the Table. Power calculations showed that we had >95% power to replicate these findings (Table III in the online-only Data Supplement).

Secondary analyses under recessive and dominant models also revealed no significant associations (Figure IIA and IIB in the online-only Data Supplement). All SNPs had \( P > 0.005 \) in all analyses.

WMH Volumes Analysis

Similarly, no SNP met our criteria for statistical significance for association with WMH volumes. Results for all SNPs in
Mutations in the **NOTCH3** gene cause CADASIL, a hereditary form of SVD. Common variants in the **NOTCH3** gene have been suggested to also confer risk of sporadic SVD. To test this hypothesis, we analyzed **NOTCH3** in an imputed genome-wide association study data set of 1350 cases and 7397 controls. We found no evidence that common variants in **NOTCH3** associated with risk of lacunar stroke or WMH.

Our observation is in contrast to a recent study in a community-dwelling elderly cohort, the Austrian Stroke Prevention study, which found 4 common variants at the **NOTCH3** gene to be associated with the presence of WMH although only in hypertensive subjects. The SNP that showed the strongest association, rs10404382, was replicated within the Cohorts for Heart and Ageing Research in Genomic Epidemiology (CHARGE) Consortium. The associations found in the Austrian Stroke Prevention study were all confined to hypertensive subjects. In the present study, we failed to replicate any of these findings in the present study, even when only hypertensive patients were studied. There might be several

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**Discussion**

Mutations in the **NOTCH3** gene cause CADASIL, a hereditary form of SVD. Common variants in the **NOTCH3** gene have been suggested to also confer risk of sporadic SVD. To test this hypothesis, we analyzed **NOTCH3** in an imputed genome-wide association study data set of 1350 cases and 7397 controls. We found no evidence that common variants in **NOTCH3** associated with risk of lacunar stroke or WMH.

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**Table. Association of Single Nucleotide Polymorphisms Reported in Previous Publications With WMH and Lacunar Stroke**

<table>
<thead>
<tr>
<th>SNP</th>
<th>MAF</th>
<th>OR (95% CI)*</th>
<th>P Value</th>
<th>OR (95% CI)*</th>
<th>P Value</th>
<th>OR (95% CI)*</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10404382</td>
<td>0.12</td>
<td>1.04 (0.97–1.11)</td>
<td>0.33</td>
<td>1.08 (0.92–1.26)</td>
<td>0.36</td>
<td>1.05 (0.87–1.27)</td>
<td>0.61</td>
</tr>
<tr>
<td>rs1043994</td>
<td>0.12</td>
<td>1.03 (0.96–1.11)</td>
<td>0.34</td>
<td>1.08 (0.92–1.26)</td>
<td>0.33</td>
<td>1.06 (0.88–1.29)</td>
<td>0.54</td>
</tr>
<tr>
<td>rs10423702</td>
<td>0.12</td>
<td>1.04 (0.97–1.11)</td>
<td>0.31</td>
<td>1.07 (0.92–1.25)</td>
<td>0.39</td>
<td>1.05 (0.87–1.27)</td>
<td>0.63</td>
</tr>
<tr>
<td>rs1043997</td>
<td>0.13</td>
<td>1.04 (0.97–1.11)</td>
<td>0.23</td>
<td>1.10 (0.95–1.28)</td>
<td>0.20</td>
<td>1.10 (0.91–1.32)</td>
<td>0.31</td>
</tr>
<tr>
<td>rs78501403</td>
<td>&lt;0.01</td>
<td>†</td>
<td>...</td>
<td>†</td>
<td>...</td>
<td>†</td>
<td>...</td>
</tr>
<tr>
<td>rs61794020</td>
<td>0.02</td>
<td>†</td>
<td>...</td>
<td>1.32 (0.83–2.10)</td>
<td>0.25</td>
<td>1.31 (0.75–2.29)</td>
<td>0.33</td>
</tr>
<tr>
<td>rs3815188</td>
<td>0.22</td>
<td>1.02 (0.95–1.09)</td>
<td>0.59</td>
<td>1.11 (0.68–1.81)</td>
<td>0.66</td>
<td>1.31 (0.73–2.35)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; MAF, mean allele frequency; OR, odds ratio; and WMH, white matter hyperintensity.

*The reported associations are adjusted for age, sex, and the first 2 ancestry informative principal components.

†These variants were not genotyped in the specific populations because of a too low MAF.
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In the Austrian Stroke Prevention study, no correction for
intraclass correlation coefficient, 0.95; 95% confidence inter-
with a good agreement between the 2 main reading centers
done using a similar semiautomatic method in all cohorts,
WMH are more frequent in patients with a history of stroke
than in healthy age-matched individuals.19 It might be that the
underlying pathology of WMH differs between patients with
a history of stroke and community-dwelling elderly free from stroke.
However, it is likely that, at least to some extent, there
is an overlap in pathology because the WMH-associated locus
17q25, which was previously identified in the CHARGE con-
sortium, was successfully replicated in the WMH populations
used in the present study.11

Third, methods to assess WMH volume differed between
studies. Grading of WMH was done in a similar manner in the
present study and Austrian Stroke Prevention study because
both used the Fazekas scale and the same cutoff for the pres-
ence of WMH.

WMH volume measurements in the present study were
done using a similar semiautomatic method in all cohorts,
with a good agreement between the 2 main reading centers
(intraclass correlation coefficient, 0.95; 95% confidence inter-
val, 0.91–0.97; n=50). Also in the Austrian Stroke Prevention
study, a semiautomatic method was used to measure the
WMH volume. In the CHARGE consortium, WMH volume
measurements were done using 2 different approaches; in
most cohorts, either an automatic or a semiautomatic method
is used, but in some cohorts, a semiquantitative rating scale
was used.

Fourth, statistical analysis differed between the studies. In
the Austrian Stroke Prevention, analyses were adjusted for
several potential confounding risk factors: age, sex, diabetes
mellitus, and cardiac disease. We repeated the analysis
for hypertension and diabetes mellitus did not significantly
change the estimates. In addition, population structure is an
important source of confounding to account for in genetic
association studies.12 In contrast to the analysis reported to
be done in Austrian Stroke Prevention study and its replica-
tion in CHARGE, we accounted for population structure in
our analysis by including 2 ancestry informative PCs in every
analysis.

Furthermore, we applied a correction for multiple testing in
the analysis of the present study, based on the effective number
of independent SNPs in the studied region (Galwey method).17
In the Austrian Stroke Prevention study, no correction for
multiple testing was used in their analyses, which enhances
the possibility of false-positive findings.

One other study investigated the association between com-
mom NOTCH3 variants and ischemic stroke and revealed an
association for 1 SNP, rs78501403.7 Unfortunately, we could
not investigate this SNP in our study because the minor allele
frequency of this SNP is generally <0.01% in white popu-
lations and therefore the SNP was not present in the 1000
genomes population to which we imputed our data to.14
Surprisingly, this SNP had a minor allele frequency of 3.3%
in the white population in this previous study. Consistent with
our finding, 1 previous study in lacunar stroke found no asso-
ciation between 2 common NOTCH3 SNPs and the presence
of WMH.8

There are several limitations in this study. We used the
approach of genotyping and then imputing all cohorts to the
1000 genomes population. Although this method provides
good performance for identifying common variants, the quali-
ity of imputation can drop at mean allele frequency <5%,
meaning we cannot rule out associations at these frequencies.
Similarly, the size of some of the WMH cohorts was small,
meaning low-frequency variants could not be assessed in this
analysis.

In summary, our results do not support a role for common
NOTCH3 variation in the risk of sporadic SVD.

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SUPPLEMENTAL MATERIAL

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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.

**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.b58gene.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 KORAgem 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.

**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.

**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.

**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, echocardiography, Holter monitoring, magnetic resonance angiography (MRA), CT-angiography (CTA) and blood tests.

**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.

**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%).

**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.

**Ischemic Stroke Genetics Study (ISGS)**

Ischemic Stroke Genetics Study (ISGS) was a 5-center, prospective, case-control study of first-ever ischemic stroke cases. 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.
Specific acknowledgements for UK Young Lacunar Stroke DNA Study (DNA Lacunar)

**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; Lakshmanan 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 Courtault). 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 Gyuier). 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>
<thead>
<tr>
<th>Centre</th>
<th>Country</th>
<th>N</th>
<th>Mean age (sd)</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Lacunar patients</td>
<td>UK</td>
<td>1013</td>
<td>57.2 (9.5)</td>
<td>720 (71.1)</td>
</tr>
<tr>
<td>DNA Lacunar controls</td>
<td></td>
<td>970</td>
<td>59.7 (4.3)</td>
<td>510 (52.6%)</td>
</tr>
<tr>
<td>Germany WTCCC2 patients</td>
<td>Germany</td>
<td>37</td>
<td>65.2 (9.6)</td>
<td>28 (75.7)</td>
</tr>
<tr>
<td>Germany WTCCC2 controls</td>
<td></td>
<td>797</td>
<td>-</td>
<td>409 (51.3)</td>
</tr>
<tr>
<td>UK WTCCC2 patients</td>
<td>UK</td>
<td>258</td>
<td>69.1 (11.7)</td>
<td>109 (42.2)</td>
</tr>
<tr>
<td>UK WTCCC2 controls</td>
<td></td>
<td>5175</td>
<td>-</td>
<td>2564 (49.5)</td>
</tr>
<tr>
<td>Leuven patients</td>
<td>Belgium</td>
<td>42</td>
<td>65.5 (13.9)</td>
<td>29 (69%)</td>
</tr>
<tr>
<td>Leuven controls</td>
<td></td>
<td>455</td>
<td>55.7 (14.5)</td>
<td>212 (46.6%)</td>
</tr>
<tr>
<td>Overall patients</td>
<td></td>
<td>1350</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall controls</td>
<td></td>
<td>7397</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: DNA Lacunar, UK Young Lacunar Stroke DNA Study; Germany WTCCC2, The Wellcome Trust Case-Control Consortium II Munich; UK WTCCC2, The Wellcome Trust Case-Control Consortium II UK; Leuven, Leuven Stroke Study
### Table II WMH study populations

<table>
<thead>
<tr>
<th>Centre</th>
<th>Country</th>
<th>N</th>
<th>Mean age (sd)</th>
<th>% Male</th>
<th>% Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milano</td>
<td>Italy</td>
<td>151</td>
<td>57 (14)</td>
<td>60%</td>
<td>57%</td>
</tr>
<tr>
<td>WTCCC2-Edinburgh</td>
<td>UK</td>
<td>64</td>
<td>68 (13)</td>
<td>50%</td>
<td>72%</td>
</tr>
<tr>
<td>WTCCC2-Munich FLAIR</td>
<td>Germany</td>
<td>447</td>
<td>66 (12)</td>
<td>66%</td>
<td>72%</td>
</tr>
<tr>
<td>WTCCC2-Munich T2</td>
<td>Germany</td>
<td>203</td>
<td>67 (12)</td>
<td>55%</td>
<td>67%</td>
</tr>
<tr>
<td>WTCCC2-Oxford Flair</td>
<td>UK</td>
<td>65</td>
<td>65 (15)</td>
<td>54%</td>
<td>65%</td>
</tr>
<tr>
<td>WTCCC2-Oxford T2</td>
<td>UK</td>
<td>75</td>
<td>67 (13)</td>
<td>59%</td>
<td>68%</td>
</tr>
<tr>
<td>WTCCC2-SGUL</td>
<td>UK</td>
<td>323</td>
<td>70 (14)</td>
<td>63%</td>
<td>77%</td>
</tr>
<tr>
<td>GENESIS 1</td>
<td>UK</td>
<td>121</td>
<td>67 (14)</td>
<td>67%</td>
<td>62%</td>
</tr>
<tr>
<td>GENESIS 2</td>
<td>UK</td>
<td>228</td>
<td>69 (15)</td>
<td>58%</td>
<td>76%</td>
</tr>
<tr>
<td>SGUL 1</td>
<td>UK</td>
<td>70</td>
<td>70 (13)</td>
<td>61%</td>
<td>61%</td>
</tr>
<tr>
<td>SGUL 2</td>
<td>UK</td>
<td>57</td>
<td>68 (14)</td>
<td>58%</td>
<td>72%</td>
</tr>
<tr>
<td>DNA Lacunar</td>
<td>UK</td>
<td>303</td>
<td>57 (9)</td>
<td>72%</td>
<td>68%</td>
</tr>
<tr>
<td>Leuven</td>
<td>Belgium</td>
<td>361</td>
<td>66 (15)</td>
<td>58%</td>
<td>59%</td>
</tr>
<tr>
<td>MGH-Affymetrix</td>
<td>US</td>
<td>476</td>
<td>67 (14)</td>
<td>60%</td>
<td>64%</td>
</tr>
<tr>
<td>MGH-Omni</td>
<td>US</td>
<td>84</td>
<td>64 (15)</td>
<td>63%</td>
<td>68%</td>
</tr>
<tr>
<td>MGH-Illumina</td>
<td>US</td>
<td>228</td>
<td>66 (15)</td>
<td>64%</td>
<td>61%</td>
</tr>
<tr>
<td>ASGC</td>
<td>Australia</td>
<td>96</td>
<td>65 (13)</td>
<td>57%</td>
<td>77%</td>
</tr>
<tr>
<td>ISGS</td>
<td>US</td>
<td>207</td>
<td>68 (14)</td>
<td>62%</td>
<td>61%</td>
</tr>
<tr>
<td>SWISS</td>
<td>US</td>
<td>111</td>
<td>66 (11)</td>
<td>48%</td>
<td>74%</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td></td>
<td>3670</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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; Leuven, Leuven Stroke Study; MGH, Massachusetts General Hospital; ASGC, Australian Stroke Genetics Collaborative; ISGS, Ischemic Stroke Genetics Study; SWISS, Sibling with Ischaemic Stroke Study*
**Table III** Estimated power in the present study to detect an association of the common SNPs in *NOTCH3* with lacunar stroke

<table>
<thead>
<tr>
<th></th>
<th>MAF</th>
<th>OR*</th>
<th>Estimated power</th>
<th>n cases needed for 80% power</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs10404382</td>
<td>0.12</td>
<td>1.75</td>
<td>&gt;99%</td>
<td>236</td>
</tr>
<tr>
<td>rs1043994</td>
<td>0.12</td>
<td>1.68</td>
<td>&gt;99%</td>
<td>282</td>
</tr>
<tr>
<td>rs10423702</td>
<td>0.12</td>
<td>1.70</td>
<td>&gt;99%</td>
<td>267</td>
</tr>
<tr>
<td>rs1043997</td>
<td>0.13</td>
<td>1.48</td>
<td>&gt;99%</td>
<td>531</td>
</tr>
</tbody>
</table>

Abbreviations: MAF, minor allele frequency; OR, odds ratio

*Odds ratio’s for the presence of white matter hyperintensities, reported in the study by Schmidt et al.*

Power calculations were conducted using the Genetic Power Calculator for a case-control study of discrete traits under an additive disease risk model and a disease prevalence of 0.2% for lacunar stroke.
Table IV Association of four common SNPs in NOTCH3 in DNA lacunar, adjusted for principal components 1 and 2 and age and sex (model 1) and adjusted for the factors in model 1 plus hypertension and diabetes (model 2).

<table>
<thead>
<tr>
<th></th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MAF</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>rs10404382</td>
<td>0.12</td>
<td>1.03 (0.84-1.27)</td>
</tr>
<tr>
<td>rs1043994</td>
<td>0.12</td>
<td>1.01 (0.83-1.25)</td>
</tr>
<tr>
<td>rs10423702</td>
<td>0.12</td>
<td>1.03 (0.84-1.27)</td>
</tr>
<tr>
<td>rs1043997</td>
<td>0.13</td>
<td>0.99 (0.81-1.21)</td>
</tr>
</tbody>
</table>
Figure I Association of common NOTCH3 variants with lacunar stroke under dominant (A) and recessive (B) models by genomic position.
Figure II Association of common \textit{NOTCH3} variants with lacunar stroke with leukoaraiosis under dominant (A) and recessive (B) models by genomic position.
Figure III-A Forest plot for the association of the single nucleotide polymorphism rs10404382 with WMH

The size of the box is inversely proportional to the estimate variance of the effect estimator.
Figure III-B Forest plot for the association of the single nucleotide polymorphism rs1043994 with WMH

The size of the box is inversely proportional to the estimate variance of the effect estimator.
Figure III-C Forest plot for the association of the single nucleotide polymorphism rs10423702 with WMH

The size of the box is inversely proportional to the estimate variance of the effect estimator.
Figure III-D Forest plot for the association of the single nucleotide polymorphism rs1043997 with WMH

The size of the box is inversely proportional to the estimate variance of the effect estimator.
**Figure IV** Forest plot for the association of the single nucleotide polymorphism rs10404382 with WMH in only hypertensive patients.

<table>
<thead>
<tr>
<th>Study</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASGC</td>
<td>1.24</td>
<td>[0.72, 2.13]</td>
</tr>
<tr>
<td>DNA Lacunar</td>
<td>1.30</td>
<td>[0.99, 1.69]</td>
</tr>
<tr>
<td>GENESIS1</td>
<td>0.76</td>
<td>[0.40, 1.41]</td>
</tr>
<tr>
<td>GENESIS2</td>
<td>1.01</td>
<td>[0.74, 1.39]</td>
</tr>
<tr>
<td>iSGS</td>
<td>1.00</td>
<td>[0.88, 1.14]</td>
</tr>
<tr>
<td>Leaven</td>
<td>1.30</td>
<td>[0.93, 1.83]</td>
</tr>
<tr>
<td>MGH-Affymetrix</td>
<td>0.88</td>
<td>[0.67, 1.11]</td>
</tr>
<tr>
<td>MGH-Illumina</td>
<td>0.80</td>
<td>[0.55, 1.16]</td>
</tr>
<tr>
<td>MGH-Omni</td>
<td>0.67</td>
<td>[0.36, 1.22]</td>
</tr>
<tr>
<td>Milano</td>
<td>1.39</td>
<td>[0.96, 1.99]</td>
</tr>
<tr>
<td>SGUL1</td>
<td>1.24</td>
<td>[0.64, 2.39]</td>
</tr>
<tr>
<td>SGUL2</td>
<td>1.05</td>
<td>[0.52, 2.13]</td>
</tr>
<tr>
<td>SWISS</td>
<td>1.06</td>
<td>[0.66, 1.69]</td>
</tr>
<tr>
<td>WTCCC2-Edinburgh</td>
<td>1.29</td>
<td>[0.44, 3.75]</td>
</tr>
<tr>
<td>WTCCC2-Munich</td>
<td>0.99</td>
<td>[0.79, 1.23]</td>
</tr>
<tr>
<td>WTCCC2-Munich</td>
<td>0.91</td>
<td>[0.64, 1.29]</td>
</tr>
<tr>
<td>WTCCC2-Oxford</td>
<td>0.40</td>
<td>[0.13, 1.28]</td>
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<tr>
<td>WTCCC2-Oxford</td>
<td>0.92</td>
<td>[0.48, 1.78]</td>
</tr>
<tr>
<td>WTCCC2-SGUL</td>
<td>1.36</td>
<td>[1.03, 1.78]</td>
</tr>
</tbody>
</table>

**Summary Estimate**  
1.05 [0.96, 1.15]

*Cochran’s Q = 21.8, p=0.24, I²=17.4%*

The size of the box is inversely proportional to the estimate variance of the effect estimator.
References


2. Purcell S, Cherny SS, Sham PC. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics*. 2003;19:149-150