Conclusions—We detected a novel region associated with IA susceptibility that was replicated in an independent Dutch sample. This region on chromosome 7 has been previously associated with ischemic stroke and the large vessel stroke occlusive subtype (including HDAC9), suggesting a possible genetic link between this stroke subtype and IA. (Stroke. 2014;45:3194-3199.)

Key Words: chromosomes, human, pair 7 • genome-wide association study • intracranial aneurysm
Subarachnoid hemorrhage (SAH) because of the rupture of an intracranial aneurysm (IA) occurs in 16000 to 17000 persons in the United States annually, over 40% die within 30 days.12 There is evidence that aneurysmal SAH has a genetic contribution. First degree relatives of an SAH patient have 2x to 6x greater frequency of an SAH compared with age-matched controls.3–6 First and second degree relatives of a patient with SAH or IA also have a greater risk of an unruptured IA (8.7%–13.9%), compared with the general population (1%).3–9 These data support a genetic contribution but could also be because of common lifestyle-related risk factors.

Genome-wide association studies identified and replicated significant associations at chromosome 4q31.23 (EDNRA), 5q31.3, 6q24.2, 8q12.1 (SOX17), 9p21.3 (CDKN2A/CDKN2B/CDKN2BAS), 10q24.32 (CNM2), 12q22, 13q13.1 (KLF/STARD13), 18q11.2 (RBBP8), and 20p12.1.10–15 We used a case–control design to identify additional loci associated with IA in a large sample of European ancestry. IA cases were enriched for a positive family history of IA, which may increase the contribution of genetic factors. Two independent replication samples were examined to confirm the most promising findings in the discovery sample.

Subjects and Methods

Discovery Sample

The discovery sample involved 2617 white IA cases and 2548 white controls identified through different studies. Some of these samples were reported as part of a previous analysis.12 IA cases were ascertained through 5 studies and controls through 5 studies. All studies obtained appropriate institutional ethics approvals (Materials in the online-only Data Supplement).

Genotyping and Quality Review

The discovery sample, with the exception of the Atherosclerosis Risk in Communities (ARIC) samples, was genotyped on the Affymetrix Axiom array. The ARIC samples were genotyped on the Affymetrix single nucleotide polymorphism (SNP) array 6.0. All released Axiom genotypes underwent a common quality review pipeline that included identification of sample duplicates, related individuals, and sex discrepancies. Before performing imputation, SNPs were excluded if there were (1) improper mapping to Genome Reference Consortium GRCh37, (2) a minor allele frequency <0.03, (3) SNP genotype call rate <95%, or (4) Hardy–Weinberg Equilibrium P<10−4 in controls. Minor allele frequency and call rates were calculated by combining all Axiom array data. From the 597,320 SNPs on the Axiom array, 464,632 were retained after this quality review. The ARIC samples genotyped on the Affymetrix SNP array 6.0 underwent the same quality review as the Axiom genotypes. From the 793,799 autosomal SNPs on the Affymetrix SNP array 6.0 that were provided by ARIC after their initial data review, a total of 626,645 were retained (Materials in the online-only Data Supplement).

Principal component analysis was performed using the SNPs genotyped on both the Axiom and Affymetric SNP array 6.0 platforms. Analyses were performed using Eigenstrat16 using data from 11 HapMap phase III populations. To ensure that the discovery sample included only white subjects, all samples clustering outside the Utah residents of northern and western European ancestry and Toscanos in Italy samples were excluded from further analysis. (Materials in the online-only Data Supplement: Figure I in the online-only Data Supplement).

Imputation and Statistical Analysis

Imputation was performed for all autosomes using IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html). All samples genotyped on the Axiom array that passed quality review (n=4060) were imputed together using the 1000Genomes haplotypes (n=1092) as the phased reference panel. Only variants with >1 minor copy across all 1000Genome populations were imputed. Original genotypes were not overwritten. ARIC samples that passed quality review (n=1132) were imputed separately using the same reference panel. All ARIC samples genotyped on the Affymetrix SNP array 6.0 were controls, whereas all cases and the remaining controls were genotyped on the Axiom array. Therefore, extensive and detailed quality review was performed to ensure that spurious association was not detected based on platform effects. SNPs imputed in all data sets were not included in the analysis sample; only SNPs genotyped on at least one of the arrays (1,195,878 SNPs) were considered for inclusion in the analysis. We used the approach described by Sinnott and Kraft17 which implements an aggressive filtering approach to remove SNPs having the potential for systematic platform differences (Materials in the online-only Data Supplement). The final data set retained 672,210 autosomal SNPs for analysis.

The genotyped and imputed SNPs were tested for association with IA susceptibility using a logistic regression model. No additional covariates such as principal components for ancestry were necessary. Analysis was performed using the SNPTEST v2 software using an additive model of SNP effect. Imputed genotypes were encoded in the logistic model as the expected allele count. For autosomal SNPs, all samples were analyzed together. For chromosome X, only SNPs that were on the Axiom array were included because the ARIC study samples only had genotypes for autosomal SNPs. Using the same quality control as above, 13071 chromosome X SNPs were used. Genomic control was applied to correct for inflation (initial lambda $\lambda=1.104$; after genomic control, $\lambda=1.0$). Between the autosomes and X chromosome SNPs, the data set included 685,281 SNPs for the association analysis. We applied a Bonferroni correction to obtain the appropriate genome-wide threshold for significance (0.05/685,281=P<7.3×10−8).

To test whether there might be more than one risk variant in a particular gene or gene region contributing to the association, conditional analyses were performed. A logistic regression model was used to include the genotype at the most significant SNP from the meta-analysis and to test for association with other SNPs in the region.

Replication and Meta-Analysis

Two independent case–control cohorts were used as replication samples. The first was a Dutch case–control sample. Patients with IA (n=786) admitted to the Utrecht University Medical Center (in The Netherlands) between 1997 and 2007 were included. This included both patients with ruptured and unruptured IA (Materials in the online-only Data Supplement). Controls were 3110 Dutch subjects recruited as part of the Nijmegen Biomedical Study and the Nijmegen Bladder Cancer Study.19,20

The second replication cohort was a case–control Finnish sample. The patients with IA (n=851), included both ruptured and unruptured IA, treated at the Helsinki and Kuopio University Hospitals (Materials in the online-only Data Supplement). Controls were 2317

Table 1. Sample Demographics for Study Cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number</th>
<th>Mean Age of Onset (SD)</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIA Study multiplex families (1/family)</td>
<td>388</td>
<td>50.7 (11.9)</td>
<td>31.4</td>
</tr>
<tr>
<td>FIA Study (general recruitment)</td>
<td>1441</td>
<td>54.1 (11.7)</td>
<td>20.1</td>
</tr>
<tr>
<td>GERFHS</td>
<td>44</td>
<td>54.7 (12.9)</td>
<td>40.9</td>
</tr>
<tr>
<td>Australia</td>
<td>118</td>
<td>53.4 (16.3)</td>
<td>34.8</td>
</tr>
<tr>
<td>UCSF</td>
<td>128</td>
<td>55.8 (12.0)</td>
<td>32.8</td>
</tr>
<tr>
<td>Poland</td>
<td>498</td>
<td>52.1 (12.9)</td>
<td>41.2</td>
</tr>
<tr>
<td>Total number of cases</td>
<td>2617</td>
<td>53.2 (12.3)</td>
<td>27.4</td>
</tr>
</tbody>
</table>

FIA indicates Familial Intracranial Aneurysm; GERFHS, Genetic and Environmental Risk Factors for Hemorrhage Stroke; and UCSF, University of California, San Francisco.
Table 2. Sample Demographics for Study Controls

<table>
<thead>
<tr>
<th>Controls</th>
<th>Number</th>
<th>Mean Age at Recruitment (SD)</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCC</td>
<td>294</td>
<td>64.0 (14.8)</td>
<td>46.9</td>
</tr>
<tr>
<td>GERFHS</td>
<td>484</td>
<td>66.3 (13.0)</td>
<td>52.7</td>
</tr>
<tr>
<td>Australia</td>
<td>154</td>
<td>50.9 (16.1)</td>
<td>39.0</td>
</tr>
<tr>
<td>Poland</td>
<td>484</td>
<td>56.2 (15.8)</td>
<td>39.7</td>
</tr>
<tr>
<td>ARIC</td>
<td>1132</td>
<td>54.3 (7.5)</td>
<td>27.9</td>
</tr>
<tr>
<td>Total number of controls</td>
<td>2548</td>
<td>57.9 (13.1)</td>
<td>37.7</td>
</tr>
</tbody>
</table>

ARIC indicates Atherosclerosis Risk in Communities; CCC, Cincinnati Control Cohort; and GERFHS, Genetic and Environmental Risk Factors for Hemorrhage Stroke.

Finnish individuals from 3 samples (Materials in the online-only Data Supplement): (1) Anonymous Finnish patients at the same hospitals as Finnish cases who gave blood samples for unrelated causes in consecutive days, (2) the Helsinki Birth Cohort Study (HBCS), and (3) The Health 2000 cohort.

The replication cohorts were genotyped on the Illumina CNV370-duo chips (Illumina Inc., San Diego, CA), and results were previously reported. Extensive quality control was performed in both subject and SNP data. Imputation was also performed in these samples (Materials in the online-only Data Supplement). The final Replication Samples included 717 Dutch cases, 3004 Dutch controls, 799 Finnish cases, and 2317 Finnish controls.

Replication was performed initially by reviewing results in each of the replication studies for the SNP on chromosome 7 that was found to be significantly associated with IA in the discovery sample. Replication was defined as a finding of association that was significant at the genome-wide level in both the replication samples, with the direction of the SNP effect the same as in the Discovery Sample.

Subsequently, meta-analysis was performed in the chromosome 7 region by combining individual SNP results from the discovery sample and the replication sample. METAL was used using an inverse-variance weighting scheme. We evaluated results to identify findings meeting genome-wide significance criteria.

Results

Discovery Sample

The final sample included 2617 IA cases and 2548 controls (Tables 1 and 2). The cases across the studies had similar age of onset and all had a preponderance of females. The controls across the studies also had a similar age at the time of recruitment.

The most significant genome-wide results included SNPs in CDKN2BAS, also known as ANRIL (rs10733376; P = 4.07 × 10^-12; odds ratio = 1.34; 95% confidence interval: 1.23–1.45) on chromosome 9 (Figures 1 and 2A) previously associated with IA, as well as with other phenotypes. An SNP on chromosome 7 also met genome-wide significance (rs10230207; P = 4.14 × 10^-8; Figures 1 and 2B). This SNP is in an intergenic region 3′ of TWISTNB, MIR3146, and TMEM196 and near SNPs previously associated with ischemic stroke and large vessel occlusive ischemic subtype. On chromosome 5, rs9687972 also reached genome-wide significance; however, no other SNPs in the same region provided similar evidence of association. This SNP was genotyped in the Axiom samples and imputed in the ARIC samples. The imputation information metric is 0.966, which indicates a good imputation; however, the genotype call rate in the Axiom samples was 95.5%, just above our threshold of 95%. The minor allele frequencies are 0.130 (in ARIC controls) and 0.115 (in Axiom controls; P value is 0.12, just above our threshold of 0.1). Therefore, this finding is likely a false-positive association.

On chromosome 7, imputed SNPs were analyzed to evaluate further the evidence of association. As expected, the imputed SNPs in high linkage disequilibrium (LD) with the most significant SNPs initially analyzed further supported the association with IA. The 3 neighboring regions on chromosome 7 which were examined (Figure 2C) were independent of each other, suggesting that there might be independent evidence of association to IA susceptibility factors within this chromosomal region. Conditional analysis was performed and as expected, based on the lack of LD between the primary region and the 2 more distant regions, conditioning on the most significant SNP (rs10230207) did not significantly reduce the evidence of association in the other regions. However, none of these other regions on chromosome 7 attained genome-wide criteria, and therefore these results remained tentative.

Results did not exceed the genome-wide threshold in any other chromosomal region. In the chromosomal regions nominated by previous studies, P < 0.01 was attained with the key SNPs nominated at chromosomes 4q31.23 and 8q12.1. In addition, although top SNPs previously reported on chromosomes 13q13.1 and 18q11.2 were not available in our sample, SNPs in LD with these SNPs attained P < 0.01 (Figure II in the online-only Data Supplement).

Replication Sample

Two independent replication samples were analyzed for evidence of association the SNPs on chromosome 7 identified in the discovery sample. The sample demographics are in Tables 3 and 4.

Initially, only the single SNP (rs10230207) which had attained genome-wide evidence of association in the discovery sample was tested for association. When analyzing this SNP, the Dutch sample provided replication (P = 0.01), whereas the
Finnish sample failed to do so ($P=0.25$). Subsequently, meta-analysis was performed across the discovery sample and the 2 replication samples (Table 5).

**Discussion**

We detected genome-wide evidence of association to a novel region on chromosome 7. The evidence of association was replicated in an independent Dutch sample with the same SNP (rs10230207) and in the same direction of effect. This region failed to show replication in the Finnish cohort, although the direction of effect was the same as in the other 2 samples and may be because of the different genetic architecture of the Finnish population. In addition, the incidence of SAH is higher in Finland compared with most other parts of the world, suggesting that unique genetic factors may segregate in the Finnish population. Another possible explanation is that the size of the replication samples did not have sufficient power to replicate the association. Of note, the association on chromosome 9 with ANRIL has been replicated across many European populations, including the Finns.

The region on chromosome 7 identified in this study was associated with other stroke phenotypes in previous genome-wide association studies. Matarín et al reported association to chromosome 7p21.1 for ischemic stroke, although the finding did not meet genome-wide significance. Support for the association of ischemic stroke to this region was also found in a Han Chinese sample. Large vessel stroke was associated with SNPs in HDAC9 on chromosome 7p21.1 ≈600 kb away from the initial reports for the more general ischemic stroke phenotype. Two other genes, TWIST1 and FERD3L, downstream of HDAC9 could not be excluded as underlying the association. Interestingly, unlike the earlier reports from Matarin and Ding, the SNPs in this region were not associated

**Figure 2.** Regional association results in the discovery sample. X axis is the physical position on the chromosome (Mb). Y axis denotes the $-\log_{10}(P$ value) for association. The most significantly associated SNP is shown with purple diamond. The extent of linkage disequilibrium (LD; $r^2$) between each SNP and the most significantly associated SNP is indicated by the color scale at top right. Larger values of $r^2$ indicate greater LD. A, chromosome 9; B, chromosome 7; and C, chromosome 7 (expanded view).

**Table 3.** Replication Sample Demographics for Study Cases

<table>
<thead>
<tr>
<th>Cases</th>
<th>Number</th>
<th>Mean Age of Onset</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch</td>
<td>717</td>
<td>54.4</td>
<td>31.2</td>
</tr>
<tr>
<td>Finnish</td>
<td>799</td>
<td>49.6</td>
<td>42.2</td>
</tr>
</tbody>
</table>

**Table 4.** Replication Sample Demographics for Study Controls

<table>
<thead>
<tr>
<th>Controls</th>
<th>Number</th>
<th>Mean Age at Recruitment</th>
<th>% Male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dutch</td>
<td>3004</td>
<td>61.7</td>
<td>62.8</td>
</tr>
<tr>
<td>Finnish</td>
<td>2317</td>
<td>60.9</td>
<td>53.7</td>
</tr>
</tbody>
</table>
with the general ischemic stroke phenotype or other subtypes, such as small vessel stroke or cardioembolic stroke. The METASTROKE Collaboration confirmed the association of large vessel stroke the region around \textit{HDAC9}.\textsuperscript{20}

Our genome-wide significant association is closest to the finding by Matarin. There is LD between our top SNP (rs10230207) and theirs ($r^2=0.921$). In our study, SNPs in and around \textit{HDAC9} did not attain genome-wide significance, but they provided modest evidence of association ($P=10^{-6}$; Table 5) that was independent of the association with rs10230207. The Dutch and Finnish sample did not have significant evidence of linkage with the SNPs in and around \textit{HDAC9}. We also did not find extensive LD ($r^2=0.117$) between our top SNP in this region and the SNP reported by the International Stroke Genetics Consortium (rs11984041). We performed a similar comparison with the METASTROKE association. We had modest evidence of association with the SNPs in the METASTROKE-associated region ($P<10^{-5}$); however, our most significant SNP was not in LD with the SNP identified in the METASTROKE study (rs2107595).

Our results along with those of other stroke consortium suggest that this region on chromosome 7 is associated with both IA and large vessel ischemic stroke subtype. Combined with our observation, one could speculate that IA, a disease of large intracranial arteries, and ischemic stroke because of large artery disease may share similar gene pathways and overlapping pathophysiology. Interestingly, the strongest genome-wide association studies evidence for IA to this point is for the region on chromosome 9p21.3, which has also been linked to large vessel ischemic stroke, myocardial infarction, and aortic aneurysm. Thus, genetic risk factors for large vessel vasculopathy may lead to different phenotypes depending on coexisting risk factors such as smoking, hypertension, hyperlipidemia, and other genetic risk factors.

A recent meta-analysis combined results from 61 candidate gene and genome-wide association studies and used association analysis to identify genes contributing to IA susceptibility.\textsuperscript{30} The overall number of samples was 32,887 IA cases and 83,683 controls. The strongest evidence of association was on chromosome 9 in \textit{CDKN2B}, chromosome 8 near \textit{SOX17}, and chromosome 4 near \textit{EDNRA}. These results were influenced by the available data, which typically did not include results for all SNPs, making it difficult to detect association to SNPs that were not identified in the initial report. In addition, by including candidate gene papers, the amount of available data was also limited, reducing the ability to detect novel associations in regions that have not yet been studied in the context of IA.

In summary, we identified and replicated in one sample a novel association with IA on chromosome 7 in a region previously reported to be associated with stroke. The associated region is near, although not in LD with \textit{HDAC9}, which has been associated with large vessel stroke. Studies to replicate this association in additional cohorts of European or other ancestry are necessary and may suggest some overlapping pathogenesis across stroke types and would provide important new insights into the causes and potential targets for stroke prevention.

Acknowledgments
We thank the staff, participants, and the families of the participants for their important contributions in this research study.

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Disclosures
Dr Laakso has received consulting fees from Orion Pharma Ltd. Dr Worrall is Associate Editor of American Academy of Neurology/Neurology. The other authors report no conflicts.

References

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**Table 5. Results of Replication Analysis in the Chromosome 7 Region**

<table>
<thead>
<tr>
<th>SNP</th>
<th>Position</th>
<th>Allele</th>
<th>Discovery Sample</th>
<th>Dutch</th>
<th>Finnish</th>
<th>Meta-Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12669789</td>
<td>18724065</td>
<td>C</td>
<td>9.52×10^{-4}</td>
<td>1.42(1.22–1.67)</td>
<td>0.714</td>
<td>1.05(0.83–1.32)</td>
</tr>
<tr>
<td>rs7798197</td>
<td>19037661</td>
<td>A</td>
<td>9.52×10^{-4}</td>
<td>1.21(1.09–1.34)</td>
<td>0.13</td>
<td>1.13(0.97–1.32)</td>
</tr>
<tr>
<td>rs10230207*</td>
<td>19611307</td>
<td>T</td>
<td>4.14×10^{-4}</td>
<td>1.27(1.17–1.38)</td>
<td>0.011</td>
<td>1.18(1.04–1.33)</td>
</tr>
<tr>
<td>rs2192476</td>
<td>19612305</td>
<td>T</td>
<td>4.53×10^{-4}</td>
<td>1.26(1.16–1.36)</td>
<td>0.010</td>
<td>1.18(1.04–1.34)</td>
</tr>
</tbody>
</table>

rs7798197 and rs12669789 are top SNPs in second and third region, respectively. 95% CI indicates 95% confidence interval for the odds ratio; and OR, odds ratio. *rs10230207 achieved genome-wide significance in the discovery sample and the SNP tested initially for replication.


Genome-Wide Association Study of Intracranial Aneurysm Identifies a New Association on Chromosome 7


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Discovery Sample
Familial Intracranial Aneurysm Study (FIA Study): Families with at least 2 members who had intracranial aneurysm(s) (IA) were ascertained through 26 clinical centers (41 sites) in North America, New Zealand, and Australia. Exclusion criteria included (i) a fusiform-shaped unruptured IA of a major intracranial trunk artery; (ii) an IA that is part of an arteriovenous malformation; (iii) a family or personal history of polycystic kidney disease, Ehlers Danlos syndrome, Marfan’s syndrome, fibromuscular dysplasia, or Moya-Moya disease; or (iv) failure to obtain informed consent from the patient or family members. All medical records and relevant accompanying data were reviewed by a Verification Committee. The FIA study was approved by the Institutional Review Boards/Ethics Committees at all clinical and analytical centers and recruitment sites.

For the present analysis, only individuals having an IA based on an intra-arterial angiogram, operative report, autopsy, or size ≥7 mm on non-invasive imaging (MRA, CTA) were considered “definite” cases. A set of independent unrelated cases obtained by selecting one individual with definite IA from each FIA family self-reported as Caucasian (n=388) was included in Discovery Sample 1. Samples from an additional 30 Caucasian cases were included in Discovery Sample 3, and 1 case was included in Discovery Sample 4. Results from Discovery Sample 1 were previously published.1

Further recruitment was undertaken as part of the FIA Study and the requirement for family history of IA was removed and both familial and sporadic IA cases were enrolled. The same exclusion criteria were in place and all cases underwent the same rigorous review from the Verification Committee. A set of 829 Caucasian IA cases was selected for genotyping in Discovery Sample 2. An additional 607 Caucasian IA cases were included in Discovery Sample 3, and 42 were included in Discovery Sample 4.

Australasian Cooperative Research on Subarachnoid Hemorrhage Study (ACROSS):
Caucasian cases and controls identified from other studies, including those from the Australasian Cooperative Research on Subarachnoid hemorrhage Study (ACROSS), which was a prospective, population-based, case-control study of SAH undertaken in three cities in Australia and one city in New Zealand during the mid-1990s.2 ACROSS included incidence cases of SAH secondary to documented or presumed ruptured IA who were frequency-matched (by sex, 10-year age strata, and city of residence) to controls selected from electoral rolls in each city. Detailed information about key exposures, such as smoking, hypertension, family history of stroke/IA, was obtained by standardized interviews with subjects (or proxies) and where possible, blood samples were obtained for storage and future DNA extraction. Samples from a total of 135 cases and 168 controls were available for genotyping in Discovery Sample 2. This study was approved by the institutional review committees at 10 sites. This sample has been included in a previous report.1

UCSF: The University of California, San Francisco recruited a prospective cohort of adult patients with spontaneous SAH due to IA who were admitted to a tertiary-care referral center in San Francisco during 2003 to 2008. Cases were confirmed by non-contrast CT and cerebral
angiogram. After excluding subjects based on FIA exclusion criteria, 184 samples from Caucasian subjects with detailed medical histories and blood banked for DNA were available for genotyping in Discovery Sample 2. This study was approved by the institutional review committee at University of California, San Francisco. This sample has been has been included in a previous report.¹

**ARIC:** Genotypic data from a set of 1148 white controls, included in Discovery Sample 2, were obtained through a collaborative agreement with the Atherosclerosis Risk in Communities (ARIC) study. In the ARIC sample, a subset of subjects who never had a stroke or TIA was matched to the cases in Discovery Sample 2 cases by sex and, where possible, by age (±5 years). However, because the age of the ARIC controls was limited to 44–66, cases younger than 39 or older than 71 at onset were matched to controls outside of the 5-year criterion. Genotyping had been performed previously using the Affymetrix SNP array 6.0.³ This sample has been has been included in a previous report.¹

**Cincinnati Control Cohort and Genetic and Environmental Risk Factors for Hemorrhage Stroke:** Controls were obtained from two population-based studies. The first was the NINDS-funded case-control Genetic and Environmental Risk Factors for Hemorrhage Stroke (GERFHS) study, which was designed to identify the important environmental and genetic risk factors for IA-related SAH as well as for spontaneous intracerebral hemorrhage. Controls identified by random-digit telephone dialing from the Greater Cincinnati/Northern Kentucky community and matched to enrolled cases by age (±5 years), gender, and race, had the same interview questions regarding environmental risk factors as FIA study participants. Another set of controls free of stroke and IA were selected from the Cincinnati Control Cohort (CCC). The subjects in this cohort were identified by random-digit dialing from the Greater Cincinnati region during 2006. These subjects had blood drawn for DNA extraction as well as extensive interviews including detailed environmental exposures as well as detailed medical history of every major disease. Both studies were approved by the Institutional Review Boards of the University of Cincinnati and all participating hospitals. 113 GERFHS and 290 CCC controls have been included in a previous report.¹ 375 GERFHS and 7 CCC controls were not previously analyzed.

**Krakow, Poland:** IA cases were recruited from patients of the Department of Neurology and the Department of Neurosurgery and Neurotraumatology of the Jagiellonian University in Krakow. Both subjects with ruptured IAs and with unruptured IA were recruited. Presence of IA was confirmed by intra-arterial angiogram, CTA, MRA or intraoperatively. A total of 504 IA patients were included. The control group included 514 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. All subjects were of European descent. Information about key demographics, family history and risk factors were obtained using a standardized questionnaire. The study was approved by the institutional review board of the Jagiellonian University. All Polish samples were included in Discovery Sample 4.

**Genotyping and Quality Review**

Genotyping of all samples except ARIC was performed using the Axiom array at the Affymetrix core labs. Genotyping was performed in four batches using similar methods and quality control.
Forty-eight internal samples were genotyped twice for quality control. This yielded a total of 4,323 samples sent for genotyping. However, only 4,249 samples with a QC (dQC) value ≥0.82 and an initial call rate of 97% were released. All released genotypes underwent a common quality review pipeline which included identification of sample duplicates, related individuals, and gender discrepancies, which resulted in the removal of 118 samples. Prior to performing imputation, SNPs were excluded if there were: (i) improper mapping to Genome Reference Consortium GRCh37; (ii) a minor allele frequency (MAF) <0.03; (ii) a SNP call <95%; (iv) a Hardy Weinberg Equilibrium (HWE) p-value <10^{-4} in control samples. MAF and call rates were calculated by combing all 4 batches together. From the 597,320 SNPs on the Axiom array, 464,632 were retained following this quality review.

Genotypic data for the ARIC samples was obtained from the Affymetric SNP array 6.0. These data also underwent quality review and SNPs were removed based on the same criteria listed above. From the 793,799 autosome SNPs on the Affymetric SNP array 6.0 that were provided by ARIC following their initial data review, a total of 626,645 were retained for imputation in this study.

A principal component analysis (PCA) was performed using Eigenstrat and data from 11 HapMap phase III populations to identify clusters using the first two eigenvectors computed using the SNPs typed on both platforms. Samples clustering with the European American (CEU and TSI) reference set were retained, and those outside this cluster which were likely to contain African, Asian, or Hispanic admixture were removed from further analysis (n=61 of the Axiom-genotyped samples); 16 non-European American samples from the ARIC set were also removed (Supplemental Figure 1).

**Imputation**

Imputation was performed for all autosomes using IMPUTE2 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html). We have employed the recommendation of Howie et al to include diverse reference panels to optimize imputation. All distinct samples genotyped on the Axiom array (n=4060) were imputed together using the 1000Genomes haplotypes (n=1092; data freeze from Nov. 2010, May. 2011, March 2012 phased haplotype release) as the phased reference panel. Only variants with more than one minor copy across all 1000Genome populations were imputed. Original genotypes were not overwritten. ARIC sample (n=1132) were imputed separately using the same reference panel. Only SNPs genotyped on at least one of the arrays (1,195,878 SNPs) were used in the analysis.

**Statistical Analysis**

The discovery sample consists of samples recruited through multiple studies, some providing both cases and controls and others providing only cases or controls. As a result, we could not analyze each study separately and combine results using meta-analysis. Rather, we have combined all data available from our discovery sample and performed detailed quality review to reduce sources of false positive results due to population stratification and inter-study differences.
Because our sample was genotyped on two platforms, with all samples genotyped in **Affymetric SNP array 6.0** being controls, extensive and detailed quality review was performed to ensure that spurious association was not detected based on platform effects. As suggested by Sinnott and Kraft, we reviewed several SNP metrics, including imputation quality (information) and differences in SNP minor allele frequency in controls genotyped on the Axiom platform, and the ARIC controls genotyped on the **Affymetric SNP array 6.0**. We removed all SNPs with low imputation quality (information score <0.30) as well as SNPs with a significant difference in minor allele frequency between the two sources of control samples (p<0.1). To further reduce the influence of rare SNPs, which would typically have less accurate imputation, we removed all SNPs with a minor allele frequency less than 5%. Using this aggressive filtering approach, we retained 672,210 SNPs for analysis. Remaining uncertainty in the imputed genotypes after application of the aggressive information score and minor allele frequency filters was modeled using the “-method score” option in SNPTEST V2. We would expect a slight loss of power in the association tests due to the uncertainty in genotypes; however, previous studies indicate this power loss is minimal, on the order of 7% of the effective sample size on average. All samples were analyzed together with genomic control applied to correct for inflation.

**Replication Sample**

Two independent samples were used to replicate the primary findings from this study.

**Dutch sample**: IA patients (n=786) were admitted to the Utrecht University Medical Center (the Netherlands) between 1997 and 2007. The population consisted of 247 men and 539 women and included both patients with ruptured (727) and patients with only unruptured (59) IA. Ruptured IA were defined by symptoms suggestive of subarachnoid hemorrhage (SAH) combined with subarachnoid blood on a computed tomography (CT) scan and a proven aneurysm at angiography (conventional angiogram, CT- or magnetic resonance (MR)-angiogram). Unruptured IA were identified by CT or MR angiography or conventional angiography in the absence of clinical or radiological signs of SAH. Patients with fusiform IA, possible traumatic SAH, and polycystic kidney disease were excluded. As controls, we included 3110 Dutch subjects, who were recruited as part of the Nijmegen Biomedical Study (n=1832) and the Nijmegen Bladder Cancer Study (n=1278).

**Finnish sample**: The Finnish cohort consisted of 880 IA patients treated at the Helsinki and Kuopio University Hospitals, and included both patients with ruptured and unruptured IA. The patients were collected from the registries of Neurosurgery, Kuopio University Hospital, and Neurosurgery, Helsinki University Hospital, solely serving their catchment populations in Eastern and Southern Finland, respectively. The sporadic IAs (sIAs) were angiographically verified and the cases of subarachnoid hemorrhage from ruptured with computed tomography (CT). Patients with fusiform IA (n=5), (not verifiable) traumatic SAH (n=81), and polycystic kidney disease (n=4) were removed. Controls were genetically matched to cases from thee sample sets: anonymous donors from Kuopio University Hospital and Helsinki, the Helsionki Birth Cohort Study (HBCS) and the Health 2000 study (H2000). Anonymous donors were individuals who were Finnish patients at the same hospitals as Finnish cases and gave blood samples for unrelated causes in consecutive days. The Helsinki Birth Cohort Study (HBCS) includes 8,760 individuals born in the Helsinki Central Hospital between 1934 and 1944. A
subset of 1676 Illumina genotyped individuals were available for the present study. The Health 2000 Cohort (H2000) includes 2,402 Finns, and of those 2,138 Illumina genotyped individuals were available for the present study.\textsuperscript{15, 16}

**Replication sample genotyping and quality review**

First, a sliding window approach was used to thin the set of SNPs to be approximately independent of each other. A sliding window of 1500 SNPs was shifted by 150 SNPs at a time along chromosomes, and in each step SNPs were filtered if any pairwise $r^2$ was $> 0.2$, resulting in 79596 independent SNPs. Pairwise IBS distances of these SNPs were used in multidimensional scaling and four first dimensions were used in matching. PLink v. 1.07\textsuperscript{17} was used for thinning and MDS analysis. R package optmatch was used to pair each case to three controls. After 1:3 matching, additionally all Eastern Finnish controls from the previous sIA study were included.\textsuperscript{17}

All case and control subjects from the Dutch samples were genotyped on the Illumina CNV370 Duo BeadChips in previous GWAS.\textsuperscript{8, 11} Some of the Finnish samples had also been previously genotyped. PLINK version 1.07 was used for quality control of both subjects and SNPs. After removal of SNPs with A/T or C/G alleles and SNPs that were not called in any individual, we performed sample QC and SNP QC.

We performed sample QC after merging cases and controls, using a subset of common, high-quality SNPs (as defined by SNPs without deviation from Hardy-Weinberg equilibrium (HWE) ($p > 0.001$), with high minor allele frequency ($> 20\%$) and with low missingness ($< 1\%$)), and performed pruning based on linkage disequilibrium ($r^2 > 0.5$). Subjects were removed based on the following three criteria: genotype missingness (subjects with a call rate below 95\% were removed), heterozygosity (subjects were excluded if the inbreeding coefficient deviated more than 3 standard deviations from the mean) and cryptic relatedness (by calculating identity-by-descent (IBD) for each pair of individuals). In each pair with an IBD proportion of at least 20\%, a subject was excluded, if it exhibited distant relatedness with multiple individuals. For case-control pairs, we removed the control subject. In the remaining pairs, the subject with the lowest call rate was excluded.

We performed principal component analysis (PCA) using EIGENSTRAT on the study subjects and HapMap-CEU subjects. We excluded SNPs from three regions with known long-distance linkage disequilibrium (LD): the major histocompatibility (MHC) region (chr6: 25.8-36 Mbp), the chromosome 8 inversion (chr8: 6-16 Mbp) and a chromosome 17 region (chr17: 40-45 Mbp). We created multi-dimensional scaling plots with the first 4 principal components (PCs), using R version 2.11.\textsuperscript{13, 18} Based on visual inspection of these plots, we excluded subjects that appeared to be outliers with respect to the CEU or the study population. After outlier removal, we recomputed principal components to include as covariates for logistic regression.

After sample QC, we excluded SNPs with more than 2\% missing genotypes, a minor allele frequency $< 1\%$, genotype missingness higher than the minor allele frequency and HWE deviation ($p < 0.001$). We performed these QC steps in each study cohort separately and again
after merging cases and controls. We also removed SNPs with a differential degree of 
missingness between cases and controls (p < 1x10^{-5}; chi-squared test).

The final sample included 717 Dutch cases, 3004 Dutch controls, 799 Finnish cases, 2317 
Finnish controls.

**Imputation, association analysis**

Genotype imputation was performed using the prephasing/imputation stepwise approach 
implemented in IMPUTE2 and SHAPEIT (chunk size of 3 Mb and default parameters).5,19 The 
imputation reference set consisted of 2,184 phased haplotypes from the full 1000 Genomes 
Project data set (February 2012; 40,318,253 variants). All genomic locations are given in NCBI 
Build 37/UCSC hg19 coordinates. Association testing was carried out in PLINK using imputed 
SNP dosages and the principal components described above as covariates.

**REFERENCES**

Association Study of Intracranial Aneurysms Confirms Role of Anril and SOX17 in 
2. Anderson C, Ni Mhurchu C, Scott D, Bennett D, Jamrozik K, Hankey G. Triggers of 
subarachnoid hemorrhage: role of physical exertion, smoking, and alcohol in the 
Australasian Cooperative Research on Subarachnoid Hemorrhage Study (ACROSS). 
*Stroke*. 2003;34:1771-1776
4. 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
(Bethesda)*. 2011;1:457-470
6. Sinnott JA, Kraft P. Artifact due to differential error when cases and controls are imputed 
from different platforms. *Hum Genet*. 2012;131:111-119
reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet*. 
2009;41:199-204
CM, et al. Susceptibility loci for intracranial aneurysm in European and Japanese 
9. Helgadottir A, Thorleifsson G, Magnusson KP, Gretarsdottir S, Steinthorsdottir V, 
Manolescu A, et al. The same sequence variant on 9p21 associates with myocardial 
2008;40:217-224
variant near the endothelin receptor type A (EDNRA) gene is associated with intracranial 


17. 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


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Supplemental Figure I: Principal component clustering plot for genotyped study subjects.
Genotyped individuals are shown for PC1 (x-axis) and PC2 (y-axis). Reference populations are:
CEU_TSI (Utah residents with Northern and Western European ancestry from the CEPH collection; Tuscans in Italy); CHB_CHD_JPT (Han Chinese in Beijing, China; Chinese in Metropolitan Denver, Colorado; Japanese in Tokyo, Japan); YRI (Yoruba in Ibadan, Nigeria). Study samples clustering
outside the area defined by the CEU and TSI reference samples were excluded from association analyses.

**Supplemental Figure II: Comparison with previously reported results from genomewide association studies.** (A) Chromosome 4q31.23; (B) Chromosome 5q31.3; (C) Chromosome 6q24.2; (D) Chromosome 8q12.1; (E) Chromosome 10q24.32; (F) Chromosome 12q22; (G) Chromosome 13q13.1; (H) Chromosome 18q11.2; (I) Chromosome 20p12.1. X-axis is the physical position on the chromosome (Mb). Y-axis denotes the \(-\log_{10}(p\text{-value})\) for association. The most significant SNP from the initial report is shown at the top of each panel. The extent of LD (as measured by \(r^2\)) between each SNP and the most significant SNP from the initial report is indicated by the color scale at top right. Larger values of \(r^2\) indicate greater LD. If the SNP was available in our sample as either a genotyped or imputed SNP, the associated p-value is shown as a purple diamond. If the SNP was not available in our sample, the position of the SNP is shown a white diamond along the X axis.

**Supplemental Figure II-A: Chromosome 4q31.23**
Supplemental Figure II-B: Chromosome 5q31.3
Supplemental Figure II-C: Chromosome 6q24.2
Supplemental Figure II-D: Chromosome 8q12.1
Supplemental Figure II-E: Chromosome 10q24.32
Supplemental Figure II-F: Chromosome 12q22
Supplemental Figure II-G: Chromosome 13q13.1
Supplemental Figure II-H: Chromosome 18q11.2
Supplemental Figure II-I: Chromosome 20p12.1