Genetic variation at *CR1* increases risk of cerebral amyloid angiopathy

A. Biffi, J.M. Shulman, J.M. Jagiella, et al.

Neurology 2012;78;334; Published online before print January 18, 2012;
DOI 10.1212/WNL.0b013e3182452b40

Genetic variation at *CR1* increases risk of cerebral amyloid angiopathy

A. Biffi, MD
J.M. Shulman, MD, PhD
J.M. Jagiella, MD, PhD
L. Cortellini, MSc
A.M. Ayres, BA
K. Schwab, BA
D.L. Brown, MD, MSc
S.L. Silliman, MD
M. Selim, MD
B.B. Worrall, MD, MSc
J.F. Meschia, MD
A. Slowik, MD, PhD
P.L. De Jager, MD, PhD
S.M. Greenberg, MD, PhD
J.A. Schneider, MD
D.A. Bennett, MD
J. Rosand, MD, MSc

Correspondence & reprint requests to Dr. Rosand: jrosand@partners.org

ABSTRACT

Objective: Accumulated evidence suggests that a variant within the *CR1* gene (single nucleotide polymorphism rs6656401), known to increase risk for Alzheimer disease (AD), influences β-amyloid (Aβ) deposition in brain tissue. Given the biologic overlap between AD and cerebral amyloid angiopathy (CAA), a leading cause of intracerebral hemorrhage (ICH) in elderly individuals, we investigated whether rs6656401 increases the risk of CAA-related ICH and influences vascular Aβ deposition.

Methods: We performed a case-control genetic association study of 89 individuals with CAA-related ICH and 280 individuals with ICH unrelated to CAA and compared them with 324 ICH-free control subjects. We also investigated the effect of rs6656401 on risk of recurrent CAA-ICH in a prospective longitudinal cohort of ICH survivors. Finally, association with severity of histopathologic CAA was investigated in 544 autopsy specimens from 2 longitudinal studies of aging.

Results: rs6656401 was associated with CAA-ICH (odds ratio [OR] = 1.61, 95% confidence interval [CI] 1.19–2.17, $p = 8.0 \times 10^{-4}$) as well as with risk of recurrent CAA-ICH (hazard ratio = 1.35, 95% CI 1.04–1.76, $p = 0.024$). Genotype at rs6656401 was also associated with severity of CAA pathology at autopsy (OR = 1.34, 95% CI 1.05–1.71, $p = 0.009$). Adjustment for parenchymal amyloid burden did not cancel this effect, suggesting that, despite the correlation between parenchymal and vascular amyloid pathology, *CR1* acts independently on both processes, thus increasing risk of both AD and CAA.

Conclusion: The *CR1* variant rs6656401 influences risk and recurrence of CAA-ICH, as well as the severity of vascular amyloid deposition. ***Neurology*® 2012;78:334–341**

GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **BA** = Brodmann area; **CAA** = cerebral amyloid angiopathy; **CAA-ICH** = cerebral amyloid angiopathy–related intracerebral hemorrhage; **CI** = confidence interval; **GOCHA** = Genetics Of Cerebral Hemorrhage on Anticoagulation; **GWAS** = genome-wide association studies; **HR** = hazard ratio; **HTN-ICH** = hypertension-related intracerebral hemorrhage; **ICH** = intracerebral hemorrhage; **MAF** = minor allele frequency; **MAP** = Rush Memory and Aging Project; **OR** = odds ratio; **PCA** = principal component analysis; **ROS** = Religious Order Study; **SNP** = single nucleotide polymorphism.

Cerebral amyloid angiopathy (CAA) is characterized by β-amyloid (Aβ) peptide deposition in the walls of arterial vessels of the cerebral cortex and cerebellum.[1,2] Like the amyloid plaques in Alzheimer disease (AD), vascular amyloid is composed of a proteolytic fragment (Aβ) of the β-amyloid precursor protein. Aβ deposition is responsible for a variety of clinical consequences, including acute intracerebral hemorrhage (ICH).[3–6] CAA-related ICH[7] accounts for between 15% and 40% of all nontraumatic ICH in elderly individuals and is associated with mortality rates of 30%–50%.[8–10]

Supplemental data at www.neurology.org

Supplemental Data

From the Center for Human Genetic Research (A.B., L.C., J.R.) and Department of Neurology (A.B., L.C., A.A., K.S., S.M.G., J.R.), Massachusetts General Hospital, Boston; Program in Medical and Population Genetics (A.B., J.M.S., L.C., P.L.D., J.R.), Broad Institute, Cambridge, MA; Program in Translational NeuroPsychiatric Genomics (J.M.S., P.L.D.), Institute for the Neurosciences, Departments of Neurology and Psychiatry, Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Department of Neurology (J.M.J.), Jagiellonian University Medical College, Krakow, Poland; Stroke Program (D.L.B.), Department of Neurology, University of Michigan Health System, Ann Arbor, MI; Department of Neurology (S.L.S.), University of Florida College of Medicine, Jacksonville; Department of Neurology (M.S.), Beth Israel Deaconess Medical Center, Boston, MA; Department of Neurology and Public Health Sciences (B.B.W.), University of Virginia Health System, Charlottesville; Department of Neurology (J.F.M.), Mayo Clinic, Jacksonville, FL; and Rush Alzheimer's Disease Center (J.A.S., D.A.B.), Department of Neurological Sciences, Rush University Medical Center, Chicago IL.

Study funding: Funding information is provided at the end of the article.

Disclosure: Author disclosures are provided at the end of the article.

The biologic overlap between CAA and AD is substantial. CAA is found in up to 40% of individuals with a clinical diagnosis of AD who come to autopsy.[11] Furthermore, there appears to be substantial overlap in the genetics of the 2 conditions. Mutations in *APP* cause both autosomal-dominant AD and autosomal-dominant hereditary CAA-ICH.[12,13] In *APOE*, common polymorphisms influence risk of both sporadic CAA and sporadic AD.[12,14]

Genome-wide association studies (GWAS) have yielded a host of novel genetic risk factors for sporadic AD.[15–17] Of these, single nucleotide polymorphism (SNP) rs6656401 within the *CR1* gene has been associated with increased Aβ deposition in brain specimens.[18] We therefore investigated whether this variant also influences risk of clinically symptomatic CAA-ICH, as well as histopathologic severity of CAA. We analyzed SNP data for CAA-ICH case and control subjects from the ongoing Genetics Of Cerebral Hemorrhage on Anticoagulation (GOCHA) study,[14,19] as well as for autopsied individuals enrolled in the Religious Order Study (ROS) and the Rush Memory and Aging Project (MAP).[18,20–23]

METHODS

GOCHA study. ***Subject recruitment.*** Subjects were drawn from an ongoing multicenter genetic association study of primary and anticoagulation-related ICH.[14,19] In brief, study subjects are consecutive patients aged ≥55 years with ICH admitted to the emergency department of participating institutions (figure 1). Baseline head CT scans were reviewed centrally for determination of ICH location.

Race/ethnicity-, age-, and gender-matched control subjects were individuals enrolled from outpatient clinical services at participating institutions and were confirmed to have no medical history of ICH, AD, or pre-enrollment cognitive impairment through in-person interview and review of medical records.[10,14,19]

Longitudinal follow-up. All patients with ICH who survived at least 90 days after the index ICH were considered eligible for follow-up longitudinal analysis. Patients and their caregivers were interviewed by telephone at 3 months after ICH, 6 months after ICH, and every 6 months thereafter.[10] Information collected included medication use, recurrent lobar or nonlobar ICH, and death. If new neurologic symptoms, ischemic stroke, ICH, or hospital admission was reported by the subject or caregiver, the relevant medical records and radiographic images were reviewed by a study investigator blinded to other clinical and genetic data to assess the presence or absence of recurrent ICH. Events qualifying for censoring of subjects' data included clinically symptomatic ICH confirmed by neuroimaging, death, or follow-up period reaching the predetermined deadline for prospective ascertainment (January 1, 2009).

Data collection and variable definition. Clinical data were recorded at index ICH admission by stroke neurologists as part of routine clinical care. Collected data included information on demographics, previous medical history, Glasgow Coma Scale score, and pre-ICH medication use. All clinical and neuroimaging data were collected by individuals blinded to genotype data.

CAA-related ICH (CAA-ICH) was defined as lobar ICH (selective involvement of cerebral cortex or underlying white matter on admission CT scan) fulfilling the Boston criteria for a diagnosis of definite/probable CAA (i.e., demonstrating CAA pathology or multiple strictly lobar hemorrhagic lesions).[7]

To provide a negative control cohort for comparison purposes, we also analyzed data from deep hemispheric ICH (involving the basal ganglia, thalamus, or brainstem), typically caused by hypertensive vasculopathy (HTN-ICH). Subjects with cerebellar ICH and mixed location ICH were excluded from the analysis. Inclusion of these subjects (n = 9) in either analysis group did not alter results (data not shown).

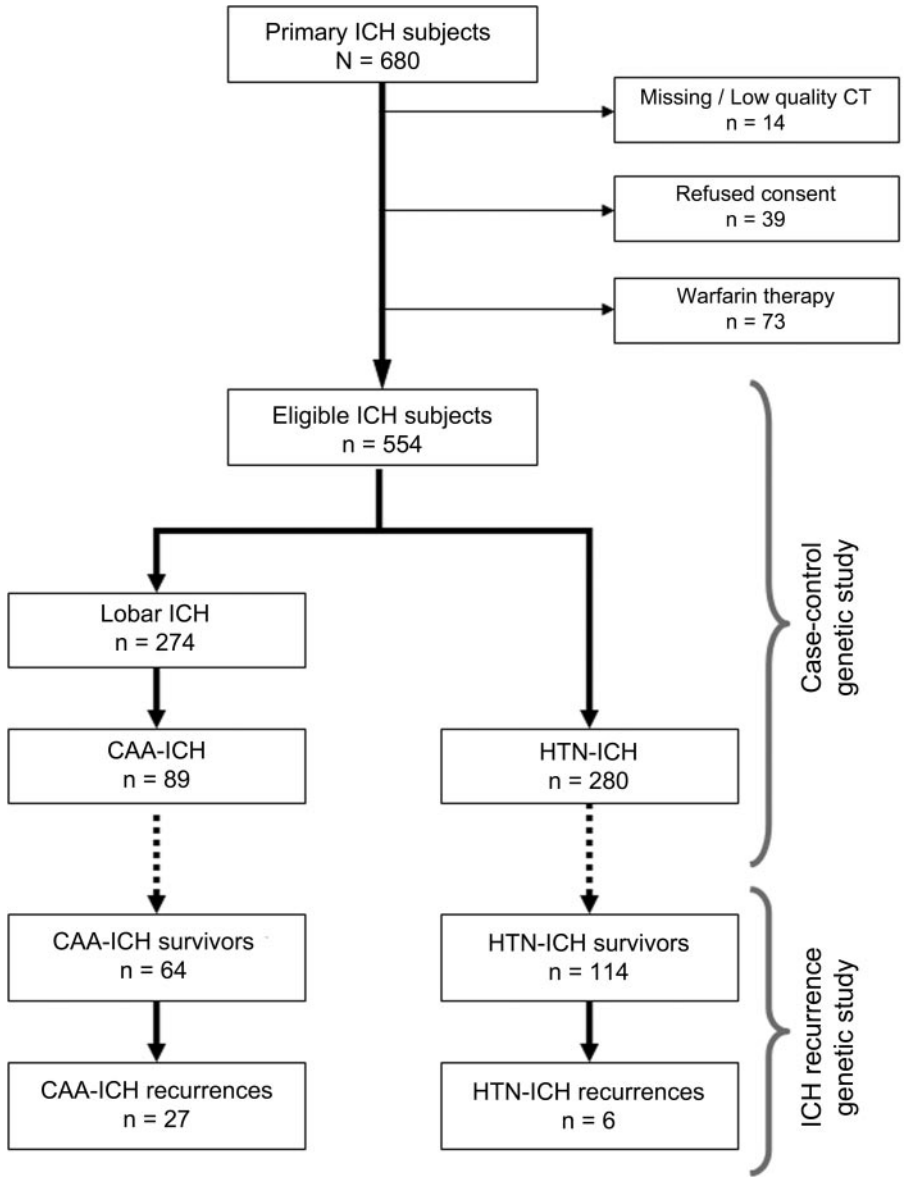
ROS and MAP. ***Subject recruitment.*** The ROS, started in 1994, enrolled older Catholic priests, nuns, and brothers, aged ≥53 years, from about 40 groups in 12 states.[20] Since January 1994, 1,132 participants, of whom 1,001 were non-Hispanic white, completed their baseline evaluation. The follow-up rate of survivors exceeds 90% as does the autopsy rate (481 autopsies of 511 deaths, of whom 457 were non-Hispanic white). Participants were free of known dementia at enrollment.

The MAP, started in 1997, enrolled older men and women (aged ≥55 years) free of known dementia from retirement communities in the Chicagoland area.[21] Since October 1997, 1,285 participants, of whom 1,118 were non-Hispanic white, completed their baseline evaluation. The follow-up rate of survivors exceeds 90%, and the autopsy rate exceeds 80% (336 autopsies of 411 deaths, of whom 320 were non-Hispanic white).

Participants in both studies agreed to annual clinical evaluations and signed both an informed consent and an Anatomic Gift Act form, donating their brains to Rush investigators at the time of death. All clinical and pathologic data were collected, and analyses were performed by study personnel blinded to genotype data. Likewise, cutoffs for CAA severity categorization were chosen according to previously published reports, without any knowledge of individuals' genetic data.[20] More detailed descriptions of these studies can be found in previously published literature.[18,20–23]

Data collection and variable definition. Brain autopsies were performed using standard techniques by investigators blinded to clinical data, as described previously.[20,22] In brief, CAA pathology was assessed by light microscopy on 20-μm immunostained sections derived from 5 brain regions, including 4 neocortical regions—midfrontal (Brodmann area [BA] 46/9), inferior temporal (BA20), angular gyrus (BA39), and calcarine cortices (BA17)—and 1 mesial temporal region and the hip-

Figure 1 Intracerebral hemorrhage (ICH) cohort sample size and study design



CAA-ICH = probable or definite cerebral amyloid angiopathy–related ICH; HTN-ICH = hypertension-related ICH in the deep brain structures.

pocampus. CAA was assessed in each region labeled with anti-Aβ (clone 6F/3-dimensional, M0872, 1:100; DAKO) and quantified using a 5-point scale (0 through 4), with 0 = none (no immunohistostaining for CAA), 1 = mild (scattered positivity in either leptomeningeal or cortical blood vessels), 2 = moderate (strong, circumferential positivity in some but not all leptomeningeal or cortical blood vessels), 3 = severe (widespread, strong, circumferential positivity in leptomeningeal and cortical blood vessels), and 4 = very severe (same as 3, but with additional changes of positivity emanating from small cortical vessels into surrounding neuropil [dysphoric change]). All brains were also examined for pathologic markers of AD.

Because CAA severity was related across regions (all $p < 0.0001$), we averaged the 5 regional scores to create an overall CAA severity score for each subject. Because the vast majority of subjects had some degree of CAA pathology (84.9%) and to make analyses easier to interpret, the overall CAA severity score was converted into a 3-level class variable predictor. We used an overall severity score of >2.5 to separate individuals with mild to moderate from those with moderate to very severe CAA, with the reference group consisting of persons with none-to-minimal CAA, defined by a score of <0.5. Separate, quantitative composite measures of neuritic and diffuse amyloid plaque pathology were calculated on the basis of counts from 5 brain regions (hippocampus, entorhinal cortex, midfrontal cortex, middle temporal cortex, and inferior parietal cortex) using the greatest density in a 1-mm^2 area of each region on modified Bielschowsky silver–stained 6-μm sections, as described previously.[24] We standardized the raw counts by dividing each person's count by the SD for that particular count and formed summary scores by averaging the standardized scores.

Genotype data. All subjects included in the present study underwent GWAS SNP genotyping using the Illumina 610-Quad

array (GOCHA) or Affymetrix GeneChip 6.0 (ROS and MAP). Patients with ICH and control subjects were both genotyped in multiple batches, and each batch included both case and control subjects in an interspersed disposition on the genotyping plates. Batch assignment for ICH case and control subjects was determined randomly, irrespective of ICH phenotype (i.e., CAA-ICH vs non–CAA-ICH). Genotypes were called using BeadStudio software, and calls were confirmed manually for SNPs in the *CR1* gene region by laboratory personnel blinded to clinical phenotype. The ROS and MAP samples were genotyped on the Affymetrix 6.0 platform in a single run using identical protocols.

Both genome-wide datasets underwent stringent quality control procedures according to a previously published protocol using PLINK version 1.07.[14,23,25] In brief, quality control of genotype data included filters for missingness, heterozygosity, and concordance between genotype-determined and reported gender. SNP quality control included filters for minor allele frequency (MAF), missingness, Hardy-Weinberg equilibrium, and differential missingness by case-control status (figure e-1 on the *Neurology*® Web site at www.neurology.org). Individual genotypes for rs6656401 were extracted from GWAS data following quality control procedures and were found to be in Hardy-Weinberg equilibrium ($p > 0.20$) in all 3 datasets (GOCHA, ROS, and MAP). Population structure was assessed by performing principal component analysis (PCA) on a subset of all SNPs selected using previously published criteria.[14,26] We assigned genotype-determined ancestry by comparing study subjects and reference populations from HapMap phase 3 data (figure e-2). To control for population stratification, all individuals analyzed were confirmed to cluster with European HapMap samples to be eligible for analysis. Furthermore, principal components 1 and 2 (PC1 and PC2) from PCA analysis were used as covariates in all multivariate models (see below).[14,25,26]

Table 1 Characteristic of subjects included in ICH case-control analysis (GOCHA study)[a]

	CAA-ICH (n = 89)[b]	HTN-ICH (n = 280)	Control subjects (n = 324)
Clinical variables			
Age, y, mean ± SD	75.1 ± 9.4	70.6 ± 12.1	73.1 ± 8.0
Gender, n (% female)	44 (49.4)	126 (45.0)	147 (45.4)
History of hypertension	56 (62.9)	237 (84.6)	216 (66.6)
Ischemic heart disease	19 (21.3)	68 (24.3)	67 (20.7)
Atrial fibrillation	19 (21.3)	58 (20.7)	71 (21.9)
Type 2 diabetes	14 (15.7)	65 (23.2)	49 (15.1)
Prior functional dependence	3 (3.4)	5 (1.7)	0 (0.0)
Prior cognitive impairment	26 (29.2)	28 (10.0)	0 (0.0)
Pre-index ICH	12 (13.5)	10 (3.6)	0 (0.0)
Pre-ICH medication use			
Antiplatelet agents	32 (36.0)	109 (38.9)	136 (42.0)
Statins	26 (29.2)	73 (26.1)	80 (24.7)
Genetic variables			
rs6656401 (A allele, MAF)	0.24	0.19	0.18
APOE ε2 (MAF)	0.18	0.10	0.10
APOE ε4 (MAF)	0.24	0.14	0.12

Abbreviations: CAA-ICH = cerebral amyloid angiopathy–related intracerebral hemorrhage; GOCHA = Genetics Of Cerebral Hemorrhage on Anticoagulation; HTN-ICH = hypertension-related intracerebral hemorrhage; ICH = intracerebral hemorrhage; MAF = minor allele frequency.
[a] All variables are reported as sample size (percentage) unless otherwise specified.
[b] Defined as lobar ICH with diagnosis of definite/probable CAA according to the Boston criteria.[1]

Statistical methods. Genotype data were analyzed using an additive model, with odds ratio (OR) or hazard ratio (HR) expressing the effect of each copy of the reference allele. To determine the association between rs6656401 and CAA-ICH or HTN-ICH risk, we used logistic regression analyses. Results from different logistic regression models were compared using the Breslow-Day test. To determine the association between rs6656401 and CAA pathology, we used ordinal logistic regression analyses. We determined univariate predictors of ICH recurrence using Kaplan-Meier plots with significance testing by the log-rank test. For individuals with multiple recurrent CAA-ICH during follow-up, data were censored at the time of the first recurrence. To determine the influence of rs6656401 on CAA-ICH recurrence, we used Cox regression analysis. The proportional hazard assumption was tested using graphical checks and Schoenfeld residual-based tests.[27]

Covariates for all multivariate models included age, sex, PC1 and PC2, number of *APOE* ε2 and ε4 alleles, and history of hypertension. CAA pathology analyses were further adjusted for AD pathology to exclude confounding due to the known association with amyloid plaque burden.

Power calculations were performed using the Genetic Power Calculator (pngu.mgh.harvard.edu/~purcell/gpc/).[28] Adjustment of effect size estimates due to the winner's curse was achieved using WINNER software (csg.sph.umich.edu/boehnke/winner/).[29] This program modifies (reduces) the observed effect size estimate in genetic association studies based on available statistical power, as determined by sample size and α threshold for significance.

All other association statistical analyses were performed using R software version 2.10.0 (The R Project for Statistical Computing, www.r-project.org). All significance tests were 2-tailed with significance threshold set at $\alpha = 0.05$.

Standard protocol approvals, registrations, and patient consents. The GOCHA study is conducted with approval of the institutional review boards of the Massachusetts General Hospital and all other enrolling institutions. The ROS and MAP studies were approved by the institutional review board of Rush University. All subjects enrolled in the present study (or their guardians) provided written informed consent before participation.

RESULTS

Characteristics of ICH case and control subjects. A total of 554 subjects with ICH presented to GOCHA study centers from 2003 to 2009, were genotyped on a GWAS array, and passed all quality control filters (table 1). Of these, 89 individuals qualified for a diagnosis of CAA-ICH, and 280 individuals were classified as having HTN-ICH (table 1). A total of 324 ICH-free control subjects of European ancestry were available for analysis. In comparison with subjects with HTN-ICH, individuals with CAA-ICH were 1) older, 2) less likely to have a history of pre-ICH hypertension or diabetes, 3) more likely to have had a prior ICH before the index event, 4) more likely to have pre-ICH cognitive impair-

Table 2 Characteristics of prospectively followed ICH survivors (GOCHA study)[a]

	CAA-ICH (n = 64)[b]	Nonlobar ICH
Clinical variables		
Age, y, mean ± SD	74.4 ± 7.8	68.4 ± 13.0
Gender, n (% female)	28 (43.8)	47 (41.3)
History of hypertension	33 (51.6)	99 (86.8)
Ischemic heart disease	9 (14.0)	26 (22.8)
Atrial fibrillation	11 (17.2)	16 (14.0)
Type 2 diabetes	3 (4.7)	29 (25.4)
Prior functional dependence	2 (3.1)	2 (1.9)
Prior cognitive impairment	8 (12.5)	14 (12.3)
Pre-index ICH	8 (12.5)	5 (4.3)
Pre-ICH medication use		
Antiplatelet agents	11 (17.2)	14 (21.9)
Statins	4 (6.3)	29 (25.4)
Genetic variables		
rs6656401 (A allele, MAF)	0.24	0.18
APOE ϵ2 (MAF)	0.18	0.09
APOE ϵ4 (MAF)	0.25	0.15
Longitudinal endpoints		
ICH recurrence: CAA-ICH	27 (42.2)	0 (0.0)
ICH recurrence: HTN-ICH	0 (0.0)	6 (5.3)

Abbreviations: CAA-ICH = cerebral amyloid angiopathy-related intracerebral hemorrhage; GOCHA = Genetics Of Cerebral Hemorrhage on Anticoagulation; HTN-ICH = hypertension-related intracerebral hemorrhage; ICH = intracerebral hemorrhage; MAF = minor allele frequency.
[a] All variables are reported as absolute number (percentage) unless otherwise specified.
[b] Subset of all lobar ICH qualifying for a diagnosis of probable CAA according to the Boston criteria.[1]

ment, and 5) more likely to possess both *APOE* ϵ2 and ϵ4 alleles (all $p < 0.05$).

A total of 178 subjects with ICH (64 with CAA-ICH and 114 with HTN-ICH) were eligible for follow-up and ICH recurrence analysis (table 2). During a median follow-up time of 34.3 months (interquartile range 15.1–57.6 months), we observed 27 recurrent CAA-ICH events and 6 HTN-ICH events. Consistent with previous reports, hemorrhage recurrence was indeed more frequent in CAA-ICH survivors (cumulative 2-year recurrence rate of 15.7% vs 3.4%, $p = 0.011$).[10]

Risk of CAA-ICH. The A allele of SNP rs6656401 was associated with risk of CAA-ICH (OR = 1.61, 95% confidence interval [CI] 1.19–2.17, $p = 8.0 \times 10^{-4}$) after multivariate analysis adjustments. No association was found for HTN-ICH (OR = 0.93, 95% CI 0.82–1.05, $p = 0.30$). Comparison of effect sizes confirmed that strength of association for rs6656401 differed in CAA-ICH and HTN-ICH (Breslow-Day $p = 0.01$).

A post hoc power calculation returned statistical power for discovery of association within the CAA-ICH subset of 41%. Based on this finding, adjustment for the winner's curse estimated an effect size for rs6656401 in CAA-ICH of OR = 1.35.

Risk of recurrent CAA-ICH. In multivariate Cox regression analyses, survivors of CAA-ICH who possessed the A allele of rs6656401 were at increased risk for recurrent CAA-ICH (HR = 1.35, 95% CI 1.04–1.76, $p = 0.024$) (figure 2). We found no evidence of association between rs6656401 and ICH recurrence in HTN-ICH survivors (HR = 1.03, 95% CI 0.57–1.87, $p = 0.91$).

Our estimated power for discovery of this genetic association was 31%. Adjustment for the winner's curse returned an effect size of HR = 1.24.

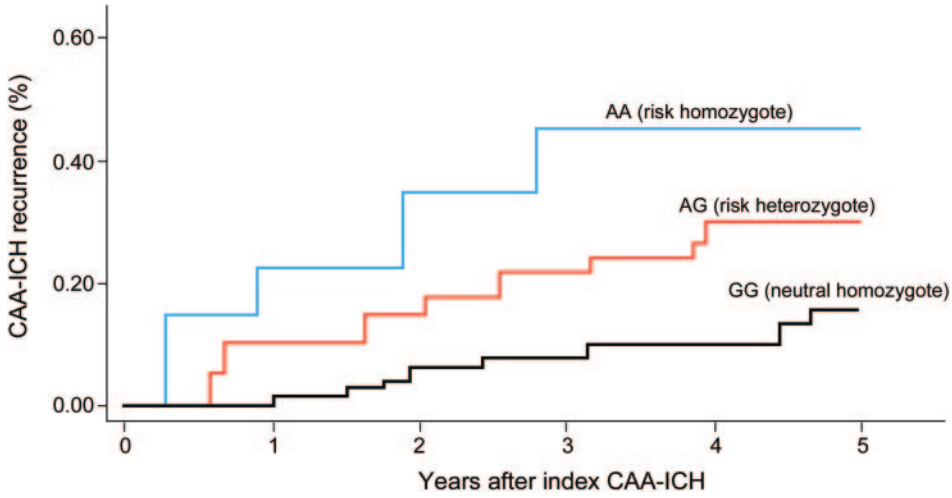
Histopathologic CAA in the ROS-MAP cohort. A total of 544 subjects from the MAP and ROS cohorts had autopsy pathology data for CAA and AD as well as genome-wide data fulfilling quality control criteria (table 3). An increase in the number of copies of the A allele at rs6656401 was associated with increasing CAA pathology burden (none to minimal vs mild to moderate vs moderate to very severe)[21–24] in ordinal logistic regression (OR = 1.27, 95% CI 1.06–1.53, $p = 0.009$). Adjustment for coexistent AD amyloid plaque burden (i.e., neuritic plaque and diffuse plaque pathology scores) did not significantly alter results (OR = 1.24, $p = 0.01$).

DISCUSSION

Results from our analyses demonstrate an association of the A allele of rs6656401 at the *CR1* locus with risk of CAA-ICH, risk of recurrent CAA-ICH, and CAA pathology burden. These findings mirror published reports linking *CR1* with parenchymal amyloid deposition in pathology samples and provide evidence of a possible biologic connection between CAA and AD.

Our study has limited statistical power (because of the limited sample size) to identify associations between rs6656401 and CAA-related phenotypes, particularly if the effect sizes typically observed for common genetic variants in common diseases are expected. The comparatively large increases in risk of CAA-ICH incidence/recurrence conferred by the rs6656401 genotype observed in the present study are therefore likely to be partially explained by the phenomenon of the winner's curse, i.e., systematic effect size overestimation by small (and therefore underpowered) studies.[29,30] Future replication and extension studies of the role of *CR1* in CAA and CAA-ICH should therefore be powered to detect

Figure 2 Kaplan-Meier plot for recurrent cerebral amyloid angiopathy–related intracerebral hemorrhage (CAA-ICH) in CAA-ICH survivors based on rs6656401 genotype status



Different lines represent different individual genotypes at rs6656401. Failure rate estimates are adjusted for age, gender, pre-enrollment ICH, warfarin use, aspirin use, number of *APOE* ϵ2 and ϵ4 alleles, and principal components 1 and 2.

smaller effect sizes than those reported in the present study.

Of note, SNP rs6656401 shows significant stratification when different ethnic groups are compared; HapMap phase 3 data reports MAF >20% for European populations, MAF ~10% for Mexican Americans, and MAF <5% for Han Chinese, Chinese American, Japanese, Indian Gujarati, and African (Kenya) reference samples (www.hapmap.org). Future studies will therefore also require application of tools for control of bias due to genetic ancestry, and power calculations will have to be tailored to specific allele frequencies in the populations being investigated.

Winner's curse adjusted effect size estimates for SNP rs6656401 in CAA are still larger than reported association results in recently published AD genome-wide studies.[15–17] One potential explanation is that *CR1* genetic variation acts on AD and CAA through different pathologic mechanisms, in whole or in part. A more likely explanation is that the phenotypic CAA classification, provided by neuroimaging (using the Boston criteria) or pathologic data, has greater accuracy than the AD diagnosis, which relies solely on clinical data in many studies.[31] Indeed, a recently published case-control association study of neuropathology-confirmed cases of AD re-

Table 3 Characteristics of ROS and MAP subjects

	CAA Severity		
	None to minimal (n = 146)	Mild to moderate (n = 280)	Moderate to very severe (n = 118)
Demographic variables			
Age at enrollment, y, mean (SD)	84.4 (7.2)	86.3 (7.1)	88.6 (6.9)
Gender, n (% female)	80 (55.0)	160 (57.0)	73 (62.0)
Pre-enrollment history of dementia, n (%)	33 (23.0)	122 (44.0)	74 (63.0)
Alzheimer disease pathology			
Neuritic plaques pathology score median (IQR)[a]	0.0 (0.0–0.00)	0.87 (0.54–1.11)	1.10 (0.88–1.31)
Diffuse plaques pathology score, median (IQR)[a]	0.0 (0.0–0.23)	0.84 (0.51–1.17)	1.02 (0.78–1.30)
Genetic variables			
rs6656401 (A allele, MAF)	0.18	0.21	0.23
APOE ϵ2 (MAF)	0.13	0.07	0.04
APOE ϵ4 (MAF)	0.06	0.15	0.27

Abbreviations: CAA = cerebral amyloid angiopathy; IQR = interquartile range; MAF = minor allele frequency; MAP = Rush Memory and Aging Project; ROS = Religious Order Study.
[a] Calculated for each pathology feature by dividing each person's count by the SD for that particular count to standardize individual subjects' values.

ported larger effect sizes than those observed in discovery analyses. These pathology-informed AD studies uncovered effect sizes for SNP rs6656401 in the range we observed for CAA, rather than the smaller estimates generated in studies of clinically diagnosed AD.[32]

The association between rs6656401 and CAA-ICH risk and recurrence is potentially confounded by the genetic effects this variant exerts on parenchymal amyloid burden, which in turn correlates with CAA severity.[18,20] However, we found a significant association between rs6656401 and increasing severity of vascular amyloid deposition in autopsy samples from initially healthy community-dwelling elderly subjects. Adjustment for AD pathology in these analyses did not cancel the observed effect, suggesting that, despite the correlation between parenchymal and vascular amyloid burden,[20] *CR1* probably acts independently on both processes, thus increasing the risk of both AD and CAA. This finding echoes evidence from genetic association studies of *APOE*, in which the ε2 allele decreases AD risk but increases risk of CAA.[12,14] Taken together, evidence from genetic association studies is consistent with multiple experimental observations in suggesting that, although etiologically related, AD and CAA represent different pathologic and clinical conditions.

Our study is limited by the challenges of assembling adequate sample sizes in diverse populations for a genetic study of CAA. We present 3 independent analyses that provide biologically consistent results, but we are not able to provide independent replication at this time. Adequate samples for replication are limited, although ongoing efforts in the international community are likely to generate such samples in the future. None of the reported associations achieve genome-wide significance ($p < 0.5 \times 10^{-8}$), which would improve the robustness of our findings. However, a large body of preexisting data in AD genetics suggests that our findings are consistent with the known or suspected biologic functions of the *CR1* gene product. Finally, because of the restrictions imposed by genotyping procedures and study design, we are not able to extend our findings to non-European ancestry populations at this time. Additional studies will be required to clarify the role of *CR1* in these populations.

AUTHOR CONTRIBUTIONS

Study concept: Dr. Biffi, Dr. Rosand. Study design: Dr. Biffi, Dr. Rosand. Acquisition of data: Dr. Biffi, Dr. Shulman, Dr. Jagiella, L. Cortellini, A.M. Ayres, K. Schwab, Dr. Brown, Dr. Silliman, Dr. Selim, Dr. Worrall, Dr. Meschia, Dr. Slowik, Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand. Statistical analysis: Dr. Biffi. Manuscript preparation: Dr. Biffi, Dr. Rosand. Manuscript review: Dr. Biffi, Dr. Shulman, Dr. Jagiella, L. Cortellini, A.M. Ayres, K. Schwab, Dr. Brown, Dr. Silliman, Dr. Selim, Dr. Worrall, Dr. Meschia, Dr. Slowik, Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand. Study supervision/coordination: Dr. Biffi, L. Cortellini, A.M. Ayres, K. Schwab, Dr. De Jager, Dr. Schneider, Dr. Bennett, Dr. Rosand. Study funding: Dr. De Jager, Dr. Greenberg, Dr. Schneider, Dr. Bennett, Dr. Rosand.

ACKNOWLEDGMENT

The authors thank the participants in the GOCHA study, the ROS, and the MAP, all research staff at GOCHA participating institutions, the Rush Alzheimer's Disease Center, and the Broad Institute.

STUDY FUNDING

The GOCHA study was supported by the Edward and Maybeth Sonn Research Fund, the University of Michigan General Clinical Research Center (M01-RR000042), the National Institute for Neurologic Disorders and Stroke (R01-NS063925, R01-NS059727, P50-NS051343, K23-NS064052, and K23-NS059774) and the National Institute on Aging (R01-AG026484). Dr. Biffi received support from the American Heart Association/Bugher Foundation Centers for Stroke Prevention Research (0775011N, 0755984T). The ROS and MAP studies are supported by NIH grants (R01-AG30146, R01-AG179917, R01-AG15819, P30-AG10161, and K08-AG0344290).

DISCLOSURE

Dr. Biffi receives research support from the American Heart Association Bugher Foundation. Dr. Shulman receives research support from the NIH/NIA, the Clinical Investigator Training Program: Beth Israel Deaconess Medical Center and Harvard/MIT Health Sciences and Technology, in collaboration with Pfizer Inc and Merck & Co, Inc., Parkinson's Study Group, Harvard NeuroDiscovery Center/Massachusetts Alzheimer's Disease Research Center, and Burroughs Wellcome Fund, Career Award for Medical Scientists. Dr. Jagiella reports no disclosures. L. Cortellini receives research support from the NIH/NINDS. A.M. Ayres receives research support from the NIH. K. Schwab receives research support from the NIH (NINDS, NIA). Dr. Brown serves on the editorial board of *Neurology*®; is supported by an NINDS Career Development Award (K23 NS051202); and has received research support from CVR Global, Inc., the University of Michigan, Michigan Department of Community Health, Blue Cross Blue Shield of Michigan Foundation, the Diocese of Corpus Christi, and the NIH. Dr. Brown has received speaker honoraria from the Hazel K. Goddess Fund for Stroke Research in Women and receives research support from the NIH (NINDS, NHLBI). Dr. Silliman serves on the speakers' bureau for and has received speaker honoraria from Biogen Idec and has received research support from Biogen Idec, Novartis, Genzyme Corporation, Schering-Plough Corp., and the NIH/NINDS. Dr. Selim serves on scientific advisory boards for Mitsubishi Tanabe Pharma Corporation and Avanir Pharmaceuticals; serves as Controversies Section Co-editor for *Stroke*; receives publishing royalties for *The Stroke Book* (Cambridge University Press, 2007) and *Neurology Emergencies* (Oxford University Press, 2011); and receives research support from the NIH. Dr. Worrall serves as an Associate Editor of *Neurology*® and on the editorial board of *Seminars in Neurology*; receives royalties from the publication of *Merritt's Neurology, 10th, 11th, and 12th eds.* (chapter author); and receives/has received research support from the NIH (NHGRI, NHLBI, NINDS) and from the University of Virginia–CTSA Pilot Project. Dr. Meschia serves on the editorial boards of *Stroke* and the *Journal of Stroke and Cerebrovascular Diseases* and receives research support from the NIH/NINDS. Dr. Slowik reports no disclosures Dr. De Jager has served on scientific advisory boards for Teva Pharmaceutical Industries Ltd. and Biogen Idec; has received speaker honoraria from Biogen Idec; serves on the editorial board of *Journal of Neuroimmunology*; and receives research support from Biogen Idec and the NIH. Dr. Greenberg serves on a data safety monitoring board for the NIH/NINDS; has received speaker honoraria from Esteve, Medtronics, Inc., and Pfizer Inc; serves on the editorial boards of *Neurology*®, *Stroke*, *Cerebrovascular Disease*, and the *Journal of Alzheimer's Disease and Other Dementias*; has served as a consultant for Roche, Janssen Alzheimer Immunotherapy, and Bristol-Myers Squibb; and has received/receives research support from the NIH and the Alzheimer's Association. Dr. Schneider served on a scientific advisory board for GE Healthcare; serves as Monitoring Editor for the

Journal Histochemistry and Cytochemistry, on the editorial board of the *International Journal of Clinical and Experimental Pathology*, and as Associate Editor for the *Journal of Alzheimer's Disease*; serves as a consultant for Avid Radiopharmaceuticals, Inc.; and receives research support from Avid Radiopharmaceuticals, Inc. and the NIH. Dr. Bennett serves on the editorial boards of *Neurology®*, *Neuroepidemiology*, and *Current Alzheimer's Research*; serves on the scientific advisory board for Vigorous Minds; serves/has served as a consultant to Schering-Plough Corp., Double Helix Development, Medivation, Inc., Danone Research B.V., and Gerson Lehrman Group; and receives research support from Danone Research B.V., the NIH, the Illinois Department of Public Health, and the Robert C. Borwell Endowment Fund. Dr. Rosand receives research support from the NIH (NINDS, NHLBI), the American Heart Association–Bugher Foundation, and the MGH Deane Institute for Integrative Research in Atrial Fibrillation and Stroke.

Received May 10, 2011. Accepted in final form September 23, 2011.

REFERENCES

1. Vinters HV, Gilbert JJ. Cerebral amyloid angiopathy: incidence and complications in the aging brain: II: the distribution of amyloid vascular changes. Stroke 1983; 14:924–928.
2. Vinters HV. Cerebral amyloid angiopathy: a critical review. Stroke 1987;18:311–324.
3. Qureshi AI, Tuhrim S, Broderick JP, Batjer HH, Hondo H, Hanley DF. Spontaneous intracerebral hemorrhage. N Engl J Med 2001;344:1450–1460.
4. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol 2010;9:689–701.
5. Vinters HV. Cerebral amyloid angiopathy: a microvascular link between parenchymal and vascular dementia? Ann Neurol 2001;49:691–693.
6. Greenberg SM, Gurol ME, Rosand J, Smith EE. Amyloid angiopathy-related vascular cognitive impairment. Stroke 2004;35:2616–2619.
7. Knudsen KA, Rosand J, Karluk D, Greenberg SM. Clinical diagnosis of cerebral amyloid angiopathy: validation of the Boston criteria. Neurology 2001;56:537–539.
8. Van Asch CJ, Luitse MJ, Rinkel GJ, et al. Incidence, case fatality, and functional outcome of intracerebral haemorrhage over time, according to age, sex, and ethnic origin: a systematic review and meta-analysis. Lancet Neurol 2010; 9:167–176.
9. O'Donnell HC, Rosand J, Knudsen KA, et al. Apolipoprotein E genotype and the risk of recurrent lobar intracerebral hemorrhage. N Engl J Med 2000;342:240–245.
10. Biffi A, Halpin A, Towfighi A, et al. Aspirin and recurrent intracerebral hemorrhage in cerebral amyloid angiopathy. Neurology 2010;75:693–698.
11. Vinters HV, Wang ZZ, Secor DL. Brain parenchymal and microvascular amyloid in Alzheimer's disease. Brain Pathol 1996;6:179–195.
12. Bekris LM, Yu CE, Bird TD, et al. Genetics of Alzheimer disease. J Geriatr Psychiatry Neurol 2010;23:213–227.
13. Revesz T, Holton JL, Lashley T, et al. Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. Acta Neuropathol 2009;118:115–130.
14. Biffi A, Sonni A, Anderson CD, et al. Variants at APOE influence risk of deep and lobar intracerebral hemorrhage. Ann Neurol 2010;68:934–943.
15. Harold D, Abraham R, Hollingworth P, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088–1093.
16. Lambert JC, Heath S, Even G, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41:1094–1099.
17. Seshadri S, Fitzpatrick AL, Ikram MA, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 2010;303:1832–1840.
18. Chibnik LB, Shulman JM, Leurgans SE, et al. CR1 is associated with amyloid plaque burden and age-related cognitive decline. Ann Neurol 2011;69:560–569.
19. Genes for Cerebral Hemorrhage on Anticoagulation (GOCHA) Collaborative Group. Exploiting common genetic variation to make anticoagulation safer. Stroke 2009;40:S64–S66.
20. Arvanitakis Z, Leurgans SE, Wang Z, et al. Cerebral amyloid angiopathy pathology and cognitive domains in older persons. Ann Neurol 2011;69:320–327.
21. Bennett DA, Schneider JA, Buchman AS, et al. The Rush Memory and Aging Project: study design and baseline characteristics of the study cohort. Neuroepidemiology 2005;25:163–175.
22. Bennett DA, Schneider JA, Arvanitakis Z, et al. Neuropathology of older persons without cognitive impairment from two community-based studies. Neurology 2006;66: 1837–1844.
23. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
24. Revesz T, Holton JL, Lashley T, et al. Genetics and molecular pathogenesis of sporadic and hereditary cerebral amyloid angiopathies. Acta Neuropathol 2009;118:115–130.
25. Biffi A, Anderson CD, Desikan RS, et al. Genetic variation and neuroimaging measures in Alzheimer disease. Arch Neurol 2010;67:677–685.
26. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet 2008;17:R143–R150.
27. Hess KR. Graphical methods for assessing violations of the proportional hazards assumption in Cox regression. Stat Med 1995;14:1707–1723.
28. 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.
29. Xiao R, Boehnke M. Quantifying and correcting for the winner's curse in genetic association studies. Genet Epidemiol 2009;33:453–462.
30. Kraft P. Curses—winner's and otherwise—in genetic epidemiology. Epidemiology 2008;19:649–651.
31. Bennett DA, De Jager PL, Leurgans SE, Schneider JA. Neuropathologic intermediate phenotypes enhance association to Alzheimer susceptibility alleles. Neurology 2009; 72:1495–1503.
32. Corneveaux JJ, Myers AJ, Allen AN, et al. Association of CR1, CLU and PICALM with Alzheimer's disease in a cohort of clinically characterized and neuropathologically verified individuals. Hum Mol Genet 2010;19: 3295–3301.