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Alzheimer's disease related single nucleotide polymorphisms and correlation with intracerebral hemorrhage incidence

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ABSTRACT

Apolipoprotein E alleles have been associated with both Alzheimer's disease (AD) and intracerebral hemorrhage (ICH). In addition, ICH is associated with a markedly high risk of subsequent dementia compared to other subtypes of stroke. We sought to evaluate if other genetic markers for AD were also associated with ICH. We examined whether published AD risk single nucleotide polymorphisms (SNPs) and haplotypes were associated with ICH utilizing genome-wide association study data from 2 independent studies (genetic and environmental risk factors for hemorrhagic stroke [GERFHS] study and genetics of cerebral hemorrhage with anticoagulation [GOCHA]). Analyses included evaluation by location of ICH. GERFHS and GOCHA cohorts contained 745 ICH cases and 536 controls for analysis. The strongest association was on 1q32 near *Complement receptor type 1 (CR1)*, where rs6701713 was associated with all ICH ($P = .0074$, odds ratio [OR] = 2.07) and lobar ICH ($P = .0073$, OR = 2.80). The 51 most significant 2-SNP haplotypes associated with lobar ICH were identified within the *Clusterin* (CLU) gene. We identified that variation within *CR1* and *CLU*, previously identified risk factors for AD, and are associated with an increased risk for ICH driven primarily by lobar ICH. Previous work implicated *CR1* and *CLU* in cerebral amyloid clearance, the innate immune system, and cellular stress response.

Abbreviations: AD = Alzheimer's disease, CAA = cerebral amyloid angiopathy, CLU = Clusterin, CR1 = complement receptor type 1, GERFHS = genetics and environmental risk factors for intracerebral hemorrhage, GOCHA = genes for cerebral hemorrhage on anticoagulation, ICH = intracerebral hemorrhage, OR = odds ratio, SNP = single nucleotide polymorphism.

Keywords: Alzheimer's disease, cerebral amyloid angiopathy, genetics, intracerebral hemorrhage

1. Introduction

Intracerebral hemorrhage (ICH) is responsible for 10% to 15% of strokes worldwide each year,[1] and is associated with high rates of mortality and morbidity.[2,3] Risk factors for ICH differ depending on the location of the hemorrhage: deep (ganglionic), lobar, cerebellar, and brainstem. Overall, the overwhelmingly biggest risk factor for ICH is hypertension, which causes deep ICH more often than lobar ICH. Cerebral amyloid angiopathy (CAA), a disease process which occurs in between 52% and 97% of Alzheimer's disease (AD) cases,[4,5] is an independent risk factor for lobar ICH.[6,7]

The risk of dementia and cognitive impairment after ICH is substantially higher compared to the general population or even acute ischemic stroke survivors. Rates of dementia are as high as 20.7% within the first year and 45% within 30 years of ICH.[8-10]

Early incident dementia following ICH has been associated with specific features of the ICH, including hematoma size and location. However, the features of later onset dementia following ICH are more aligned with risk factors for neurodegenerative disease: lower education, more frequent mood symptoms, and greater white matter disease burden.[9] Another study found that lobar ICH was associated with a higher risk for developing dementia after ICH, compared to non-lobar ICH.[11] Because superficial siderosis and more cortical microbleeds were also risk factors for post-ICH dementia, CAA was proposed as the contributing factor.[11] It is possible that ICH patients developed dementia from the ICH

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The data that support the findings of this study are available from a third party, but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are available from the authors upon reasonable request and with permission of the third party.

Data is available by request from Dr Daniel Woo from a researcher or research team with a written request detailing their proposed research.

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itself or that risk factors for dementia were more common in ICH patients.

Given the overlap between ICH and AD pathophysiology and related risk factors, we hypothesized that single nucleotide polymorphisms (SNPs) previously associated with AD, specifically those in the vicinity of genes involved in amyloid and lipid metabolism, would also be associated with ICH. In particular we expected to find that the genetic associations would be location dependent. To examine these hypotheses, we tested genome wide significant SNPs in the *Sortilin Related Receptor-1*, *Clusterin (CLU)*, *Complement receptor type 1 (CR1)*, *Phosphatidylinositol Binding Clathrin Assembly Protein*, *Bridging Integrator-1, ATP-binding cassette sub-family A member 7, Cas Scaffold Protein Family Member 4, and Spondin-1* genes for association in 2 cohorts of ICH subjects.

2. Material and Methods

2.1. SNP selection

To identify SNPs and regions of interest associated with AD, we performed a comprehensive PubMED literature search using the search term "genome wide association study," "genes," "alleles," "genetic risk," and "Alzheimer's disease." Studies were included if genome wide significant variants were identified including meta-analyses of previously reported genome wide association studies with novel loci. AD risk SNPs and associated regions were then selected if the individual studies' predetermined cutoff for statistical significance was met. If a gene with a SNP was determined to affect amyloid or lipid metabolism by the authors based on literature review, then the SNP and chromosome location were included in our statistical analysis. Based on the above criteria, 27 SNPs were identified in 8 genes. If the SNP occurred outside of a gene, then the most proximal gene was used.

Because of the close physical proximity of many of the candidate genes identified and potential linkage disequilibrium, we also performed 2-SNP haplotype analysis for the 27 SNPs identified.

2.2. Description of selected cohorts

The Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS) study is a prospective, demographically matched case-control study of white and black ICH patients living within 50 miles of University of Cincinnati.[12] Hemorrhages associated with trauma, brain tumor, encephalitis, endarterectomy, hemorrhagic cerebral infarction, or thrombolytic treatment of ischemic stroke did not meet study criteria.[12-14] Patients with ICH associated with anticoagulation, primary intraventricular hemorrhage, or prior history of ischemic stroke were included.[12-14] Study neurologists reviewed clinical and neuroimaging information for each patient and made the final determination of case eligibility. Controls for the GERFHS study were identified by random digit dialing to match cases by age (±5 years), race, and gender.[12] In the GERFHS genome wide association study cohort, 52 cases and 3 controls with dementia/AD were removed based on interview and abstraction data, as well as dementia specific medications to make this determination

The Genetics of Cerebral Hemorrhage with Anticoagulation (GOCHA) Study is a case-control study of ICH. Enrolled cases included acute ICH subjects aged > 55 years presenting to the Massachusetts General Hospital and several other institutions.[15] Data are available on dbGAP and via the cerebrovascular disease knowledge portal.[16] Exclusion criteria included trauma, brain tumor, hemorrhagic transformation of an ischemic stroke, vascular malformation, or any other perceived cause of secondary ICH. Controls were enrolled from the same population that gave rise to the cases and included individuals aged > 55 years attending ambulatory clinics.[15]

The quality control analyses for GERFHS and GOCHA have been previously described.[14,15] The inclusion criteria for these 2 studies are very similar, with GERFHS being more inclusive based on age. Genotype data were merged using PLINK, and genome-wide imputation was performed on the combined data using IMPUTE and the phase 1 integrated reference panel, version 3. Genetic association testing was computed using SNPTEST and SNPLASH.

All human subjects participating in the described research have signed an informed consent prior to being enrolled into GERFHS and GOCHA. The procedures employed were reviewed and approved by the appropriate institutional review committees.

2.3. Statistical analysis

Analyses were computed for ICH and stratified by location (lobar, non-lobar). Stratification by ICH location was done because lobar ICH is traditionally associated with CAA, itself associated with AD, and non-lobar ICH is traditionally associated with hypertension.[1] Demographic characteristics are reported as mean ± SD or n (%), and comparisons between cases and controls used chi-squared tests for categorical and the Wilcoxon rank sum test for continuous measures. Genetic model association testing was computed for each SNP, adjusting for age, gender, and ancestry (i.e., 2 principal components) as previously described.[15] Genotypic uncertainty was taken into account for imputed SNPs. A 2-marker haplotype test was computed using the SNPGWA module of SNPLASH. Best guess genotypes were used for imputed SNPs.

A Bonferroni correction was computed to account for multiple testing. To determine the number of independent tests, principal components analysis was performed using all good-quality SNPs in the regions of interest, separately for the all-ICH, lobar, and non-lobar groups. The number of principal components that explained 95% of the variation in the region was used for the Bonferroni correction; 976 for all-ICH, 638 for lobar, and 747 for non-lobar. Using the most conservative estimate of 976 independent tests, we applied a study-wide Bonferroni correction threshold of P-value 6.0×10^{-5}.

A power analysis was computed to estimate the odds ratio (OR) detectable for the allele frequency and genetic model. Our study had 80% power to detect a 4.1% difference in minor allele frequency assuming a type 1 error rate of $\alpha = 0.05$ for a SNP with minor allele frequency of 5% in the control group.

3. Results

A total of 745 cases and 536 controls were available for analysis. Table 1 presents the demographic characteristics of the case-control cohort.

Tables 2 and 3 summarizes the tests of association for the reference SNPs. The strongest a priori single SNP association was on 1q32 near *CR1*, where rs6701713 was associated with ICH (P = .0074, OR = 2.07) and lobar ICH (P = .0073, OR = 2.80)

Table 1

Demographic characteristics of cases and controls.

	Cases	Controls	*P* value†
Subjects, n	745	536	
Age, mean (SD)	69.0 (13.8)	67.7 (13.6)	.1566
Female, n (%)	347 (46.6)	268 (50.0)	.2264
Hypertension, n (%)	325 (64.0)	250 (50.2)	.00001
Lobar ICH, n (%)	271 (36.4)	-	
Non-lobar ICH, n (%)	420 (56.4)	-	

ICH = intracerebral hemorrhage.

† Differences in age tested using Wilcoxon ranked sum test and the categorical data tested using chi-square test for contingency tables.

under a recessive model. Although not statistically significant, a trend was observed for non-lobar ICH ($P = .06$). The previously reported AD and CAA associated SNP, rs6656401, was significant for ICH ($P = .016$, OR = 2.03) but not for either lobar ($P = .45$) or non-lobar ($P = .10$) ICH. The strongest 2-SNP haplotype association across the *CR1* region was rs10779350-rs80209101 ($P = 4.4 \times 10^{-5}$).

Although none of the a priori AD-risk SNPs in the *CLU* gene region on 8p21 were statistically significant, the analysis of the extended region identified 2-SNP haplotypes that reached study-wide significance in lobar ICH (rs1254927-rs75347213, $P = 5.9 \times 10^{-5}$, Table 4). Seven more 2-SNP haplotypes showed a trend towards significance ($P < 7.4 \times 10^{-6}$), 3 more in lobar and 3 in non-lobar (Table 4) ICH. The associated SNP did not explicitly contain the a priori AD-risk SNPs and therefore cannot be considered direct replication, but they do implicate the region. In addition, the 51 and 11 most significant p-values for the 2-SNP haplotype were within the *CLU* gene region for lobar ICH and non-lobar ICH, respectively.

Beyond the *CR1* and *CLU* regions, no other a priori SNPs met statistical significance, even without multiple comparisons adjustment (Table 2, all $P > .05$). The most suggestive evidence of association was on 11q24 with rs1699102 (*SORL1*: ICH $P = .07$, OR = 1.17; lobar $P = .04$, OR = 1.26). Exploring more extensively the AD-risk regions also failed to identify any individual SNPs meeting the Bonferroni criteria of 6.0×10^{-5}.

4. Discussion

Our study found the SNPs rs3818361 and rs6701713 in the *CR1* gene were associated with increased risk across all ICH but is driven primarily by lobar ICH. Interestingly, *CR1* has been associated with severity of CAA pathology at autopsy as well as risk of ICH related to CAA.[17] CAA is associated with increased risk of lobar ICH consistent with our findings of the *CR1* region as a risk factor for lobar, but not non-lobar ICH. Whether or not CAA is driving the changes that we saw in our study is not known.

Abnormal amyloid deposition in neuronal and glial tissue is a prominent pathophysiologic finding in AD.[18] The proposed mechanism for CAA is the deposition of amyloid β-protein in cerebral arteries.[19] Amyloid deposition leads to a cascade of events resulting in vascular dysfunction which in turn causes lobar ICH and microhemorrhage.[19] The apolipoprotein E epsilon 4 allele[20] has been demonstrated to alter amyloid β-protein aggregation and clearance,[21] has been associated with lobar ICH in Caucasian populations,[13] and is a major genetic risk factor for AD and lobar ICH.[22]

Unlike the strong relationship between lobar ICH and AD, the relationship between deep ICH and AD has not been established. In contrast to lobar ICH, deep ICH is predominantly due to arteriosclerosis in the setting of hypertension, with alterations in lipid metabolism playing a role.[23,24] Alterations in lipid metabolism have been observed in numerous stages of AD pathophysiology.[25,26] Furthermore, more severe cerebral atherosclerosis and arteriolosclerosis have been associated with an increased the risk for AD- associated dementia.[27] The neurovascular hypothesis of AD suggests vascular dysfunction caused by various risk factors lead to a breakdown of vascular integrity causing reduced cerebral blood flow resulting in reduced clearance of beta-amyloid, which results in neuronal dysfunction and neurodegeneration.[28]

Table 2

Summary of all ICH association results for Alzheimer's disease risk SNPs related to amyloid processing and cholesterol metabolism.

								All ICH			
Gene		SNP	Chr	Position	Minor Allele	Other Allele	MAF Ctrl	MAF Case	*P* value		OR	95%CI
CR1		rs6656401	1	207692049	A	G	0.19	0.19	.0160	*r*	2.03	1.14-3.60
CR1	*i*	rs3818361	1	207784968	A	G	0.19	0.19	.0077	*r*	2.07	1.21-3.53
CR1	*i*	rs6701713	1	207786289	A	G	0.19	0.19	.0074	*r*	2.07	1.22-3.54
BIN1	*i*	rs7561528	2	127889637	A	G	0.31	0.33	.2391	*r*	1.25	0.86-1.81
BIN1	*i*	rs6733839	2	127892810	T	C						
BIN1	*i*	rs744373	2	127894615	G	A						
CLU	*i*	rs7012010	8	27448729	C	T	0.28	0.28	.9731		1.00	0.84-1.20
CLU	*i*	rs2279590	8	27456253	T	C	0.41	0.41	.4869	*d*	1.09	0.86-1.39
CLU	*i*	rs7982	8	27462481	A	G	0.39	0.39	.5303	*d*	1.08	0.85-1.36
CLU		rs11136000	8	27464519	T	C	0.39	0.40	.4627	*d*	1.09	0.87-1.37
CLU	*i*	rs1532278	8	27466315	T	C	0.39	0.39	.6213	*d*	1.06	0.84-1.34
CLU	*i*	rs867230	8	27468503	C	A						
CLU	*i*	rs9331888	8	27468862	G	C						
SPON1		rs11023139	11	14224346	A	G	0.05	0.05	.8925	*d*	0.97	0.66-1.44
PICALM		rs3851179	11	85868640	T	C	0.37	0.36	.5777		0.95	0.81-1.13
SORL1	*i*	rs117260922	11	121367627	A	G						
SORL1	*i*	rs641120	11	121380965	A	G	0.42	0.44	.5350	*d*	1.08	0.85-1.38
SORL1	*i*	rs143571823	11	121429476	T	C						
SORL1		rs11218343	11	121435587	C	T	0.04	0.04	.4041	*d*	1.20	0.78-1.83
SORL1		rs3781834	11	121445940	G	A	0.02	0.03	.1801	*d*	1.46	0.84-2.54
SORL1	*i*	rs1699102	11	121456962	C	T	0.31	0.35	.0686		1.17	0.99-1.39
ABCA7	*i*	rs3764650	19	1046520	G	T						
ABCA7	*i*	rs115550680	19	1050420	G	A						
ABCA7	*i*	rs3752246	19	1056492	G	C	0.18	0.17	.5700		0.94	0.76-1.17
ABCA7	*i*	rs4147929	19	1063443	A	G	0.18	0.17	.2496	*r*	0.67	0.34-1.33
EXOC3L2	*i*	rs597668	19	45708888	C	T	0.17	0.16	.4973	*d*	0.92	0.72-1.18
CASS4	*i*	rs7274581	20	55018260	C	T	0.08	0.09	.3776	*d*	1.14	0.85-1.54

The following SNPs (gene) did not pass quality control in our data: rs6733839 (BIN1), rs744373 (BIN1), rs867230 (CLU), rs9331888 (CLU), rs117260922 (SORL1), rs3764650 (ABCA7), rs115550680 (ABCA7). SNP rs143571823 (SORL1) is monomorphic. SNPs with no results were imputed poorly or monomorphic in our data.

95%CI = 95% confidence interval, ABCA7 = ATP-binding cassette sub-family A member 7, BIN1 = Bridging Integrator-1, CASS4 = Cas Scaffold Protein Family Member 4, Chr = chromosome, CLU = Clusterin, CR1 = complement receptor type 1, *d* = dominant (otherwise additive model), *i* = imputed, ICH = intracerebral hemorrhage, MAF = minor allele frequency, OR = odds ratio, *PICALM* = *Phosphatidylinositol Binding Clathrin Assembly Protein*, *r* = recessive, SNP = single nucleotide polymorphism, SPON1 = Spondin-1.

Table 3

Comparison of Lobar and Non-Lobar ICH results for Alzheimer's disease risk SNPs related to amyloid processing and cholesterol metabolism.

								Lobar					Non-Lobar				
Gene		SNP	Chr	Position	Minor allele	Other allele	MAF Ctrl	MAF case	*P* value		OR	95%CI	MAF case	*P* value		OR	95%CI
CR1		rs6656401	1	207692049	A	G	0.19	0.19	.4463	*d*	0.89	0.65-1.21	0.17	.1005	*d*	0.80	0.61-1.05
CR1	*i*	rs3818361	1	207784968	A	G	0.19	0.20	.0077	*r*	2.79	1.31-5.94	0.17	.0556	*d*	0.77	0.58-1.01
CR1	*i*	rs6701713	1	207786289	A	G	0.19	0.20	.0073	*r*	2.80	1.32-5.96	0.17	.0554	*d*	0.77	0.58-1.01
BIN1	*i*	rs7561528	2	127889637	A	G	0.31	0.35	.0439	*r*	1.64	1.01-2.64	0.32	.6502	*r*	1.11	0.71-1.73
BIN1	*i*	rs6733839	2	127892810	T	C											
BIN1	*i*	rs744373	2	127894615	G	A											
CLU	*i*	rs7012010	8	27448729	C	T	0.28	0.27	.6405		0.94	0.74-1.2	0.29	.6400		1.05	0.85-1.29
CLU	*i*	rs2279590	8	27456253	T	C	0.41	0.42	.3658	*d*	1.16	0.84-1.59	0.40	.3079	*r*	0.83	0.58-1.19
CLU	*i*	rs7982	8	27462481	A	G	0.39	0.40	.3280	*d*	1.17	0.86-1.58	0.39	.5834	*r*	0.90	0.63-1.29
CLU		rs11136000	8	27464519	T	C	0.39	0.41	.2551	*d*	1.19	0.88-1.62	0.39	.5728	*r*	0.90	0.64-1.28
CLU	*i*	rs1532278	8	27466315	T	C	0.39	0.40	.4168	*d*	1.14	0.83-1.55	0.39	.6421	*r*	0.92	0.64-1.32
CLU	*i*	rs867230	8	27468503	C	A											
CLU	*i*	rs9331888	8	27468862	G	C											
SPON1		rs11023139	11	14224346	A	G	0.05	0.04	.6067	*d*	0.87	0.52-1.47	0.05	.9647	*d*	1.01	0.65-1.58
PICALM		rs3851179	11	85868640	T	C	0.37	0.36	.4894		0.92	0.74-1.15	0.37	.5504	*r*	0.89	0.6-1.31
SORL1	*i*	rs117260922	11	121367627	A	G											
SORL1	*i*	rs641120	11	121380965	A	G	0.42	0.41	.7274	*d*	0.95	0.69-1.3	0.45	.1873	*d*	1.21	0.91-1.6
SORL1	*i*	rs143571823	11	121429476	T	C											
SORL1		rs11218343	11	121435587	C	T	0.04	0.03	.6952	*d*	0.89	0.49-1.6	0.05	.1932	*d*	1.38	0.85-2.22
SORL1		rs3781834	11	121445940	G	A	0.02	0.02					0.03	.0785	*d*	1.76	0.94-3.29
SORL1	*i*	rs1699102	11	121456962	C	T	0.31	0.36	.0409		1.26	1.01-1.58	0.34	.1164		1.17	0.96-1.43
ABCA7	*i*	rs3764650	19	1046520	G	T											
ABCA7	*i*	rs115550680	19	1050420	G	A											
ABCA7	*i*	rs3752246	19	1056492	G	C	0.18	0.18	.8675		0.98	0.74-1.29	0.16	.5441	*d*	0.92	0.69-1.22
ABCA7	*i*	rs4147929	19	1063443	A	G	0.18	0.18	.9767		1.00	0.77-1.32	0.16	.2437		0.86	0.67-1.11
EXOC3L2	*i*	rs597668	19	45708888	C	T	0.17	0.16	0.4967	*d*	0.89	0.64-1.24	0.16	0.6539	*d*	0.94	0.7-1.25
CASS4	*i*	rs7274581	20	55018260	C	T	0.08	0.09	0.7217	*d*	1.08	0.72-1.61	0.09	0.4183	*d*	1.15	0.82-1.63

95%CI = 95% confidence interval, ABCA7 = ATP-binding cassette sub-family A member 7, BIN1 = Bridging Integrator-1, CASS4 = Cas Scaffold Protein Family Member 4, CLU = Clusterin, CR1 = complement receptor type 1, *i* = imputed, ICH = intracerebral hemorrhage, MAF = minor allele frequency, OR = odds ratio, *PICALM* = *Phosphatidylinositol Binding Clathrin Assembly Protein*, SNP = single nucleotide polymorphism, SPON1 = Spondin-1.

Table 4

Two-SNP haplotype analysis for haplotypes approaching statistical significance after Bonferroni correction.†

Gene	SNP1	Chromosome	Position	SNP2	Position	*P* value	*R2*††
All ICH							
CR1	rs10779350	1	207834524	rs80209102	207834661	4.4×10^{-5}	0.491786
Lobar ICH							
CLU	rs12542927	8	27017381	rs75347213	27017476	5.9×10^{-5}	0.005737
CLU	rs138648154	8	27023258	rs7822741	27023370	6.2×10^{-5}	0.007316
CLU	rs74969269	8	27032866	rs2218567	27032937	7.3×10^{-5}	0.005695
CLU	rs7822741	8	27023370	rs186608173	27023680	7.3×10^{-5}	0.001479
Non-lobar ICH							
CLU	rs78457827	8	27041762	rs78062570	27041867	6.5×10^{-5}	0.000266
CLU	rs118010112	8	27048453	rs117510344	27048498	6.5×10^{-5}	1
CLU	rs117510344	8	27048498	rs7010650	27048577	6.5×10^{-5}	0.000186

CLU = Clusterin, CR1 = complement receptor type 1, ICH = intracerebral hemorrhage, SNP = single nucleotide polymorphism.

† Study-wide Bonferroni threshold for statistical significance $P = 6 \times 10\text{-}5$

†† All haplotype SNP pairs have linkage disequilibrium D'>0.95.

CR1 encodes Complement C3b/C4b Receptor 1 protein, which is involved in immune clearance of opsonized pathogens on erythrocytes. There is also data to suggest that CR1 is present on microglia where the receptor plays a role in the clearance of amyloid beta in AD.[29] Decreased clearance of amyloid due to SNPs in *CR1* may result in increased amyloid deposition in blood vessel walls, supported by previous pathology studies demonstrating SNPs in *CR1* were associated with greater CAA burden. Additionally, *CR1* variant rs6656401 has been shown to influence risk and recurrence of CAA related ICH, as well as the severity of vascular amyloid deposition.[17]

Another potential mechanism for increased risk for ICH is dysregulation of the innate immune system. CR1 protein is believed to regulate the complement cascade on many levels, mainly reducing activation of complement by a variety of mechanisms. Thus, decreased function of the CR1 protein may result in over-activation of the innate immune system.[29] Over activity of the complement system has been implicated in coronary artery disease as well as hypertension in renal disease. Interestingly, SNPs rs3818361 and rs6701713 in *CR1* were associated with a lower risk of non-lobar ICH, though this did not meet statistical significance.

We did identify several statistically significant haplotypes which did not contain the pre-specified risk allele, but were within one of our genes of interest, the *CLU* gene. While not confirming a prior association, the potential that different variations within the same gene cause the same phenotypic end point is possible. Seven of the 8 haplotypes which reached statistical significance corresponded to the *CLU* gene located at 8p21.1.

CLU is widely expressed in cells throughout the body, with multiple functions determined by its domains, post-translational modifications (alternative splicing, glycosylation, sialylation), and surrounding environment.[30] The major form of CLU is secreted into physiological fluids, but truncated forms have been identified which are targeted to the nucleus. In vitro systems suggest that CLU functions in membrane lipid recycling, apoptotic cell death, and as a stress-induced secreted chaperone protein, amongst others.[30]

CLU's role in AD is complex. The secreted form of CLU binds to and inhibits plaque formation by preventing soluble forms of Aβ from sedimentation. In vivo experiments show that it prevents the proteolytic degradation of Aβ, which leads to the oligomerization of soluble Aβ and triggers even worse cytotoxic effects.[31] The effects of CLU on localization of amyloid deposition may be salient to its relationship with ICH. A *CLU* knockout mouse model found a marked decrease in plaque deposition in the brain parenchyma but a striking increase in CAA within the cerebrovasculature. Despite the several-fold increase in CAA severity, *CLU* knockout mice had significantly less hemorrhage and inflammation.[32] Authors proposed that in the absence of CLU, amyloid clearance shifts to perivascular drainage pathways, resulting in fewer parenchymal plaques but more CAA because of loss of CLU chaperone activity.[32]

We hypothesize 2 pathophysiologic mechanisms likely contributing to the shared risk between AD and ICH caused by *CR1* and *CLU* polymorphisms. The first involves impaired clearance of amyloid from the brain and cerebrovasculature, and the second involves immune system dysfunction with impaired regulation of the innate immune system via CR1 and impaired neuronal/glial stress response via CLU. Examining how therapies that enhance amyloid clearance affect *CR1* and *CLU* variants could better elucidate these mechanisms

Our study has several limitations. As patients were not examined prior to ICH, it is unclear how many patients may have had undiagnosed dementia prior to stroke. In addition, potential controls with dementia are less likely to enroll into research studies. If a greater number of ICH cases compared to controls had undiagnosed dementia, with AD being the most common type of dementia, it would result in falsely elevated association between AD associated SNPs and ICH. Another limitation is likely overlap between the cases enrolled in our study and those in Biffi et al, as both consisted of subjects from the GOCHA cohort.[17] This is relevant because both studies found associations with *CR1*. Our study expands the number of SNPs relating to *CR1* and further elucidates the relationship between *CLU* and ICH.

In summary, our study demonstrated increased risk of all cause ICH and lobar ICH in SNPs rs3818361 and rs6701713 of the *CR1* gene. Haplotype analysis demonstrated several pairwise associations within the *CLU* gene. Both genes encode proteins vital to amyloid clearance, with *CR1* also significantly involved in the innate immune system. Evaluation of the effects of *CR1* and *CLU* SNPs on recurrent ICH and recovery from ICH are needed.

Author contributions

DW and JR contributed to study concept and design. MEC, MM, and CDL analyzed the data. RPS and SLD contributed to the drafting and editing of this manuscript. All authors brought major revisions in significant portions of the manuscript for intellectual content.

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