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# Association of Coagulation and Inflammation Related Genes and Factor VIIc Levels with Stroke: The Cardiovascular Health Study

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## Abstract

**Background—**Thrombosis and inflammation are critical in stroke etiology, but associations of coagulation and inflammation gene variants with stroke, and particularly factor VII levels are inconclusive.

**Objectives—**To test the associations between 736 single nucleotide polymorphisms (SNP) between tagging haplotype patterns of 130 coagulation and inflammation genes, and stroke events in the 5,888 participants ≥ 65 of the observational Cardiovascular Health Study cohort.

**Patients—/Methods:** With 16 years of follow-up, age and sex-adjusted Cox models were used to estimate associations of SNPs and factor VIIc levels with future stroke.

**Results—**815 strokes occurred in 5,255 genotyped participants without baseline stroke (748 ischemic strokes, 586 among whites). Among whites, 6 SNPs were associated with stroke with a nominal $p < 0.01$: rs6046 and rs3093261 (F7 gene); rs4918851 and rs3781387 (HABP2 gene); rs3138055 (NFKB1A) and rs4648004 (NFKB1). Two of these SNPs were associated with factor VIIc levels (units = percent activity): rs6046 ($\beta = -18.5$, $p = 2.38 \times 10^{-83}$) and rs3093261 ($\beta = 2.99$, $p = 3.93 \times 10^{-6}$). Adjusting for age, sex, race, and cardiovascular risk factors, the association of factor VIIc quintiles (Q) with stroke were (HR: 95% CI): Q1 (reference); Q2 (1.4; 1.1, 1.9); Q3 (1.1; 0.8, 1.5); Q4 (1.5; 1.1, 2.0); Q5 (1.6; 1.2, 2.2). Associations between SNPs and stroke were independent of factor VIIc levels.

**Conclusions—**Variation in factor VII-related genes and factor VIIc levels were associated with risk of incident ischemic stroke in this elderly cohort, suggesting a potential causal role for factor VII in stroke etiology.

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### Keywords

Stroke; Genetic Epidemiology; factor VII; hemostasis; inflammation; cardiovascular disease risk

Stroke is a major cause of morbidity and mortality in the developed world; in the United States, one in six men and one in five women suffer a stroke in their lifetime with stroke being responsible for 17% of all deaths[1]. Thrombosis plays a key role in ischemic stroke; after disruption of the vessel wall, thrombus is formed and either disrupts blood flow at the site of injury or breaks off and embolizes to where the occlusion occurs[2,3]. Inflammation is also associated with ischemic stroke pathophysiology and may relate to changes in the composition of blood or of the vessel wall[2]. Risk factors for stroke are not as well characterized as for myocardial infarction (MI), and few prospective studies have evaluated associations of hemostatic and inflammation biomarkers with stroke risk[4].

Hemostasis and thrombosis related proteins have long been prime biomarker candidates for stroke risk but many are difficult to measure due to high within-person variability and difficultly standardizing assays. Thus, measurement of gene variants may reveal associations that cannot be determined by assessing phenotypes.

We studied polymorphisms in hemostasis and inflammation related genes in relation to stroke risk in the Cardiovascular Health Study (CHS) cohort, and evaluated whether the protein products of genes related to stroke (where possible) were associated with stroke and other cardiovascular disease (CVD) outcomes. Further, we assessed whether these protein products mediated any of the association between the SNPs and CVD. Findings may provide insights into the pathophysiology of stroke which can be exploited for risk stratification, new interventions for primary prevention, or perhaps novel treatment approaches.

## Methods

### Cohort

The CHS is a prospective, observational cohort study of risk factors for and consequences of CVD in elderly adults 65 years or older as detailed previously[5]. Exclusion criteria at baseline include being wheelchair-bound, under active treatment for cancer, institutionalization, or inability or refusal to give informed consent. Among those approached, 9.6% were ineligible, and 57% participated [6]. The cohort originally enrolled 5201 men and women between 1989 and 1990 with a supplemental cohort of 687 African-Americans enrolled between 1992 and 1993. Written informed consent was obtained from all participants in accordance with Institutional Review Board guidelines from each site.

### Genotyping

Genotyping was supported through the Thrombosis Genetics, Myocardial Infarction and Stroke in Older Adults (TGEN) ancillary study[7]. We selected for analysis 736 single nucleotide polymorphisms (SNPs) of minor allele frequency ≥5% from 130 autosomal candidate hemostasis and inflammation-related genes (supplemental table S1). SNPs were located between 5 kb upstream of the transcription start site to 3 kb downstream of the transcription end site of the 130 genes and selected using the LDselect algorithm[7,8]. Detailed genotyping methods have been published elsewhere[7]. Genotyping was attempted on the 5,759 (of 5,888) individuals who provided informed consent for genetic studies.

### Outcomes and Follow-up

Participants were contacted biannually, alternating between telephone interviews and clinic examinations through 1999; telephone interviews continue to the present time. All deaths were reviewed and classified by a committee using information from death certificates, autopsy and coroners' forms, hospital records, and interviews with physicians and next of kin[6,9]. Incident stroke and MI events were determined by a committee based on medical record review, in-person examinations, laboratory and imaging data, telephone interviews (when in-person examinations were not possible), or reports from proxies of participants using established protocols[5,9]. Periodic searches of the Health Care Financing Administration Medicare utilization files were compiled to identify events missed by other methods. The cerebrovascular adjudication committee, a physician review panel that included neurologists, internists, and neuroradiologists, adjudicated all suspected stroke events and classified strokes as ischemic, hemorrhagic, or unknown using all available information [10]. Adjudication of outcomes was complete through June 2005 for this analysis.

### Laboratory methods

Phlebotomy was performed the morning of enrollment after an 8 to 12 hour fast[11]. Factor VII coagulant activity (VIIc) was measured using factor VII-deficient plasma and human placenta-derived thromboplastin (Thromborel S, Behring Diagnostics, Marburg, Germany) on the Coag-A-Mate X2 (Organon-Teknika, Durham, NC). The sample was citrated plasma (32g/L) processed at room temperature; coefficients of variation were between 5.89% and 6.16%. Units are expressed as "%" of normal pool. Cholesterol and creatinine were measured using standard methods with coefficients of variation (CVs) of 2.52% and 3.58% respectively. High sensitivity C-reactive protein (CRP) was measured using a validated in-house enzyme-linked immunosorbent assay[12].

### Definitions

Race was defined using participant self-report from a list (white, black, American Indian/Alaskan native, Asian/Pacific Islander, or other). Baseline stroke and MI were identified by participant self-report and confirmed by medical record review using standardized criteria[13,14]. Diabetes was defined as a fasting glucose >126mg/dL or treatment with insulin or oral hypoglycemic medications. Hypertension was defined as a blood pressure greater than 140/90 mmHg on enrollment or taking antihypertensive medications with a physician report of hypertension. Estimated glomerular filtration rate was calculated using the 4-variable Modification in Diet in Renal Disease equation; an estimated glomerular filtration rate <60ml/min/1.73m$^2$ on enrollment was defined as chronic kidney disease (CKD) [15].

### Statistical Methods

Analyses were done using StataSE version 8.2 (College Station, TX). In initial models, associations of SNPs (coded additively as 0/1/2 copies of minor allele) with incident stroke were assessed using age- and sex-adjusted Cox proportional hazard models to account for residual confounding. For the purpose of SNP discovery, we considered a result statistically significant if $p<0.01$. Subsequent analyses were driven by the findings from these initial analyses; after the initial SNP identification, we used more stringent corrected p-values for significance threshold holding based on the number of significance tests. In later models we stratified by stroke type; ischemic or hemorrhagic. We requested a look-up from the Cohorts for Heart and Aging Research in Genetic Epidemiology consortium (CHARGE) for any SNPs we found significant and proxy SNPs with an $r^2$ >0.9. The methodologies used in the CHARGE consortium are described in detail elsewhere[16,17].

Due to different haplotype frequencies, all models using genetic data were run in the non-minority cohort only. As four of the six SNPs associated with stroke were in or near the factor VII (F7) gene or the factor VII activating protease gene (HABP2), we assessed the association between these four SNPs and factor VIIc levels using linear regression (p for significance <0.01). As warfarin affects factor VIIc levels, baseline users of warfarin were excluded from analyses including factor VIIc.

Continuing our focus on F7 (whose gene product, factor VIIc is measured in CHS), we then assessed the association of quintiles of factor VIIc levels and allele frequency of the four F7 and HABP2 SNPs with cardiovascular disease risk factors. P-values for trend were calculated using t-tests, $\chi^2$ tests, or Wilcoxon rank-sum tests as appropriate. We used staged Cox-proportional hazard models to evaluate the association between factor VIIc levels and incident ischemic stroke, hemorrhagic stroke, and myocardial infarction, including covariates based on prior established CVD risk factors and the cross sectional associations of factor VIIc with covariates. Model A adjusted for demographic variables: age (continuous), sex, and race (white versus minority). Model B, in addition to the covariates in Model A, adjusted for cardiovascular risk factors: smoking (current versus never or former), diabetes (yes, no), hypertension (yes, no), systolic blood pressure (continuous), baseline CVD (stroke in MI models, MI in stroke models), HDL and LDL cholesterol (continuous), body mass index (continuous) and pre-baseline cancer (yes, no). A final model (Model C) added novel CVD risk factors to Model B, CRP (natural log transformed) and CKD (yes/no).

In a final analysis to assess for mediation, we evaluated the association of factor VIIc levels and F7 SNPs for stroke together in the same Cox proportional hazard models using the covariates from Model B above.

## Results

Through June 2005 (up to 15 years of follow-up), the 5,759 participants without baseline stroke from the original and minority cohorts experienced 815 incident strokes: 748 were ischemic and 106 were hemorrhagic (overlap due to incident strokes of different type). Through the follow-up period, the 4,382 CHS white participants without baseline stroke experienced 713 incident strokes: 586 ischemic and 79 hemorrhagic. Through June 2005 there were 736 incident MI in the entire cohort.

Of the 736 SNPs examined, 6 were associated with stroke with nominal p-values <0.01 (Table 1). One SNP (rs6046) was located within the coding region and another (rs3093261) within the 3′ flanking region of F7 (between F7 and factor X gene) on chromosome 13. Two SNPs (rs4918841 and rs3781387) were located within introns of HABP2 on chromosome 10. Two SNPs were located near inflammation-related genes: rs3138055 within the 3′ flanking region of I-kappa-B-alpha (NFKBIA) on chromosome 14 and rs4648004 within intron of NF-kappa-B p50 subunit (NFKB1) on chromosomes 4. Supplemental tables S2 and S3 present the findings from the SNP look-up in the CHARGE consortium. Within the CHARGE consortium, none of the 6 SNPs we identified replicated for all-cause stroke or ischemic stroke, however rs3138055 (or any proxy for this SNP) was not assessed in CHARGE, and rs4648004 was measured through a proxy SNP only (rs3960787, p-value 0.02 for ischemic stroke).

Table 2 presents age- and sex-adjusted hazard ratios for associations of the 6 SNPs with ischemic and hemorrhagic stroke among white participants. Using a corrected p- value of 0.004 ($\alpha = 0.05/12$; 6 SNPs X 2 outcomes), 3 of the 6 SNPs were associated with ischemic stroke; both of the F7 SNPs (rs6046; HR 0.73, p = 0.002; rs3093261; HR 1.21, p = 0.002)

and one of the HABP2 SNPs (rs4918841; HR 0.75, p = 0.0002). None of the 6 SNPs were associated with hemorrhagic stroke or MI (in an exploratory analysis; all p >0.004, Table 2).

Table 3 shows that the two F7 SNPs (rs6046 and rs3093261) were associated with factor VIIc levels in CHS whites; each copy of the minor allele of rs6046 was associated with 18.5 percentage point lower factor VII level ($p = 2.38 \times 10^{-83}$) and each copy of the minor allele of rs3093261 was associated with a 2.99 percentage point higher factor VIIc level ($p = 3.93 \times 10^{-6}$). HABP2 SNPs were not associated with factor VIIc levels. The two F7 SNPs (rs6046 and rs3093261) were associated with factor VIIc quintiles (both p <0.001).

Given the association of the F7 SNPs with stroke, we assessed the association of factor VIIc levels with cardiovascular disease risk factors (Table 4). Factor VIIc was lower with greater age, in males versus females, and in blacks than whites. Factor VIIc was higher with hypertension, higher HDL and LDL cholesterol, greater BMI, presence of CKD, and higher CRP. Factor VIIc was not associated with smoking status or diabetes.

Table 5 presents associations between factor VIIc quintiles and ischemic stroke, hemorrhagic stroke and MI in the three models described in the methods section. Quintile 1 served as the reference group. The top 2 quintiles of factor VIIc were associated with ischemic stroke in all models, with a 36% increased risk for both quintiles 4 and 5 versus quintile 1 in Model C. There was no significant association in any model between factor VIIc quintiles and hemorrhagic stroke or MI. Results were similar coding estimated glomerular filtration rate as a continuous variable.

In the traditional cardiovascular disease risk factor model (Model B), we assessed the mediation by the F7 SNPs (rs6046, rs3093261) on the association between factor VIIc quintiles and our primary outcome, stroke, by adding both factor VIIc quintiles and the SNPs in the same proportional hazard model (Table 6). The $4^{th}$ (HR 1.46; 95% CI 1.08, 1.98) and $5^{th}$ (HR 1.58; 95% CI 1.15, 2.16) quintile of factor VIIc were associated with stroke when no SNPs were added into the model. When rs6046 (the SNP with the largest association with factor VIIc level) was added to the model, the HR for stroke for the $4^{th}$ (HR 1.28, 95% CI 0.93, 1.76) and $5^{th}$ (1.34; 95% CI 0.96, 1.87) quintiles of factor VIIc were lower. With singular adjustment for rs3093261 alone, only minimal change in the factor VIIc HRs were observed. When both SNPs were added to the model, factor VIIc quintiles were no longer associated with stroke risk (Table 6). The association of rs6046 (HR 0.71; 95% CI 0.58, 0.87) and rs3093261 (HR 1.17; 95% CI 1.04, 1.32) changed minimally when factor VIIc quintiles were added into the model (Model B); rs6046 (HR 0.75; 95% CI 0.60, 0.93) and rs3093261 (HR 1.15; 95% CI 1.02, 1.30).

## Discussion

Of 736 SNPs from 130 candidate inflammation and hemostasis genes, 6 were associated with stroke among whites in this elderly cohort: two SNPs were located within or near F7 (rs6046, rs3093261); two were within introns of HABP2 (rs4918841, rs3781387); and two were near inflammation-related genes (rs3138055, NFKBIA and rs4648004, NFKB1). The two F7 SNPs were associated with factor VIIc levels (rs6046 and rs3093261). Factor VIIc levels were also significantly associated with ischemic stroke, but not MI or hemorrhagic stroke. Addition of both F7 SNPs into a model for total stroke attenuated the association of factor VIIc with stroke, but factor VIIc did not attenuate the association of the two F7 SNPs with stroke.

These data extend prior studies on the genetics of stroke risk. In a recent genome-wide association study from the Cohorts for Heart and Aging Research in Genetic Epidemiology (CHARGE) consortium of 2,194,468 SNPs, only 2 SNPs on chromosome 12 near the NINJ2

gene were associated with stroke with genome-wide significance [16]. NINJ2 encodes an adhesion molecule expressed in glia. The CHARGE meta-analysis included 19,602 Caucasian individuals and 1,544 incident stroke cases (including data from CHS). Though CHS was part of the CHARGE consortium, their dataset used a shorter follow-up period than in this analysis and only included 459 incident stroke events from CHS. None of the associations in the current study of stroke were close to genome-wide significant; the lowest p-value was 0.02 for rs3960787 (a proxy for rs4648004, NFKB1), with all other p-values greater than 0.10. Further, per our protocol, we did not assess any SNPs from NINJ2 as we concentrated on 736 SNPs from 130 inflammation and hemostasis-related genes. Not surprisingly, the genome-wide association study did not detect any of the associations we observed, as our lowest p-value was $7\times10^{-4}$ for rs4918851, which was well above the threshold selected for the genome-wide statistical significance in CHARGE ($p < 5\times10^{-8}$).

Other studies of the SNPs and genes we identified here have reported mixed results. The F7 gene encodes coagulation factor VII, an essential component of hemostasis at sites of vessel injury. The rs6046 SNP has long been known to affect factor VII levels and has been studied in relation to risk of stroke, MI and venous thrombosis in case-control studies[18–21]. While the consequences of rs6046 on factor VIIc levels are well documented, the reason for the lower factor VIIc levels is unclear. The minor haplotype of rs6046 results in a guanine to adenine substitution in codon 353 of the F7 gene product resulting in glutamine to arginine substitution [22]. Whether this SNP in-of-itself reduces factor VIIc levels or is a marker for a hypofunctioning phenotype requires further investigation [23]. In the Framingham Heart study, using a stepwise selection algorithm to select SNPs associated with factor VII levels, rs6046 fell out of the model with inclusion of rs1755685 (within the 5′ flanking/promoter region of F7). These SNPs are in linkage disequilibrium ($r^2 = 0.79$), and the stepwise selection algorithm makes no assumptions on function, only which variable produces a better model fit for analysis [19]. In a recent meta-analysis of 1,537 cases of ischemic stroke and 3,133 controls, the rs6046 SNP showed no overall association with stroke (OR 0.9; 95% CI 0.4, 1.9), though some of the individual studies did show associations [20]. In terms of MI, in the Framingham Heart Study with only 155 CVD events (the majority MI), rs6046 was associated with factor VII levels, but no F7 SNPs were associated with the combined CVD endpoint[19]. In another case-control study, rs6046 was associated with MI in patients with established coronary artery disease[18]. In a recent analysis of the Women's Genome Health Study, rs6046 was associated with a lower risk of idiopathic venous thrombosis in women[21]. Differences among studies may reflect heterogeneity of the phenotypes of stroke, MI and venous thrombosis. In our elderly population, we hypothesize that stroke may represent a less heterogeneous phenotype than in younger populations. SNP rs3093261 is in the 3′ flanking region of F7; between the F7 gene and factor X genes, with an $r^2 = 0.137$ between rs6046 and rs3093261[24]. When both rs6046 and rs3093261 were in the same model for stroke risk, rs3093261 was not significantly associated with stroke ($p = 0.13$), and so this association may represent a weak linkage with rs6046 or another SNP. We are not aware of any other data for rs3093261, factor VII levels, and CVD risk.

The association of factor VII with stroke has been controversial. Theoretically, factor VII is a strong hemostasis candidate protein for vascular disease risk[25]. In primary hemostasis, factor VII plays a key role in vascular wall injury and exogenous administration of activated factor VII has thrombotic complications including stroke, MI, and venous thrombosis[26–28]. Epidemiologic studies, including in the CHS have shown mixed results on the association between factor VIIc and cardiovascular disease risk[2,29,30]. The first study to suggest an association was the Northwick Park Study cohort which showed an association between fatal cardiovascular disease and factor VII levels[30]. Prior analyses in CHS have had fewer stroke events or combined stroke and transient ischemic attack as an endpoint[2,31]. Factor VII levels in the Atherosclerosis Risk in Communities cohort (a

younger cohort) were not associated with stroke risk with 268 ischemic strokes[29]. Our analysis revealed a modest association between factor VII and stroke, and many other studies would be underpowered based on the association we observed and often used a combined CVD endpoint (which may attenuate associations due to a lack of association of factor VIIc with MI seen here). While arterial diseases in diverse vascular beds share many common risk factors, emerging evidence suggests each has a unique risk factor profile. Further, the quality of the literature examining novel risk factors and biomarkers for stroke is sparse compared to the equivalent literature for myocardial infarction [32]. Our current analysis represents the largest prospective study we are aware of relating factor VII levels and cardiovascular risk in an elderly population. Further basic science studies are needed to determine the reasons for differing risk factors in diverse vascular beds.

HABP2 encodes a heterodimer serine protease which has a variety of effects on coagulation and inflammation genes including cleaving fibrinogen and activating pro-urokinase and factor VII[33]. The HABP2 gene has been a target of study for CVD risk with the Marburg I and II polymorphisms being associated with stroke, MI, and venous thrombosis[33–35]. Marburg I and II SNPs were not evaluated here as their gene frequencies were less than 0.05, but we found two SNPs within introns of HABP2 were associated with stroke risk. Neither of these SNPs has specifically been linked with stroke risk previously and are not in linkage disequilibrium [24]. Several hypotheses link variants of HABP2 with vascular diseases; one espouses a decoupling of activation of the fibrinolytic system (pro-urokinase to urokinase) with activation of factor VII and fibrinogen, while another postulates increased smooth muscle cell proliferation [33]. Neither of the HABP2 SNPs studied here were associated with factor VII levels after adjustment for multiple testing. The protein product for HABP2 can also be measured in blood but was unavailable for this study, so we could not assess the correlation between HABP2 SNPs, factor VII-activating protease levels, and stroke risk[36].

We observed nominal associations between stroke and 2 SNPs near inflammation- related genes; rs4648004 (NFKB1, p50 subunit) and rs3138055 (NFKBIA). NF-κB is a complex multimeric transcription factor regulating hundreds of inflammation and cellular apoptotic proteins, which is upregulated in brain ischemia models[37]. NFKBIA transcribes a component of the Inhibitor of KappaB Kinase[37]. Animal models suggest these proteins play a key role in cerebral ischemia and specific inhibitors have been investigated in stroke models with mixed results[37].

Despite having a well-characterized cohort with a large number of validated strokes, a major limitation of our study was the small number of non-whites; our results may not generalize to other races or ethnicities. Further, we were limited by the need to balance between the number of SNPs and genes assessed and the potential for false-positive results. For SNP discovery we used a $p < 0.01$, greater than the most conservative method (Bonferroni correction) which would require a $p < 6.7 \times 10^{-5}$. The application of a Bonferroni threshold, which does not factor in the correlation between the test statistics for the SNPs that are in linkage disequilibrium, is strictly agnostic to biological information about the genes being studied. We note that three of the genes (F7, HABP2 and NFKB1) that contain, or are near SNPs, that were nominally associated with stroke have strong circumstantial evidence relating them to cardiovascular disease. We also note that there were strong internal consistencies between the genotypes of the F7 SNPs and the phenotypes of the F7 gene product and stroke; rs6046 was associated with lower factor VIIc levels and a lower risk of ischemic stroke, and rs3093261 was associated with higher factor VIIc levels and a higher risk of ischemic stroke. When both F7 SNPs were added into a model with factor VIIc levels, the association of factor VIIc with stroke was partly mediated by the genotypes. The

genotypes likely better reflect factor VIIc levels over time than the one-time determination of factor VIIc levels.

In summary, 6 SNPs within 4 hemostasis and inflammation-related genes (F7, HABP2, NFKBIA, NFKB1) were nominally associated with stroke risk. Two F7 SNPs that were associated with stroke were also associated with factor VII levels, which in turn were associated with ischemic stroke risk. The F7 SNPs mediated the association of factor VII levels with stroke. The consistency and the rigorous methods used to acquire these data provide strong supporting evidence that in this elderly population, factor VII may play an etiological role in stroke. Future work needs to confirm these findings and further elucidate the role of inflammation and hemostasis in stroke risk.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Addendum

NAZ drafted the manuscript. All coauthors helped design and interpret the analyses and provided critical revision of the manuscript for scientific content. APR secured grant funding and APR and LL performed the analyses.

**Table 1**

Coagulation and Inflammation Gene SNPs Associated with Risk of Incident Stroke in CHS Whites

| SNP # | Gene Symbol | Gene Name | Chromosome | Alleles | location | Nominal p-value* |
|---|---|---|---|---|---|---|
| rs4918851 | HABP2 | Factor VII activating protease | 10 | A/C | intron | 0.0007 |
| rs3138055 | NFKBIA | I-kappa-B-alpha | 14 | C/T | 3′ flanking | 0.0047 |
| rs3781387 | HABP2 | Factor VII activating protease | 10 | G/A | intron | 0.0051 |
| rs3093261 | F7 | Factor VII | 13 | T/C | 3′ flanking | 0.0051 |
| rs6046 | F7 | Factor VII | 13 | A/G | coding | 0.0054 |
| rs4648004 | NFKB1 | NF-kappa-B p50 subunit | 4 | G/A | intron | 0.0092 |

*736 SNPs evaluated, significance threshold set a p<0.01 in age- and sex-adjusted Cox proportional hazard models.

**Table 2**

Age- and Sex-adjusted Hazard Ratio of SNPs for Incident Stroke among CHS Whites by Stroke Type

| Gene Symbol | SNP # | Ischemic Stroke (n=586) | | Hemorrhagic Stroke (n=79) | | Myocardial Infarction (n= 669) | |
|---|---|---|---|---|---|---|---|
| | | HR** (95% CI) | p-value* | HR** (95% CI) | p-value* | HR** (95% CI) | p-value* |
| HABP2 | rs4918851 | 0.75 (0.64, 0.87) | 0.0002 | 1.08 (0.73, 1.60) | 0.69 | 1.01 (0.88, 1.15) | 0.94 |
| F7 | rs6046 | 0.73 (0.60, 0.89) | 0.002 | 0.74 (0.44, 1.25) | 0.26 | 1.01 (0.85, 1.19) | 0.94 |
| F7 | rs3093261 | 1.21 (1.07, 1.36) | 0.002 | 0.88 (0.64, 1.22) | 0.46 | 0.99 (0.88, 1.11) | 0.88 |
| HPBP2 | rs3781387 | 0.77 (0.63, 0.93) | 0.006 | 0.91 (0.55, 1.51) | 0.71 | 1.01 (0.86, 1.19) | 0.91 |
| NFKB1A | rs3138055 | 0.83 (0.72, 0.95) | 0.007 | 0.82 (0.57, 1.18) | 0.29 | 1.00 (0.89, 1.14) | 0.95 |
| NFKB2 | rs4648004 | 1.14 (1.01, 1.28) | 0.03 | 1.40 (1.02, 1.92) | 0.04 | 0.95 (0.85, 1.07) | 0.40 |

* p <0.004 defined as significant (α= 0.05/12)

** Per each additional copy of the minor allele

**Table 3**

Unadjusted Correlation of Stroke-Associated SNPs and Baseline Factor VIIc Level Among CHS Whites (N=4,286)

| Gene | SNP | β | Std error | $R^2$ | p-value* |
|---|---|---|---|---|---|
| F7 | rs6046 | −18.5% | <0.01 | 0.084 | $2.38 \times 10^{-83}$ |
| F7 | rs3093261 | 2.99% | 0.64 | 0.005 | $3.93 \times 10^{-06}$ |
| HABP2 | rs3781387 | 2.06% | 0.95 | 0.001 | 0.031 |
| HABP2 | rs4918851 | 0.912% | 0.77 | 0.0003 | 0.24 |

*p < 0.01 defined as significant (α= 0.05/4)

**Table 4**

Baseline Associations of Factor VIIc Quintiles with Cardiovascular Risk Factors and Stroke-Associated SNPs

| | Factor VIIc Quintiles | | | | | |
|---|---|---|---|---|---|---|
| | **Q1** | **Q2** | **Q3** | **Q4** | **Q5** | |
| **N** | **1,148** | **1,121** | **1,142** | **1,170** | **1,178** | **p-value (trend)** |
| **Factor VII Range, %** | **41 – 99** | **100 – 113** | **114 – 126** | **127 – 144** | **145 – 346** | |
| **Number of Strokes** | **136** | **173** | **145** | **172** | **189** | |
| **Person-years follow-up** | **10.5** | **11.2** | **11.6** | **11.5** | **11.7** | |
| Age (years ±SD) | 73.3 ±5.8 | 73.1±5.6 | 72.9 ±5.6 | 72.8 ±5.5 | 72.0 ±5.2 | <0.001 |
| Male (n (%)) | 759 (66) | 622 (55) | 475 (42) | 362 (31) | 225 (19) | <0.001 |
| Black (n, %) | 283 (25) | 213 (19) | 143 (13) | 137 (12) | 99 (8) | <0.001 |
| Current smoking (n, %) | 136 (12) | 145 (13) | 135 (12) | 147 (13) | 125 (11) | 0.33 |
| Diabetes (n, %) | 347 (30) | 305 (27) | 327 (29) | 350 (30) | 375 (32) | 0.19 |
| Hypertension (n, %) | 475 (41) | 474 (42) | 463 (41) | 551 (47) | 578 (49) | <0.001 |
| Systolic BP (mmHg ±SD) | 135 ±22 | 136 ±22 | 136 ±21 | 137 ±23 | 139 ±22 | <0.001 |
| HDL cholesterol (mg/dL ±SD) | 51 ±14 | 53 ±15 | 54 ±15 | 56 ±16 | 57 ±18 | <0.001 |
| LDL cholesterol (mg/dL ±SD) | 118 ±33 | 124 ±31 | 132 ±34 | 136 ±36 | 140 ±39 | <0.001 |
| BMI ($kg/m^2$ ±SD) | 26.1 ±4.5 | 26.4 ±4.7 | 26.5 ±4.7 | 26.8 ±4.7 | 27.5 ±4.8 | <0.001 |
| Chronic Kidney Disease* (n (%)) | 63 (5.7) | 55 (5.1) | 58 (5.3) | 68 (6.0) | 99 (8.7) | <0.001 |
| CRP (mg/L ±SD) | 3.6 ±6.9 | 3.1 ±6.6 | 3.5 ±6.4 | 3.5 ±5.0 | 4.3 ±5.4 | 0.001 |
| rs3093261 (minor allele frequency) | 0.29 | 0.35 | 0.38 | 0.38 | 0.41 | <0.001 |
| rs6046 (minor allele frequency) | 0.25 | 0.15 | 0.10 | 0.08 | 0.05 | <0.001 |

*Estimated Glomerular Filtration rate <60ml/min/1.73$m^2$

**Table 5**

Adjusted Hazard Ratio for Factor VIIc Quintiles and Cardiovascular Outcomes

| Outcome | Model* | Factor VIIc Quintile | | | | | p for trend |
|---|---|---|---|---|---|---|---|
| | | Q1 | Q2 | Q3 | Q4 | Q5 | |
| Ischemic | A | ref | 1.32 (1.02, 1.71) | 1.11 (0.84, 1.45) | 1.40 (1.07, 1.81) | 1.60 (1.22, 2.08) | 0.001 |
| Stroke | B | ref | 1.31 (1.01,1.69) | 1.05 (0.80, 1.38) | 1.32 (1.01, 1.73) | 1.46 (1.11, 1.92) | 0.016 |
| (n=748) | C | ref | 1.29 (1.00, 1.66) | 1.06 (0.81, 1.38) | 1.36 (1.04, 1.77) | 1.36 (1.03, 1.79) | 0.03 |
| Hemorrhagic | A | ref | 1.43 (0.77, 2.67) | 0.81 (0.40, 1.65) | 0.81 (0.40, 1.67) | 1.03 (0.51, 2.08) | 0.51 |
| Stroke | B | ref | 1.50 (0.81, 2.79) | 0.80 (0.39, 1.66) | 0.91 (0.44, 1.90) | 1.06 (0.51, 2.22) | 0.61 |
| (n=106) | C | ref | 1.38 (0.74, 2.59) | 0.65 (0.30, 1.40) | 0.66 (0.30, 1.45) | 0.72 (0.32, 1.59) | 0.11 |
| Myocardial | A | ref | 0.95 (0.75, 1.18) | 1.01 (0.80, 1.27) | 1.07 (0.85, 1.35) | 1.12 (0.88, 1.42) | 0.22 |
| Infarction | B | ref | 0.89 (0.71, 1.11) | 0.95 (0.75, 1.20) | 0.98 (0.77, 1.25) | 0.98 (0.78, 1.27) | 0.81 |
| (n = 736) | C | ref | 0.86 (0.68, 1.08) | 0.94 (0.75, 1.19) | 0.95 (0.74, 1.22) | 0.96 (0.74, 1.25) | 0.95 |

*Model A adjusted for age, sex, and race

Model B adjusted for age, sex, race, ever smoker, diabetes, hypertension, baseline CVD, pre-baseline cancer, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, and BMI

Model C adjusted for age, sex, race, ever smoker, diabetes, hypertension, baseline CVD, pre-baseline cancer, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, BMI, CRP, and CKD

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**Table 6**

Impact of Factor VII Gene SNPs on Association of Factor VIIc with Stroke in Whites

| Model | HR (95% CI) for Stroke by Factor VIIc Quintile | | | | |
|---|---|---|---|---|---|
| | Q1 | Q2 | Q3 | Q4 | Q5 |
| Model B alone | Reference | 1.42 (1.06, 1.91) | 1.12 (0.82, 1.53) | 1.46 (1.08, 1.98) | 1.58 (1.15, 2.16) |
| Model B + rs6046 | Reference | 1.31 (0.97, 1.77) | 1.00 (0.72, 1.37) | 1.28 (0.93, 1.76) | 1.34 (0.96, 1.87) |
| Model B + rs3093261 | Reference | 1.39 (1.03, 1.87) | 1.09 (0.79, 1.48) | 1.41 (1.04, 1.92) | 1.52 (1.11, 2.08) |
| Model B + rs6046 + rs3093261 | Reference | 1.23 (0.97, 1.56) | 0.92 (0.71, 1.18) | 1.15 (0.89, 1.48) | 1.21 (0.92, 1.57) |

*Adjusted for age, sex, race, ever smoker, diabetes, hypertension, baseline CVD, pre-baseline cancer, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, and BMI