Original Article

DOI 10.3349/ymj.2011.52.1.20
pISSN: 0513-5796, eISSN: 1976-2437

Yonsei Med J 52(1):20-25, 2011

YMJ

Association of the *Adiponectin* Gene Variations with Risk of Ischemic Stroke in a Korean Population

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Received: April 7, 2010
Revised: May 26, 2010
Accepted: June 1, 2010
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· The authors have no financial conflicts of interest.

Purpose: Stroke is the second leading cause of death and a major cause of morbidity and mortality worldwide. Evidence of variations in *adiponectin* (*AdipoQ*) genes that are associated with ischemic stroke has not been consistent, and it is unclear whether the same loci contribute to these associations in the Korean population. Using a Korean population, we tested ischemic stroke-associated *AdipoQ* markers. **Materials and Methods:** In a preliminary genome-wide association study using 320 250 k Affymetrix NSP chips, *AdipoQ* was found to be associated with ischemic stroke in Koreans. To study of *AdipoQ*, a further 673 ischemic stroke patients and 267 unrelated individuals without a history of stroke or transient ischemic attack were examined in a case-control study. **Results:** Six polymorphisms (rs182052G > A, rs16861205G > A, rs822391T > C, rs822396A > G, rs12495941G > T and rs3774261A > G) that had a minor allele frequency of over 1% were strongly associated with stroke ($p < 0.05$). Two of these, rs822391T > C and rs822396A > G showed this association on both dominant and additive logistic regression analysis after adjusting for age and sex. The haplotypes ht 1 (AGGCGG and AAGTAG) were significantly associated with susceptibility to stroke. **Conclusion:** Our findings show that polymorphisms in *AdipoQ* are associated with risk for ischemic stroke in the Korean population. This study lends further support to the putative role of *AdipoQ* in stroke.

Key Words: *AdipoQ*, ischemic, stroke, Koreans

INTRODUCTION

Stroke is the second leading cause of death worldwide and a major cause of morbidity and mortality. According to the Korea National Statistical Office, stroke was the second most common cause of death in 2007 (http://www.nso.go.kr).

The clinical phenotype of stroke is broadly divided into ischemic and hemorrhagic stroke. Ischemic stroke constitutes 80% to 90% of all cases.[1-3] Ischemic stroke is divided into 2 principal types: thrombotic and embolic. Thrombotic stroke can also be subdivided into large-vessel occlusion and small-vessel occlu-

sion, correlating with the location of the blockage within the brain. Therefore, stroke does not appear to be defined as a single disease or one distribution.[4] The proportion of small-vessel occlusion is relatively high in Korea as compared to Western countries.[5] The identification of a reliable genetic risk factor for stroke is important.

Adiponectin is also called *GBP-28*, *apM1*, *AdipoQ*, and *Acrp30*.[6] It was identified as an adipocytokine, a 244-amino-acid protein, the product of the *AdipoQ* gene, which is highly expressed in adipose cells.[7] *AdipoQ*, a novel adipose tissue-specific protein, is a structural homolog of collagen VIII and X, complement factor C1q, and tumor necrosis factor-α (TNFα).[7-10] *AdipoQ* regulates the homeostasis of glucose, energy storage, and fatty acid metabolism[11,12] and acts as an anti-inflammatory[13] and antiatherogenic plasma protein.[14,15]

AdipoQ is associated with obesity, metabolic syndrome, type 2 diabetes mellitus, hypertension, and coronary artery diseases. Furthermore, low levels of plasma adiponectin have been linked to ischemic cerebrovascular disease (CVD).[15-23] In comparing type 2 diabetes patients to controls, it can be seen that *AdipoQ* mRNA levels are reduced in adipose tissue, although they are normal levels in circulating adiponectin.

The *adiponectin* (*AdipoQ*) gene is located on chromosome 3q27.[24-26] *AdipoQ* gene variations are associated with changes in plasma adiponectin concentration. Variations in the *AdipoQ* gene also correlate with ischemic stroke in some populations. In American,[27] Japanese,[28] Greece[29] and European[30] populations, *AdipoQ* is associated with ischemic stroke. The aim of the present study was to investigate this association in a Korean population.

We used 320 250 k Affymetrix NSP chips (Affymetrix Inc., Santa Clara, CA, USA) in a stroke case and control association study. In this genome-wide association chip experiment, many single nucleotide polymorphisms (SNPs) in several genes, including *AdipoQ*, were associated with ischemic stroke. We queried the GeneCards, Online Mendelian Inheritance in Man (OMIM), and PubMed databases to identify stroke-related genes and compared them with the SNPs from the preliminary experiments.

To test whether *AdipoQ* was a candidate gene that was linked to the Korean population, we obtained 10 tag SNPs of the *AdipoQ* gene. Then, we performed an Illumina Golden Gate experiment using samples from 940 Koreans to determine any association with the markers. In this study, we identify the markers that are associated with the *AdipoQ* gene in the Korean population.

MATERIALS AND METHODS

Subjects

A preliminary experiment examined 160 normal healthy subjects and 160 stroke patients who were recruited from the Korean Institute of Oriental Medicine (KIOM). The follow-up experiment examined 673 ischemic stroke cases and 267 healthy controls recruited from KIOM. Informed consent was obtained from all participants through KIOM, and our research was approved by the research ethical committee of KIOM.

Candidate gene and marker selection

In the preliminary experiment with 320 Affymetrix 250 k NSP chips, SNPs in genes that were associated with the combined phenotype of ischemic stroke were identified ($p < 0.01$), and we selected several SNPs within the 100-kb upstream and downstream regions (using the Affymetrix annotation file; Mapping205K_Nsp.na22.annot.csv). We searched the GeneCards, OMIM, and PubMed databases and found 768 stroke-related genes compared with the SNPs in the preliminary experiment.

In a follow-up association analysis (the Illumina Golden Gate experiment), we selected 10 tag SNPs in the *AdipoQ* gene, which was one of the candidate genes related to ischemic stroke. The 10 tag SNPs were selected if they had an efficiency of more than 80% using Haploview and HapMap Japanese data (http://www.hapmap.org).

Statistical analysis

Differences in clinical characteristics between the 2 groups were determined by a Student's t-test for continuous variables [age, body-mass index (BMI)] and a Chi-squared test for categorical variables (sex, hypertension, diabetes mellitus, and hypercholesterolemia). The Chi-squared test was used to measure Hardy-Weinberg equilibrium (HWE) for comparing allele and genotype frequencies between cases and controls. The relative risk for stroke associated with genotype in the case-control analysis was calculated with odds ratios (ORs) [95% confidence interval (CI)] using logistic regression models and corresponding *p* values, while controlling for age and sex as covariates [software: PLINK (v1.03, http://pngu.mgh.harvard.edu/~purcell/plink/)].

Three alternative models for the minor alleles-codomi-

nant, dominant, and recessive-were applied in our analyses. For each OR, we calculated 95% CIs. Association analysis of the haplotypes was performed using Haploview (v 4.1). Logistic regression models were used to estimate odds ratios that assessed the association between *AdipoQ* haplotypes and stroke, adjusting for sex and age [software: PLINK (v1.03)]. A 2-tailed p value < 0.05 was significant.

RESULTS

The distribution of stroke types in our study is shown in Table 1. The proportion of small-vessel occlusion cases was relatively high, and that of cardiac embolism was low, as in a previous study.[5] The principal characteristics of the subjects in the *AdipoQ* gene association study are shown in Table 2. Stroke patients experienced a higher prevalence of hypertension, diabetes mellitus, and hypercholesterolemia than the normal controls. This profile was similar to that of a previous study in Koreans, in which hypertension was present in 60% to 70%, diabetes mellitus in 25% to 30%, and hypercholesterolemia in 15% to 20% of stroke patients.[5] BMI was not significantly different between the two groups. However, these risk factors were not adjusted for because of the large number of similar missing values within each group.

From the selected candidate genes, we identified 10 independent tag SNPs in the *AdipoQ* gene using public databases. The 10 SNPs had a minor allele frequency (MAF) of ≥ 0.05 and a Hardy-Weinberg equilibrium p value of ≥ 0.00001. Six SNPs were significantly related to strokers 182052, rs16861205, rs822391, rs822396, rs12495941, and rs3774261 (Table 3)-as determined by the chi-squared test ($p < 0.05$). The 6 polymorphisms were further examined in a more rigorous evaluation of association by multivariable logistic regression analysis, adjusting for sex and age. Genotypes were assessed as either dominant, recessive, or additive. Two SNPs (rs822391T > C and rs822396A > G) were more highly associated with risk of stroke in the dominant and additive analyses.

The genomic positions of the 10 SNPs in the *AdipoQ* gene are shown in Fig. 1A. We performed haplotype-based conditional logistic regression analysis, adjusting for sex and age (Table 4). The haplotypes AGGCGG and AAGTAG in ht1 showed the most preventive effect in stroke (p = 0.029 and 0.050, respectively).

Table 1. Ischemic Stroke Subtypes

Subtypes	n (%)
Large-vessel	160 (23.77)
Small-vessel	483 (71.77)
Cardioembolic	30 (4.46)

DISCUSSION

There are few studies of the association between the *AdipoQ* gene and stroke. Several studies have linked *AdipoQ* gene variations to the risk of stroke. Five *AdipoQ* genetic variations (rs266729, rs182052, rs822396, rs2241766, and rs1501299) were examined for their association with incident myocardial infarction or ischemic stroke in a Caucasian population;[27] in this study, rs266729 and rs182052, and the haplotype G-A-G (comprising rs266729, rs182052, and rs822396) were correlated with a risk of ischemic stroke, irrespective of diabetes. The rs266729C > G polymorphism of the *AdipoQ* gene has been associated with atherothrombotic cerebral infarction in Japanese individuals with a metabolic syndrome.[28]

AdipoQ gene variations are associated with plasma adiponectin concentrations, and metabolic syndrome is a risk factor for cardiovascular disease and also associated with plasma adiponectin concentrations. Therefore, *AdipoQ* gene

Table 2. Clinical Profiles of the Subjects

Clinical profile	Normal controls	Stroke patients
Number of subjects	267	673
Age (mean ± SD)*	59.9 ± 9.8	67.1 ± 12.8
Sex (male / female)*	110 / 157	366 / 307
BMI, kg/m^2 (mean ± SD)	24.3 ± 3.2	23.6 ± 4.1
Hypertension, %*	2.2	63.9
Diabetes mellitus, %*	1.1	27.9
Hypercholesterolemia, %*	7.1	19.9

BMI, body-mass index.
* p value < 0.05 for the difference between stroke patients and normal controls.

Table 3. Association Analyses of stroke with *AdipoQ* Polymorphism

rs ID	Region		Patient			Normal			Additive		Dominant		Recessive	
			Genotype		Freq.	Het.	Genotype		Freq.	Het.	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)	*p*
rs182052	G>A	Intron	GG	GA	AA		AA	AG	GG							
			168	344	161	0.511	0.500	69	142	56	0.532	0.499	1.13 (0.92 - 1.38)	0.25	1.10 (0.80 - 1.54)	0.53	1.25 (0.89 - 1.77)	0.20
rs16861205	G>A	Intron	GG	GA	AA		GG	GA	AA							
			413	235	25	0.350	0.334	179	75	13	0.281	0.307	0.15 (0.89 - 1.48)	0.27	1.28 (0.95 - 1.72)	0.10	0.75 (0.38 - 1.50)	0.42
rs822391	T>C	Intron	TT	TC	CC		TT	TC	CC							
			559	106	8	0.158	0.165	236	30	1	0.112	0.113	1.56 (1.05 - 2.34)	0.03*	1.55 (1.02 - 2.38)	0.04*	3.2 (0.40 - 25.71)	0.43
rs822396	A>G	Intron	AA	AG	GG		AA	AG	GG							
			558	107	8	0.159	0.166	236	30	1	0.112	0.113	1.58 (1.05 - 2.37)	0.03*	1.57 (1.03 - 2.40)	0.04*	3.2 (0.40 - 25.71)	0.43
rs12495941	G>T	Intron	GG	GT	TT		GG	GT	TT							
			272	306	95	0.3455	0.465	103	131	33	0.491	0.466	0.10 (0.81 - 1.23)	0.99	0.93 (0.69 - 1.24)	0.60	1.17 (0.76 - 1.78)	0.48
rs3774261	A>G	Intron	AA	AG	GG		AA	AG	GG							
			242	317	114	0.471	0.482	102	117	48	0.438	0.480	1.02 (0.83 - 1.26)	0.81	1.10 (0.82 - 1.48)	0.52	0.93 (0.64 - 1.35)	0.70

CI, confidence interval.
*p value < 0.05 for the difference between stroke patients and normal controls.

Fig. 1. Gene map and linkage disequilibrium blocks in the *AdipoQ* gene. (A) Map of *AdipoQ* gene on chromosome 3q27. (B) LD Blocks.

variations may be linked to susceptibility to ischemic stroke.

In our study, we found evidence for the association of *AdipoQ* gene variations/haplotypes with ischemic stroke in the Korean population. We found that rs822391T > C and rs822396A > G were related to an increased risk for ischemic stroke in the dominant and additive analyses. Even though we do not know the exact inheritance mode of *AdipoQ* gene, according to the association results, we may drop the *AdipoQ* recessive inheritance in stroke causation or susceptibility. Furthermore, a haplotype block (comprising rs182052, rs16861205, rs822391, rs822396, rs12495941, and rs3774261) that contained the haplotypes AGGCGG and AAGTAG was associated with ischemic stroke.

We identified associated genes in a genome-wide commercial SNP chip experiment and compared them with candidate genes from public databases in non-Korean populations, such as GeneCards, OMIN, and PubMed. Furthermore, we verified these results by analyzing 10 SNPs in the *AdipoQ* gene in 940 subjects. Similar, significant genetic risk factors for ischemic stroke were also observed in American,[27] Japanese,[28] Greek,[29] and general European[30] populations.

Stroke is the second leading cause of death in Koreans and a major cause of morbidity and mortality around the

Table 4. Haplotype of LD Blocks

Ht 1	rs16861194 (A > G)	rs182052 (A > G)	rs16861205 (G > A)	rs822391 (T > C)	rs822396 (A > G)	rs12495941 (G > T)	Frequency			*p* value	Adjusted	
							Overall	Patients	Controls		OR	*p* value
1	A	G	G	T	A	T	0.364	0.364	0.363	0.957	0.982	0.868
2	A	A	G	T	A	G	0.278	0.266	0.311	0.050*	0.791	0.048*
3	G	A	A	T	A	G	0.204	0.210	0.189	0.304	1.18	0.220
4	A	G	G	C	G	G	0.082	0.091	0.060	0.028*	1.63	0.025*
5	A	G	G	T	A	G	0.051	0.050	0.053	0.818	1.06	0.817
6	G	A	G	T	A	G	0.015	0.014	0.019	0.471	0.569	0.182

Ht 2	rs3774261 (A > G)	rs1063537 (C > T)	Frequency			*p* value	Adjusted	
			Overall	Patients	Controls		OR	*p* value
1	G	C	0.400	0.403	0.394	0.741	1.01	0.901
2	A	C	0.314	0.317	0.308	0.706	1.06	0.644
3	A	T	0.282	0.278	0.293	0.515	0.929	0.533

LD, linkage disequilibrium; OR, odds ratio.
*p value < 0.05 for the difference between stroke patients and normal controls.

world, and the role of inflammation may be important.[27] *AdipoQ* acts as an anti-inflammatory[13] and antiatherogenic plasma protein.[14,15] *AdipoQ* gene variations are associated with obesity, metabolic syndrome, type 2 diabetes mellitus, hypertension, coronary artery disease, and ischemic CVD. The five population studies-in Americans,[27] Japanese,[28] Greece,[29] European[30] and Koreans (our study)-suggest that polymorphisms in *AdipoQ* increase susceptibility to stroke. Therefore, further functional molecular biology and genetic studies are needed to explain the mechanics of this link.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Cerebrovascular Disease Oriental Medicine Project of KIOM (Korean Institute of Oriental Medicine) and in part by a grant (2010-0012810) from the National Research Foundation of Korea.

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