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

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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. 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. The proportion 
of small-vessel occlusion is relatively high in Korea as com-
pared to Western countries. The identification of a reliable 
 genetic risk factor for stroke is important.

Adiponectin is also called GBP-28, apM1, AdipoQ, and 
Acrp30. It was identified as an adipocytokine, a 244-amino-
acid protein, the product of the AdipoQ gene, which is 
highly expressed in adipose cells. 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). AdipoQ regulates the homeostasis of 
glucose, energy storage, and fatty acid metabolism and 
acts as an anti-inflammatory protein.

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). 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. 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, Japanese, Greek and European 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 \textit{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 \textit{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 \textit{AdipoQ} gene using public databases. The 10 SNPs had a minor allele frequency (MAF) of \( \geq 0.05 \) and a Hardy-Weinberg equilibrium \( p \) value of \( \geq 0.00001 \). Six SNPs were significantly related to strokes 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 \textit{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 \( (p = 0.029 \text{ and } 0.050, \text{ respectively}) \).

**DISCUSSION**

There are few studies of the association between the \textit{AdipoQ} gene and stroke. Several studies have linked \textit{AdipoQ} gene variations to the risk of stroke. Five \textit{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;\(^5\) 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 \textit{AdipoQ} gene has been associated with atherothrombotic cerebral infarction in Japanese individuals with a metabolic syndrome.\(^28\)

\textit{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, \textit{AdipoQ} gene

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**Table 1. Ischemic Stroke Subtypes**

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

**Table 2. Clinical Profiles of the Subjects**

| Clinical profile                        | Normal controls | Stroke patients |
|-----------------------------------------|-----------------|-----------------|
| Number of subjects                      | 267             | 673             |
| Age (mean \( \pm \) SD)*               | 59.9 \( \pm \) 9.8 | 67.1 \( \pm \) 12.8 |
| Sex (male / female)*                    | 110 / 157       | 366 / 307       |
| BMI, kg/m\(^2\) (mean \( \pm \) SD)    | 24.3 \( \pm \) 3.2 | 23.6 \( \pm \) 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 | Genotype | Patient | Normal | Additive | Dominant | Recessive |
|-------|--------|----------|---------|--------|----------|----------|-----------|
| G > A | Intron | GG | GA | AA | Genotype | Freq. | Het. | OR (95%CI) | p | OR (95%CI) | p | OR (95%CI) | p |
| rs182052 | 1.13 (0.92 - 1.38) | 0.532 | 0.499 | 0.25 | 1.10 (0.80 - 1.54) | 0.53 | 1.25 (0.89 - 1.77) | 0.20 |
| rs16861205 | 1.00 (0.60 - 1.60) | 0.281 | 0.307 | 0.27 | 1.25 (0.95 - 1.62) | 0.10 | 0.75 (0.38 - 1.50) | 0.42 |
| rs22391 | T > C | Intronic | TT | TC | CC | Genotype | Freq. | Het. | OR (95%CI) | p | OR (95%CI) | p | OR (95%CI) | p |
| rs22396 | 1.58 (1.05 - 2.37) | 0.112 | 0.113 | 0.03* | 1.57 (1.03 - 2.40) | 0.04* | 3.2 (0.40 - 25.71) | 0.43 |
| rs12495941 | 1.00 (0.80 - 1.23) | 0.112 | 0.113 | 0.03* | 1.57 (1.03 - 2.40) | 0.04* | 3.2 (0.40 - 25.71) | 0.43 |

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.**

**Map of AdipoQ gene on chromosome 2q23 (BID Blocks).**

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

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**Table 4. Haplotype of LD Blocks**

| Ht 1  | rs1686194 (A > G) | rs182052 (A > G) | rs16861205 (G > A) | rs822391 (T > C) | rs822396 (A > G) | rs12495941 (G > T) | Frequency | p value | Adjusted OR | p value |
|-------|------------------|------------------|--------------------|-----------------|-----------------|-------------------|-----------|---------|-------------|---------|
|       |                  |                  |                    |                 |                 |                   | Overall   | Patients | Controls    |         |
| 1     | A                | G                | G                  | T               | A               | T                 | 0.364     | 0.364   | 0.363       | 0.957   |
| 2     | A                | A                | G                  | T               | A               | G                 | 0.278     | 0.266   | 0.311       | 0.050*  |
| 3     | G                | A                | A                  | T               | A               | G                 | 0.204     | 0.210   | 0.189       | 0.304   |
| 4     | A                | G                | G                  | C               | G               | G                 | 0.082     | 0.091   | 0.060       | 0.028*  |
| 5     | A                | G                | G                  | T               | A               | G                 | 0.051     | 0.050   | 0.053       | 0.818   |
| 6     | G                | A                | G                  | T               | A               | G                 | 0.015     | 0.014   | 0.019       | 0.471   |

| Ht 2  | rs3774261 (A > G) | rs1063537 (C > T) | Frequency | p value | Adjusted OR | p value |
|-------|------------------|-------------------|-----------|---------|-------------|---------|
|       |                  |                   | Overall   | Patients | Controls    |         |
| 1     | G                | C                  | 0.400     | 0.403   | 0.394       | 0.741   |
| 2     | A                | C                  | 0.314     | 0.317   | 0.308       | 0.706   |
| 3     | A                | T                  | 0.282     | 0.278   | 0.293       | 0.515   |

LD, linkage disequilibrium; OR, odds ratio.

*p value < 0.05 for the difference between stroke patients and normal controls.
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