Association between rs1761667 CD36 polymorphism and risk of stroke in Korean patients with type 2 diabetes

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To the Editor: As the prevalence of stroke in the general population is two to three times less than among patients with type 2 diabetes mellitus (T2DM), among whom stroke accounts for 11.0% of all deaths, stroke is clearly a major macrovascular complication that increases the mortality and morbidity of patients with T2DM.[1] The pathophysiology of stroke consists of complicated processes involving oxidative stress, endothelial dysfunction, and pro-inflammatory response. CD36 is a B scavenger receptor that is involved in inflammation, innate immunity, and lipid metabolism and has been implicated in the pathophysiology of atherosclerosis.[2] However, there is no study evaluating the effect of CD36 polymorphism in patients with T2DM according to disease duration. Therefore, the present study evaluates the association between the rs1761667 CD36 single nucleotide polymorphism (SNP) and stroke in Korean patients with T2DM according to disease duration.

A total of 759 patients with T2DM who visited the Chungbuk National University Hospital from January 2015 to December 2018 were included in the present analysis. Diagnoses of diabetes mellitus (DM) were performed according to the American Diabetes Association guidelines. Diabetic complications, both microvascular (such as retinopathy, nephropathy, and peripheral neuropathy) and macrovascular (including cardiovascular disease, stroke, and peripheral artery disease), were evaluated. CD36 (rs1761667) polymorphisms were detected using TaqMan probe-based real-time polymerase chain reaction (PCR). A mixture of PCR primers and fluorescent probes for rs1761667 (ThermoFisher TaqMan™ SNP Genotyping Assay, Cat# 4371353, Waltham, MA, USA) was used for PCR amplification with a genotyping master mix (ThermoFisher, Cat# 4371353) in a 25 μL reaction. Reactions were cycled using the following parameters: preheating at 95°C for 10 min,

followed by 40 cycles at 95°C for 15 s, and 60°C for 60 s. Fluorescence was detected at the end of every cycle, and genotype data were automatically generated with a BioRad CFX96 Real-Time PCR system (BioRad, Hercules, CA, USA). Genotyping results were presented as GG, AG, or AA. The probability of Hardy-Weinberg equilibrium was tested using the Chi-squared test. Data were expressed as the mean ± standard deviation for continuous variables or as percentages for categorical variables. Baseline characteristics were compared with Student's *t* test for continuous variables and the Chi-squared test for categorical parameters. The Chi-squared test was used to evaluate differences in prevalence according to genotype. Multiple logistic regression analyses were performed to evaluate the risk factors associated with strokes such as age, body mass index (BMI), hypertension, hemoglobin A1c (HbA1c), and low-density lipoprotein (LDL)-cholesterol. All statistical analyses were performed using SPSS for Windows (22.0; IBM Corp., Armonk, NY, USA). Significance was set as $P < 0.05$. All participants provided written informed consent. This study was approved by the Institutional Review Board of the Chungbuk National University Hospital (No. 2018-03-034-001). The current study was conducted according to the guidelines administered by the *Declaration of Helsinki*.

The genotypic distribution of CD36 met the Hardy-Weinberg equilibrium ($P = 0.946$). The frequency of the CD36 genotypes in the study subjects was as follows: GG, 51.5% ($n = 391$); AG, 40.4% ($n = 307$); and AA, 8.0% ($n = 61$). There were no significant differences in the baseline characteristics between individuals with GG and AG + AA genotypes. The mean age and BMI were 61.4 ± 12.4 [19.0–91.0] years and 25.8 ± 3.8 [15.8–41.1] kg/m², respectively. The mean duration of T2DM was 10.5 ± 7.9 years, and 55.2% of the study sample was

comprised of men. There were differences in clinical characteristics between men and women. Therefore, the subgroup analysis was performed according to the duration of T2DM and sex. No statistically significant differences were noted in the baseline characteristics in the female group according to the duration of DM.

There was a higher prevalence of stroke among men with the AG + AA genotypes than those with the GG genotype (16.3% *vs.* 10.2%, respectively; $P = 0.049$). A subgroup analysis performed according to the duration of diabetes revealed a significantly higher prevalence of stroke among patients with a duration of T2DM of <10 years and the AG + AA genotypes compared with their counterparts with the GG genotype (18.0% *vs.* 4.8%, $P = 0.001$) [Figure 1]. Multiple logistic regression analysis was performed for male patients with a DM duration of ≤10 years. The A allele was significantly associated with a higher risk of stroke (odds ratio 3.710, 95% confidence interval 1.148–9.709, $P = 0.008$) after having adjusted for risk factors including age, BMI, hypertension, HbA1c, and LDL-cholesterol. The prevalence of stroke did not differ significantly according to genotype among men with DM that had lasted for >10 years (15.7% *vs.* 16.0%,
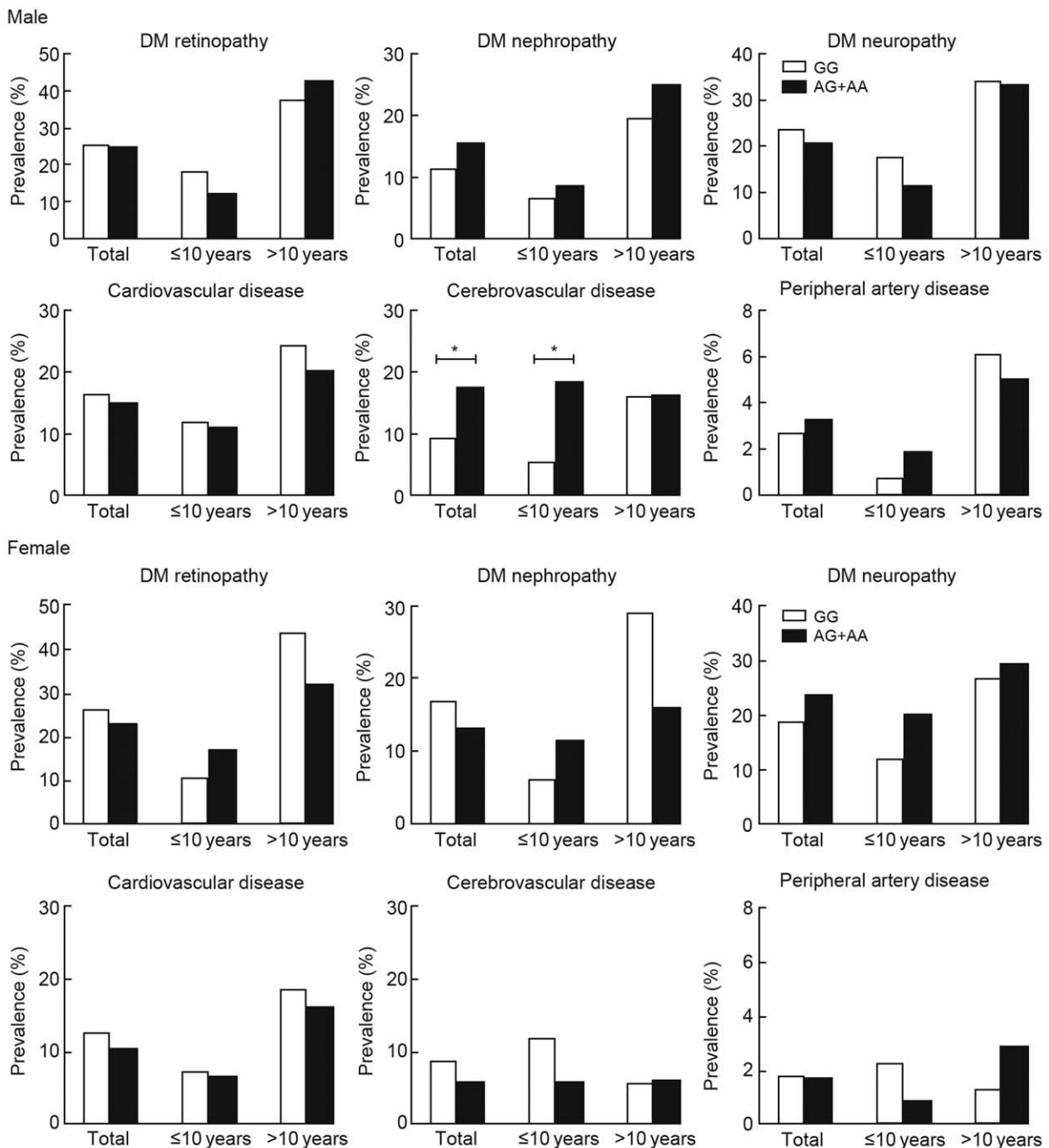

Figure 1: CD36 genotypes and prevalence of diabetic complications according to sex and duration of diabetes. DM: Diabetes mellitus.

$P = 0.946$) or among women (AG + AA *vs.* GG, 8.1% *vs.* 5.9%, $P = 0.435$). No association between cardiovascular disease, peripheral artery disease, or rs1761667 CD36 SNP in T2DM patients of either sex was found. Regarding microvascular complications, there was no significant association with rs1761667 CD36 SNP for either sex.

This study found an association between the CD36 rs1761667 SNP and stroke among Korean men with T2DM; specifically, a significantly higher prevalence of stroke was found among those with the AG + AA genotypes whose histories of diabetes spanned <10 years compared with their counterparts with the GG genotype. Stroke worsens the prognosis of patients with T2DM and occurs at a younger age among patients with T2DM than among non-diabetic patients. CD36 expression is up-regulated in the presence of hyperglycemia. A few studies have considered the association between the CD36 polymorphism and stroke: Ikram *et al*[3] found that the CD36 (rs3211928) polymorphism was significantly associated with stroke in Caucasian populations, Zhang *et al*[4] reported that CD36 (rs1761667 and rs3211928) polymorphisms may indicate a genetic susceptibility to ischemic stroke among Chinese Han populations. The present study expands upon such prior findings by being — to the best of our knowledge — the first to study the relationship between CD36 rs1761667 SNP and stroke in an Asian population with T2DM.

Several studies have showed that CD36 is in acute stroke pathology. A previous study reported that polymorphisms in the *CD36* gene might link to abnormal lipid metabolism through decreasing in oxidized LDL uptake, which in turn diminishes the capacity of lipid clearance and increases the overall risk of atherosclerosis and the occurrence of stroke.[5]

The main limitation of this study is that we did not evaluate direct CD36 expression according to the genotype of CD36 as in other polymorphism studies. Because of the retrospective study design, there was a possibility for recall bias or missing data. This might be contributable to the lower prevalence of stroke in this study. Furthermore, this study was unable to demonstrate the influence of the CD36 rs1761667 SNP on the clinical course of the stroke. The exact mechanism by which the CD36 rs1761667 SNP is associated with CD36 expression and stroke warrants further investigation.

However, the present study had several strengths. First, the number of subjects included was relatively large compared with previous studies. Second, we performed a comprehensive evaluation of the diabetic complications, including both microvascular and macrovascular complications, according to disease duration.

To conclude, the present study demonstrated that the rs1761667 CD36 SNP is associated with stroke in Korean patients with T2DM, especially in male patients with relatively short duration of DM. Studies in large populations that consider various parameters related to cerebrovascular disease are needed to validate our findings.

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Conflicts of interest

None.

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