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Correlation of matrix metalloproteinase-2 single nucleotide polymorphisms with the risk of small vessel disease (SVD)

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ABSTRACT

Background: Maladjustment of matrix metalloproteinases (MMPs) results in cerebral vasculature and blood–brain barrier dysfunction, which is associated with small vessel disease (SVD). This study was to aim at evaluating correlations between matrix metalloproteinase-2 and 9 single nucleotide polymorphisms and the risk of SVD.
Methods: A total of 178 patients with SVD were enrolled into this study via Nanjing Stroke Registry Program (NSRP) from January 2010 to November 2011. SVD patients were further subtyped as isolated lacunar infarction (ILI, absent or with mild leukoaraiosis) and ischemic leukoaraiosis (ILA, with moderate or severe leukoaraiosis) according to the Fazekas scale. 100 age- and gender-matched individuals from outpatient medical examination were recruited as the control group. The genotypes of MMP-2-1306 T/C and MMP-9-1562 C/T were determined by the TaqMan method.
Results: Of 178 SVD patients, 86 and 92 patients were classified as ILI and ILA, respectively. Comparison analysis between SVD patients and controls revealed a significant correlation between SVD and hypertension, as well as a prevalence of hypertension in ILA. Further genotype analysis showed that the frequency of MMP-2-1306 CC genotype was higher in ILA patients than in controls ($P = 0.009$, χ^2 test; $P = 0.027$, the multiple test with Bonferroni correction). Finally, logistic regression analysis with adjustment of age, sex and vascular risk factors showed that the MMP-2-1306 T/C polymorphism was an independent predictor for ILA (OR: 2.605; 95% confidence interval [CI], 1.067–6.364; $P = 0.036$).
Conclusion: Our findings suggest that the MMP-2-1306 T/C polymorphism is a direct risk factor for ILA.

1. Introduction

About 30% ischemic stroke is associated with small-vessel disease (SVD), which mainly involves the perforating arteries with a diameter less than 200 μm [1]. Several studies showed an impairment of blood–brain barrier (BBB) integrality in SVD patients [2,3]. Based on previous reports, SVD is divided into two subtypes, isolated lacunar infarction (ILI, absent or with mild leukoaraiosis) and ischemic leukoaraiosis (ILA, with moderate or severe leukoaraiosis) [4].

There is emerging evidence that matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, play a key role in the impairment of blood–brain barrier [5,6]. It is thought that the expression of MMP-2 and MMP-9 is regulated mainly at the transcription level, and single nucleotide polymorphisms (SNPs) in the promoter region of MMP-2 and MMP-9 gene are shown to be transcriptional regulators. Moreover, MMP-2-1306 T/C and MMP-9-1562 C/T in the promoter regions are functional SNPs, thus potentially affecting the expression of MMP-2 and MMP-9 protein [7,8]. The common C → T transition at −1306 disrupting a Sp1-type promoter site (CCACC box) causes a strikingly lower promoter activity [9]. In contrast, the presence of the −1562 T allele is associated with decreased capacity of a transcription repressor to bind to the promoter region, and as a result leading to an increase in gene expression [10].

However, it remained to be confirmed whether there is a correlation between MMP polymorphisms and the risk of SVD in clinical genetic studies. In this study, we genotyped MMP-2-1306 T/C and MMP-9-1562 C/T in a relatively large cohort of SVD patients and controls, and

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assessed the correlation of matrix metalloproteinase-2 and 9 single nucleotide polymorphisms with the risk of SVD.

2. Subjects and methods

2.1. Subjects

A total of 178 SVD patients were enrolled via Nanjing Stroke Registry Program (NSRP) from January 2010 to November 2011 in this study. 100 age- and gender-matched individuals from outpatient periodical medical examination were recruited as the control group. SVD is defined as the clinical lacunar syndrome with a compatible lesion on magnetic resonance (MR) [11,12]. The enrollment criteria for SVD patients included: (1) Chinese ethnicity; (2) aged 18 years or older; (3) first-ever stroke within 7 days; and (4) written informed consent obtained. The exclusion criteria included: (1) severe (>50%) ipsilateral extracranial artery stenosis; (2) potential cardiac sources of embolism; (3) presence of subcortical infarction larger than 15 mm in diameter or any cortical infarction or asymptomatic lacunar infarction by MRI; (4) a history of neurological disorders or brain injury; (5) contraindications to cranial MRI; and (6) severe renal failure or severe cardiac failure or various types of tumor or rheumatic disease or dementia. The enrollment criteria for the healthy controls included: (1) age- and gender-matched; (2) Chinese ethnicity; (3) no signs or symptoms related to neurological impairment; (4) no history of stroke or transient ischemic attack; and (5) no moderate or severe leukoaraiosis (Fazekas grade ≥ 3). The exclusion criteria were same as those for SVD patients. This study was approved by the Institutional Review Board of Jinling Hospital. Patients or their legally authorized representatives signed informed consent.

2.2. Clinical assessment

Detailed demographic and clinical data were collected. Hypertension was defined as self-reported high blood pressure (≥140/90 mm Hg) or use of antihypertensive medications before stroke, or clinically monitored high blood pressure (≥140/90 mm Hg) the second week after stroke. Diabetes was defined as a fasting glucose ≥ 125 mg/dL or use of antidiabetic medication. Coronary artery disease was defined as a history of myocardial infarction, coronary artery bypass grafting, coronary angioplasty, or stenting or angina pectoris. Hyperlipidemia was defined as a total cholesterol level >5.17 mmol/L or the current use of lipid-lowering treatment or both [13]. Smoking status was defined as either a current smoker or not.

2.3. Magnetic resonance imaging and SVD subtyping

MRI scan was performed using a 3-T system (Magnetom Avanto; Siemens, Erlangen, Germany) with a 12-channel head coil. DWI sequence was examined on MRI to determine a 15 mm cut-off [11,12]. Leukoaraiosis in the enrolled patients was graded with the Fazekas scale, which reflects the severity of white matter lesion associated with cerebral ischemia. This scale provided 3-point grade (0–3) for the respective imaging change of two different areas, periventricular and deep white matter regions. Thus the total grade (0–6) was the sum of the scale for the two areas [14]. SVD patients were further subtyped as ILI (absent or with mild leukoaraiosis, Fazekas grade ≤ 2), and ILA (with moderate or severe leukoaraiosis, Fazekas grade ≥ 3) [4,12]. Subtypes of SVD patients were determined based on MRI scan in combination with clinical symptoms. For the 100 healthy controls, MRI scan was performed to exclude asymptomatic lacunar infarction, which lacked clinical symptoms but displayed some focal lesions with a roughly same density as cerebrospinal fluid on MRI and a maximum diameter less 15 mm [15].

2.3.1. Genotyping

Genomic DNA was isolated from peripheral blood lymphocytes using a phenol–chloroform extraction method. Genotyping was performed to determine MMP-2-1306 T/C and MMP-9-1562 C/T polymorphisms using TaqMan SNP method. One MMP-2-1306 T/C probe (CCAGCACTCTACCTCT) was labeled at the 5′-terminal with VIC (Applied Biosystems' proprietary dye) and at the 3′-terminal with TAMRA; the other probe (CCAGCACTCTACCTCT) was labeled at the 5′-end with 6-carboxyfluorescein (FAM) and at the 3′-end with TAMRA. The MMP-2-1306 T/C primers were as follows: forward, 5′- GCCATTGTCAATGTTCCCTAAAACA-3′; reverse, 5′- TGACTT CTGAGCTGAGACCTGAA-3′. One MMP-9-1562C/T probe (TAGGCG TGCGCCAC) was labeled at the 5′-end with VIC and labeled at the 3′-end with TAMRA; the other probe (ATAGGCATGCGCCAC) was labeled at the 5′-end with FAM and at the 3′-end with TAMRA. The MMP-9 primers for genotyping and sequencing were as follows: forward, 5′- AGCCTCCCGAGTAGCTGGTAT-3′; reverse, 5′- GCCTGGTCAA CGTAGTGAAACC-3′. Real time TaqMan PCR and genotyping analyses were performed in an Mxpro 3000 real-time PCR system (Stratagene, Palo Alto, CA, USA) according to the manufacturer's standard protocol. Data were analyzed with MxPro software, version 4.10 (Stratagene).

2.3.2. Statistics

Statistical analysis was performed by using the Statistical Package for the Social Sciences version 11.0 software (SPSS). Chi square test were used to compare baseline characteristics between SVD patients and controls. Unpaired *t* test or ANOVA (analysis of variance) was used to analyze normally distributed data among groups; if necessary, multiple tests with Bonferroni correction was applied. Mann–Whitney U test was used to compare the difference in leukoaraiosis score between these groups. The binary logistic regression model (adjusted OR) were used to analyze the independent association between SNP polymorphisms and ILA after adjustment of age, sex and vascular risk factors (hypertension, diabetes mellitus, smoking, hyperliptidemia and coronary heart disease). Hardy–Weinberg equilibrium test was chosen to examine genotype distributions. Values of $P < 0.05$ were deemed as statistically significant.

3. Results

3.1. Differences in basic characteristics between SVD patients and controls

Of the 178 SVD patients, 86 and 92 patients were subtyped as ILI and ILA, respectively. The characteristics of SVD patients and controls are shown in Table 1. Hypertension (70.8% vs 21.0%; $P < 0.01$) and hyperlipidemia (38.2% vs 25.0; $P < 0.01$) is more common in SVD patients than in healthy controls. Other factors, such as gender, DM, smoking status, and history of heart disease had a similar distribution between SVD patients and the controls. Further subtype analysis revealed that hypertension is more prominent in ILA patients ($P < 0.01$, compared to the control group), whereas hyperlipidemia is more prevalent in ILI patients ($P < 0.01$, compared to the control). Also, there were some distribution differences in age, hypertension and hyperlipidemia between the two subtypes.

3.2. Genotype distributions

All genotypic distributions were in concordance with Hardy–Weinberg equilibrium in SVD patients (including ILA and ILI subgroups) and controls. Genotype frequencies for MMP-2-1306 T/C and MMP-9-1562 C/T polymorphisms are shown in Table 2. The genotype frequencies of MMP-2-1306 T/C and MMP-9-1562 C/T polymorphisms in SVD patients were similar to those in the controls. However, further subgroup analysis demonstrated that the MMP-2-1306 CC genotype was more common in ILA as compared with controls (82.6% versus 66.0%,