Association of a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 polymorphisms with severity of coronary stenosis in type 2 diabetes mellitus

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INTRODUCTION

Diabetes mellitus (DM) is a major risk factor for coronary artery disease (CAD). It has been known that cardiovascular disease (CVD) is the most important complication and approximately 75% of type 2 diabetic patients die from CVD.[1] This observation can be explained by several mechanisms including concomitant risk factors such as hypertension, dyslipidemia, hyperglycemia, obesity, and metabolic syndrome which are responsible for endothelial dysfunction.[2] Recently, many studies have suggested that proteins or factors relating to endothelial dysfunction were considered to be associated with DM.[3] von Willebrand factor (vWF) is an important biomarker of endothelial dysfunction. It plays...
a crucial role in primary hemostasis and thrombus formation by mediating platelet adhesion and aggregation. Upon released from endothelial cells, ultralarge vWF multimers which are the most thrombogenic are cleaved into the less active forms by a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13). Reduction in ADAMTS13 activity and elevated vWF antigen are associated with thrombotic thrombocytopenic purpura and other thrombotic diseases such as ischemic stroke, acute myocardial infarction, and CAD.

High-circulating vWF concentrations are known to be associated with DM, which mainly caused by endothelial dysfunction. It has been found to be a link to the development of microvascular and macrovascular diseases and a risk marker for death in diabetic patients. On the other hand, there are few studies reporting ADAMTS13 levels in DM and the results were inconsistent. Skeppholm et al. have suggested that ADAMTS13 activity was significantly lower in diabetic patients and a decrease in ADAMTS13 activity was also associated with an increased risk of cardiovascular events and renal disease in type 2 DM patients. In contrast, the recent study has demonstrated that ADAMTS13 activity was elevated in type 1 DM patients with retinopathy. Although vWF is released from endothelial cells, increased circulating vWF may not only due to elevated endothelial secretion or endothelial damage but may also partly be related to a reduction of ADAMTS13 function. Many ADAMTS13 and vWF polymorphisms have been reported to be associated with impaired ADAMTS13 activity as well as increased vWF antigen and activity. Thus, vWF and ADAMTS13 genes may have an important role in genetic susceptibility to the thrombotic development or vascular complication in DM.

So far, there is no study investigating the possible relationship of known ADAMTS13 and vWF polymorphisms with DM and the progression of the plaque in coronary artery of the patients with macrovascular complication. In addition, the association between ADAMTS13 and DM is controversy. Therefore, those associations were explored in this study. Genetic variations of ADAMTS13 and vWF associated with impaired ADAMTS13 activity and increased levels of vWF antigen and activity were selected as the candidate polymorphisms. The polymorphisms were investigated in type 2 DM patients with CAD complication. This genetic association study could possibly provide the new insights into the role of ADAMTS13 and vWF polymorphisms in thrombus formation and vascular complication of DM in Thai population.

MATERIALS AND METHODS

Subjects
A total of 171 participants who attended to the Cardiac Catheterization Unit, Queen Sirikit Heart Center of the Northeast Hospital, Khon Kaen University, for cardiovascular checkup (2008–2010) were recruited in this study. Patients with acute coronary syndrome, left ventricular hypertrophy, heart failure, cardiomyopathy, valvular heart disease, deep vein thrombosis, liver disease, renal disease, and inflammatory disease were excluded. Type 2 DM was diagnosed according to the criteria of the World Health Organization. According to the coronary angiographic results, the DM participants (n = 87) were diagnosed as CAD if the angiographic results of coronary arteries showing ≥50% stenosis (single-vessel disease or multi-vessel disease). The individuals without DM and presenting none or <50% stenosis were classified as control group (n = 84). The severity of coronary stenosis was determined using the numbers of main vessel stenosis ≥50% together with Gensini score, which was calculated from the degree of luminal narrowing and its geographic importance of coronary artery. Patients with Gensini score ≥220 were classified as high score, while those presenting the score <20 were defined as low–medium score. Other clinical variables including age, sex, CAD risk factors, and use of medicines were obtained from medical records. Individuals presenting one or more of the following parameters: total cholesterol (TC) ≥6.2 mmol/L, triglyceride ≥2.3 mmol/L, high-density lipoprotein-cholesterol (HDL-C) <1.0 mmol/L, and low-density lipoprotein-cholesterol (LDL-C) ≥2.3 mmol/L or use of lipid-lowering drugs were diagnosed as dyslipidemia. Individuals whom had blood pressure ≥140/90 mmHg and/or used anti-hypertensive drugs were defined as hypertension. Individuals with body mass index (BMI) ≥25 kg/m² were classified as obesity. Metabolic syndrome was considered if any three in five risk factors were presented: (i) waist circumference >90 cm in male and >80 cm in female, (ii) TG >1.7 mmol/L or on lipid-lowering drug treatment, (iii) HDL-C <1.0 mmol/L in male and <1.3 mmol/L in female, (iv) blood pressure >130/85 mmHg or use of anti-hypertensive medications, and (v) fasting blood glucose (FBG) >5.6 mmol/L or use of glucose-lowering drugs. The study was approved by the Khon Kaen University Ethics Committee for Human Research (HE510414). Signed consent form was obtained from each participant after explanation of the study.

Laboratory measurements
Blood samples were obtained after 12 h of fasting before undergoing coronary angiography. Four milliliters of blood samples was collected in 3.2% tri-sodium citrate and immediately placed on ice. Platelet poor plasma was further separated within 30 min by centrifugation at 1500 g at 4°C for 15 min. All plasma samples were stored in small aliquots at −80°C until use. vWF antigen and activity were measured by an in-house sandwich enzyme-linked immunosorbent assay and collagen-binding assay (CBA), respectively.
while ADAMTS13 activity was determined by residual CBA as previously described. The other blood chemical analysis serum high-sensitivity C-reactive protein (hs-CRP) concentration was examined by immunonephelometry using BN ProSpec® System (Siemens Healthcare Diagnostics Products GmbH, Germany).

**Polymorphism analysis**

Genotyping of ADAMTS13 and vWF polymorphisms including ADAMTS13 Q448E (rs2301612), rs2073932, rs652600, rs4962153, and vWF V1565 L (rs1800385) was performed by polymerase chain reaction-restriction fragment length polymorphism technique as described in the previous study.

**Statistical analysis**

Statistical analysis was performed using the SPSS version 17.0 (SPSS Inc., IL, USA). Kolmogorov–Smirnov was applied to test whether data were normally distributed. The continuous values were expressed as mean ± standard deviation (SD). If the values were not normal distribution, they were transformed to logarithm before comparison and presented as geometric mean ± SD. Independent sample t-test and one-way analysis of variance were used to compare continuous values between two or more than two groups, respectively. The χ²-test was performed for comparison of categorical variables such as CAD risk factors and genotype frequencies. The Hardy–Weinberg equilibrium was evaluated by χ²-test, and all genotype distributions observed in this study were compatible with Hardy–Weinberg equilibrium. Jonckheere–Terpstra test was used to analyze the probability of trend for a continuous variable across an ordinal group. Multivariate logistic regression analysis was performed to evaluate the association of genotypes with DM and severity of coronary stenosis. Statistical significance was considered when P < 0.05.

**RESULTS**

Individuals in DM group presented higher proportions of hypertension, metabolic syndrome, as well as Gensini score, systolic blood pressure, triglyceride, FBG, insulin levels, homeostasis model assessment of insulin resistance (HOMA-IR), and white blood cell count as compared to controls. No significant differences between both groups were observed for BMI, TC, LDL-C, hs-CRP levels, and number of white blood cells [Table 1]. vWF antigen was likely to increase in DM compared to control group (94.5 ± 40.4 [n = 87] vs. 83.1 ± 39.6 IU/dL [n = 84], P for trend = 0.032). However, no significant differences of ADAMTS13 activity (72.3 ± 18.1 [n = 87] vs. 73.1 ± 17.9% [n = 84], P = 0.775), vWF activity (99.6 ± 28.8 [n = 87] vs. 94.7 ± 25.7 IU/dL [n = 84], P = 0.249), and vWF antigen/ADAMTS13 activity ratio (1.2 ± 1.0 [n = 87] vs. 1.0 ± 0.6 [n = 84], P = 0.113) were observed between both groups.

Frequencies of QE genotype and E allele carrier of ADAMTS13 Q448E, AG genotype and G allele carrier of ADAMTS13 rs2073932, as well as AA genotype of ADAMTS13 rs652600 were significantly higher in DM individuals than those in control group (P < 0.05) [Table 2]. To evaluate the association of genetic variations with DM, multivariate logistic regression analysis was performed. The results demonstrated that frequencies of QE genotype and E allele carrier of the Q448E, AG genotype and G allele carrier of the rs2073932, as well as AA genotypes of the rs652600 were found to be independently associated with DM after adjustment for gender, age, hypertension, and metabolic syndrome [Table 2].

To classify the severity of coronary stenosis in DM individuals with CAD, the number of stenotic vessels and Gensini score...
were considered. The significant differences were found in frequencies of E and G alleles of the Q448E and rs2073932 in DM with multi-vessel disease and high Gensini score when compared to controls (P < 0.05). However, there were no significant differences of other genotypes in those with single-vessel disease when compared to controls [Table 3]. Furthermore, multivariate logistic regression analysis was performed to investigate the relationship between ADAMTS13 and vWF polymorphisms with severity of coronary stenosis. The results demonstrated that after adjustment for sex and age, the E allele carrier of the Q448E and the AA genotype of the rs652600 were significantly associated with an increased risk of multi-vessel disease (odds ratio [OR] [95% confidence interval (CI)] = 2.2 [1.0, 4.8], P = 0.047 and 3.2 [1.0, 10.0], P = 0.043, respectively) [Figure 1a]. In addition, this study also revealed the significant association between the minor allele carrier of the Q448E and rs2073932 with high Gensini score when compared to the control group (OR [95% CI] = 2.3 [1.1, 4.9], P = 0.035 and 2.3 [1.1, 5.1], P = 0.033, respectively) [Figure 1b].

vWF activity was significantly increased in the EE and GG genotypes of ADAMTS13 Q448E and rs2073932 when compared to heterozygous and wild type (P < 0.05 and P < 0.01, respectively) as shown in Table 4. However, no association was found between ADAMTS13 polymorphisms with ADAMTS13 activity, except vWF V1565L (P < 0.01) [Table 4]. According to the association of ADAMTS13 polymorphisms and DM risk, additional assessment of the relationship between those polymorphisms and other biochemical parameters such as FBG, insulin, and HOMA-IR was performed. The results demonstrated that patients carrying the E, G, and A alleles of the Q448E, rs2073932, and rs652600 had significantly elevated FBG and HOMA-IR [Table 4], when compared to individuals carrying wild-type genotype. Furthermore, the result also showed a negative correlation between ADAMTS13 activity and FBG in individuals carrying the EE and GG genotype of the Q448E and rs2073932 (r = −0.567, P = 0.022 and r = −0.712, P = 0.001, respectively).

**DISCUSSION**

The present study evaluated whether ADAMTS13 and vWF levels were associated with type 2 DM and severity of coronary stenosis through the determination...
of ADAMTS13 activity, vWF antigen, and activity along with their polymorphisms in DM and control individuals. The results demonstrated that vWF antigen was likely to increase in DM when compared to the controls which was consistent with the previous studies.[34] Moreover, this is the first investigation of the relationship between ADAMTS13 polymorphisms and type 2 DM in the Thai population. The results showed that minor E and G alleles of ADAMTS13 Q448E and rs2073932 and the AA genotype of rs652600 polymorphisms were independently associated with type 2 DM in this population. However, the mechanism that links genetic variations of ADAMTS13 to risk of DM is remained unclear. To date, many genome-wide association studies have investigated the novel genetic susceptibility to type 2 DM. Previous studies found that the rs10811661 of cyclin-dependent kinase inhibitor 2A/B (CDKN2A/B) gene was associated with impaired insulin secretion and type 2 DM.[23] As CDKN2A/B gene (9p21.3) is located near ADAMTS13 gene (9q34), it is possible that ADAMTS13 polymorphisms may be in linkage disequilibrium with this polymorphism. Other explanations may be explored from the results of this study showing the association of the minor alleles of ADAMTS13 Q448E, rs2073932, and rs652600 with increase in FBG and HOMA-IR, which may indicate the hyperglycemic condition, and the negative correlation of ADAMTS13 activity with FBG in individuals possessing the minor alleles of those polymorphisms. Taking this into account, this may in part contribute to the association of ADAMTS13 polymorphisms with DM.

An elevated vWF antigen in diabetic patients is associated with increased risk of thrombosis and cardiovascular events. In addition, the imbalance between vWF and ADAMTS13 levels was associated with promoting the atherosclerosis progression by increasing leukocyte rolling and adhesion.[24] Therefore, alterations of ADAMTS13 and vWF levels by ADAMTS13 polymorphisms may partly contribute to atherosclerosis in type 2 DM. So far, however, no study has examined the possible role of the ADAMTS13 polymorphisms with atherosclerotic lesion progression in type 2 DM. This present study demonstrated that individuals carrying the minor E and G alleles of Q448E

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Control (n=84)</th>
<th>DM with CAD</th>
<th>Gensini score</th>
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<tbody>
<tr>
<td><strong>ADAMTS13 gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q448E QQ</td>
<td>43 (51.2)</td>
<td>14 (31.8)</td>
<td>8 (44.4)</td>
</tr>
<tr>
<td>QE</td>
<td>34 (40.5)</td>
<td>24 (54.5)</td>
<td>7 (16.7)</td>
</tr>
<tr>
<td>EE</td>
<td>7 (8.3)</td>
<td>6 (13.5)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>E allele</td>
<td>41 (48.8)</td>
<td>30 (68.2)*</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>rs2073932 AA</td>
<td>41 (48.8)</td>
<td>14 (31.8)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>AG</td>
<td>34 (40.5)</td>
<td>24 (54.5)</td>
<td>7 (38.9)</td>
</tr>
<tr>
<td>GG</td>
<td>9 (10.7)</td>
<td>6 (13.6)</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>G allele</td>
<td>43 (51.2)</td>
<td>30 (68.2)*</td>
<td>11 (61.6)</td>
</tr>
<tr>
<td>rs652600 GG</td>
<td>37 (44.0)</td>
<td>13 (29.5)</td>
<td>7 (38.9)</td>
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<tr>
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<td>37 (44.0)</td>
<td>21 (47.7)</td>
<td>8 (44.4)</td>
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<tr>
<td>AA</td>
<td>10 (11.9)</td>
<td>10 (22.7)</td>
<td>3 (16.7)</td>
</tr>
<tr>
<td>A allele</td>
<td>47 (56.0)</td>
<td>31 (70.5)</td>
<td>11 (61.1)</td>
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<tr>
<td>rs4962153 GG</td>
<td>65 (77.4)</td>
<td>36 (81.8)</td>
<td>14 (77.8)</td>
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<tr>
<td>GA</td>
<td>15 (17.9)</td>
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<td>1 (5.6)</td>
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<tr>
<td>A allele</td>
<td>19 (22.6)</td>
<td>8 (18.2)</td>
<td>4 (22.2)</td>
</tr>
<tr>
<td>vWF gene V1565L</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VV</td>
<td>67 (79.8)</td>
<td>38 (88.4)</td>
<td>17 (94.4)</td>
</tr>
<tr>
<td>VL</td>
<td>17 (20.2)</td>
<td>5 (11.6)</td>
<td>1 (5.6)</td>
</tr>
</tbody>
</table>

Chi-square test was performed for comparison of frequencies of each genotype among the study groups. *P<0.05 compared to control group.

DM=Diabetes mellitus; CAD=Coronary artery disease; MVD=Multi vessel disease; SVD=Single vessel disease; vWF=von Willebrand factor; ADAMTS13=A disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13
and rs2073932 as well as the AA genotype of rs652600 were associated with increased risk of multi-vessel disease and high Gensini score. These results suggested that ADAMTS13 polymorphisms may affect the occurrence and progression of vascular lesion in type 2 DM. Based on this finding, they may reduce ADAMTS13 activity or increase vWF antigen and/or activity. This study revealed the relationship of homozygous variants of Q448E and rs2073932 as well as the AA genotype of rs652600 which may reduce ADAMTS13 activity or increase vWF activity which was consistent with our previous study.[24] To date, some studies have shown that vWF activity was positively correlated with Gensini score[25] and vascular endothelial growth factor,[26] which associated with increased atherosclerosis progression.[27] Therefore, the association between ADAMTS13 polymorphisms and vWF activity may in turn support their roles in promoting atherosclerosis progression in type 2 DM individuals. However, it should be noted that the sample size in this study was relatively small especially in each subgroup which may reduce the statistical power of tests. Thus, further investigation with a larger sample size is needed to elucidate the exact mechanism and association of ADAMTS13 polymorphisms with type 2 DM.

CONCLUSION

The present study has demonstrated the association of ADAMTS13 polymorphisms with type 2 DM and severity of coronary stenosis. These relationships suggested the importance of genetic variations of ADAMTS13 which may eventually be used as the atherosclerotic risk assessment in diabetic individuals.

Acknowledgments

This study and Lasom S. were supported by the grant from the National Research University, Office of the Higher Education Commission, Ministry of Education Thailand, the National Research Council of Thailand, and the Cardiovascular Research Group, Khon Kean University.

Financial support and sponsorship

This manuscript and Lasom S. were supported by the grant from the National Research University, Office of the Higher Education Commission, Ministry of Education Thailand, the National Research Council of Thailand, and the Cardiovascular Research Group, Khon Kean University.

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Table 4: Association of genetic variations with von Willebrand factor activity and antigen, a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 activity, and von Willebrand factor antigen/activity ratio in a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 ratio

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>vWF activity (IU/dL)</th>
<th>vWF antigen (IU/dL)</th>
<th>ADAMTS13 activity (%)</th>
<th>vWF/ADAMTS13 ratio</th>
<th>FBG (mmol/L)</th>
<th>Insulin (µU/mL)</th>
<th>HOMA-IR</th>
</tr>
</thead>
</table>

One-way ANOVA was performed to compare continuous values among genotypes. Values in parentheses represent number of subjects. Values of FBG, insulin and HOMA-IR are presented as geometric mean±SD. †P<0.05 and ‡P<0.01; *P for trend<0.041. FBG=Fasting blood glucose; HOMA-IR=Homeostasis model assessment of insulin resistance; ANOVA=Analysis of variance; SD=Standard deviation; vWF=von Willebrand factor; ADAMTS13= A disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13.
**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**