Original Article

Polymorphism of 270 A > G in BRAP is Associated with Lower Ankle-Brachial Index in a Taiwanese Population

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Aims: The single nucleotide polymorphism (SNP) rs11066001 of BRAP has been shown to be associated with myocardial infarction (MI), coronary atherosclerosis and carotid atherosclerosis, but it is not clear whether it also plays a role in peripheral artery disease (PAD). The ankle-brachial index (ABI) is often used as a non-invasive measure of PAD; therefore, the aim of this study was to test for a relationship between the SNP rs11066001 and ABI.

Methods: A total of 537 high-risk subjects with a family history of MI or stroke completed a health survey, including a physical examination, blood test and measurement of ABI. Among them, 523 subjects had the genotypes. Association analyses between the genotype of BRAP and ABI were performed by multiple linear and logistic regressions with adjustment for covariates.

Results: We found that the GG genotype is significantly associated with a lower ABI value. For the lowest ABI tertile, the GG genotype had an OR of 2.87 (p = 0.018) when compared with the middle ABI tertile, and OR of 2.92 (p = 0.015) when compared to the highest ABI tertile. Women with the GG genotype had a lower ABI value than men with the same genotype (p = 0.012). Accordingly, women carrying this GG risk genotype may have a higher risk for PAD.

Conclusion: Our findings provide additional evidence that support the genetic effect of BRAP on diverse cardiovascular phenotypes.


Key words: BRAP, Polymorphism, Ankle-brachial index, Peripheral artery disease, Genotype

Introduction

The BRCA-1 associated protein (BRAP) gene is located on chromosome 12q24.12 with a total length of 43.8 kb. BRAP was originally identified as a cytoplasmic protein that recognizes the nuclear localization signal of breast cancer suppressor protein ¹). BRAP was not known to be involved in cardiovascular disease (CVD) until our recent work, where a SNP rs11066001 in intron 3 was found to be significantly associated with myocardial infarction (MI) in Japanese and Chinese populations ²). The minor G allele of SNP rs11066001 was demonstrated to cause a higher BRAP expression level than the common A allele ²). The findings were further replicated by an independent study using Japanese and Korean populations ³). In addition, our group showed an association between SNP rs11066001 and carotid atherosclerosis (unpublished data) and coronary stenosis (unpublished data). BRAP protein participates in the lymphotoxin-α (LTA)-associated inflammatory pathway ²) via interaction with the binding protein of LTA ², ⁴, ⁵). Several
proinflammatory genes and their associated proteins, such as C-reactive protein (CRP), have been reported to be associated with CVD. We also demonstrated that up-regulation of BRAP can enhance nuclear factor - κB (NF-κB) nucleus translocation and increase the expression of inflammatory cytokines in vascular smooth muscle cells (unpublished data); therefore, BRAP may play an important role in the atherosclerotic process by influencing the inflammatory cascades.

The ankle-brachial index (ABI) is often used as a non-invasive measure for peripheral artery disease (PAD). PAD is a common arterial disease often caused by atherosclerosis. The early symptom of PAD is intermittent claudication, but patients with PAD often ignored this warning symptom. Usually a patient knows his/her PAD because of foot gangrene, which is a late complication of PAD; therefore, ABI is very important because it helps to identify asymptomatic PAD patients. In fact, ABI has been clearly defined for PAD diagnosis. Previous studies have documented that ABI can also predict the risk for other CVD, such as stroke and MI. Subjects with low ABI values have been shown to carry a higher risk for CVD. The Multi-Ethnic Study of Atherosclerosis (MESA) documented that a lower ABI value was significantly associated with a high score of coronary artery calcium and thick intima-media in the internal carotid artery. Subjects with high cardiovascular risks could have PAD years before the event of MI or stroke. Accordingly, ABI can be used for early diagnosis of PAD, and predict the risk of future MI and stroke events.

**Aim**

BRAP has been shown to play a role in MI, the extent of coronary stenosis and carotid atherosclerosis, but it is not clear whether it also plays a role in PAD. The aim of this study was to test for a relationship between the SNP rs11066001 of BRAP and ABI.

**Methods**

**Study Population**

We enrolled 537 high-risk subjects with a family history of MI or stroke at Kaohsiung Medical University Hospital between January 2006 and September 2010. An eligible subject needs to have one first-degree relative who has had documented MI or stroke history or two second-degree relatives with MI or stroke. All participants provided written informed consent and the study protocols and methods were approved by the local Institutional Review Board (IRB).

Each participant completed a self-administered structure questionnaire which covered demographic information, medical history, and medication data. Persons who had quit smoking during the previous one year were classified as ever smokers. Body mass index (BMI) was calculated as weight (kg)/height (m²). Total cholesterol, triglyceride, and high-density lipoprotein (HDL) levels were measured from venous blood after fasting for at least 6 hours. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg, a self-reported history of hypertension or taking anti-hypertensive medications. Diabetes was defined as fasting blood glucose ≥ 126 mg/dL, a self-reported history of diabetes or receiving hypoglycemic medications.

**Genotyping of BRAP**

Blood was collected for biochemistry analyses and genetic studies. Genomic DNA was isolated from whole blood using the salting-out method, which was standardized with the Puregene kit from Gentra (Research Triangle, NC). SNP rs11066001 (270A>G at intron 3) at BRAP was genotyped by TaqMan (Applied Biosystems, Foster City, CA) as described elsewhere.

**Measurement of Ankle Brachial Index (ABI)**

Non-invasive ABI was measured in resting supine participants using the COLIN VP 1000 (Colin, Komaki, Japan). The validity and reproducibility of the device have been reported. After entering personal data, blood pressure cuffs were placed on patient arms and ankles. Phonocardiography was placed at the second intercostal space at the left margin of the sternum. Right and left ABI were recorded automatically for about one minute. The right ABI between the arm and ankle were separately recorded automatically for about one minute. The right ABI was directly calculated as the right ankle systolic blood pressure divided by the largest of the right or left brachial systolic blood pressure. Similar measures were used for left ABI calculation. The minimal ABI value between right and left ABI data was selected as the final ABI value for each subject.

**Statistical Analysis**

Allele frequency was estimated by direct genotype counting. Hardy-Weinberg equilibrium (HWE) was tested using the χ² test. Continuous variables are presented as the means ± standard deviations (SDs). The demographic data were compared across three different genotypes using ANOVA, χ² test or Fisher’s exact test. Simple linear regression was used to test for
a relationship between ABI and traditional risk factors. Multiple linear regression analysis was performed using quantitative data of ABI with adjustment for other covariates. We also divided the ABI values into three categories, the lowest tertile <1.08, the middle tertile 1.08-1.14 and the highest tertile ≥1.14. Multiple logistic regression analysis was used to analyze the relationship between categorized ABI and SNP rs11066001 of BRAP. The sex-specific genetic effect on ABI was evaluated by ANOVA. Post-hoc comparisons were made by Tukey-Kramer tests. A two-tailed p value <0.05 was considered significant. Statistical analyses were performed with JMP software (version 8.0; SAS Institute Inc., Cary, NC).

### Results

A total of 537 participants were finally included in the present study. The range of ABI for the total participants was 0.82-1.32, where ABI data are normally distributed. The demographic information, biochemical data, disease history, are shown in Table 1. The mean age of our study participants was 54.6±9.7 (range 26-85) years old. The biochemistry data were not significantly different among the three genotypes of BRAP (Table 1).

Univariate analyses demonstrated that men had a higher ABI than women (mean ABI in men: 1.12, women: 1.09, p<0.0001). BMI was significantly associated with ABI (p=0.0005). Except for diabetes and triglyceride, we found borderline significance between ABI and age, smoking, and total cholesterol (p=0.044-0.099) (Table 2). All traditional risk factors were included in the multiple regression models and logistic models. Among our study participants, 523 subjects had genotype data (i.e. genotype call rate of 97.4%). The frequency of minor allele G was 0.29, and the number of subjects in each genotype was 42 (8.0%) for GG, 216 (41.3%) for GA and 265 (50.7%) for AA. Genotype distribution of this SNP was in HWE.

### Table 1. Comparison of baseline characteristics of subjects among different genotypes of SNP rs11066001 at BRAP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total (n = 523)</th>
<th>GG (n = 42)</th>
<th>GA (n = 216)</th>
<th>AA (n = 265)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>54.6±9.7</td>
<td>51.9±9.0</td>
<td>54.5±9.3</td>
<td>55.0±10.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Sex, Female (n, %)</td>
<td>282 (53.9)</td>
<td>23 (54.8)</td>
<td>121 (56.0)</td>
<td>138 (52.1)</td>
<td>0.68</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.9±3.5</td>
<td>24.6±3.7</td>
<td>24.6±3.5</td>
<td>25.1±3.5</td>
<td>0.37</td>
</tr>
<tr>
<td>Overweight (BMI ≥25)</td>
<td>234 (45.1)</td>
<td>18 (42.9)</td>
<td>88 (41.1)</td>
<td>128 (49.2)</td>
<td>0.20</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>203.1±37.7</td>
<td>197.2±37.5</td>
<td>203.4±36.5</td>
<td>203.8±38.8</td>
<td>0.57</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>137.3±84.6</td>
<td>123.4±66.7</td>
<td>136.7±85.0</td>
<td>140.0±86.9</td>
<td>0.49</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>52.4±14.5</td>
<td>52.7±13.4</td>
<td>52.1±14.5</td>
<td>52.7±14.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>186 (36.7)</td>
<td>15 (36.6)</td>
<td>76 (36.2)</td>
<td>95 (37.1)</td>
<td>0.98</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>76 (15.0)</td>
<td>5 (12.2)</td>
<td>36 (17.1)</td>
<td>35 (13.7)</td>
<td>0.51</td>
</tr>
<tr>
<td>Smoker (n, %)</td>
<td>57 (11.0)</td>
<td>2 (4.9)</td>
<td>27 (12.5)</td>
<td>28 (10.6)</td>
<td>0.38</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125.3±17.0</td>
<td>123.9±16.3</td>
<td>124.8±17.3</td>
<td>125.9±16.8</td>
<td>0.67</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.8±11.0</td>
<td>75.2±10.7</td>
<td>75.1±11.3</td>
<td>76.6±10.8</td>
<td>0.30</td>
</tr>
<tr>
<td>Pulse pressure-bronchial (mmHg)</td>
<td>49.5±10.6</td>
<td>48.7±10.9</td>
<td>49.7±10.5</td>
<td>49.4±10.6</td>
<td>0.83</td>
</tr>
<tr>
<td>Pulse pressure-ankle (mmHg)</td>
<td>65.7±14.2</td>
<td>61.6±11.3</td>
<td>66.2±15.4</td>
<td>66.0±13.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Ankle-brachial index (ABI)</td>
<td>1.10±0.07</td>
<td>1.08±0.07</td>
<td>1.10±0.07</td>
<td>1.11±0.07</td>
<td>0.010*</td>
</tr>
<tr>
<td>Female (n=282)</td>
<td>1.09±0.07</td>
<td>1.05±0.07</td>
<td>1.09±0.06</td>
<td>1.09±0.07</td>
<td>0.010*</td>
</tr>
<tr>
<td>Male (n=241)</td>
<td>1.12±0.07</td>
<td>1.11±0.05</td>
<td>1.12±0.07</td>
<td>1.13±0.07</td>
<td>0.409</td>
</tr>
</tbody>
</table>

*p value was calculated by Fisher’s exact test

<table>
<thead>
<tr>
<th>Variables</th>
<th>β</th>
<th>SE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.017</td>
<td>0.003</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.003</td>
<td>0.0009</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>-0.0002</td>
<td>0.00008</td>
<td>0.044*</td>
</tr>
<tr>
<td>Smoking status</td>
<td>0.008</td>
<td>0.005</td>
<td>0.093</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.0005</td>
<td>0.0003</td>
<td>0.099</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.006</td>
<td>0.004</td>
<td>0.172</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>-0.00003</td>
<td>0.00004</td>
<td>0.418</td>
</tr>
</tbody>
</table>

*p<0.05

Abbreviations: HDL, high density lipoprotein

### Table 2. The relationship between ABI value and traditional risk factors

**Abbreviations:** HDL, high density lipoprotein
The ABI values were significantly different among the three genotypes ($p=0.010$ from ANOVA test) (Table 1). Although the mean ABI decreased with the number of risk G alleles, there was no statistical difference between AA and GA genotypes, which suggests that the G allele exerts a recessive effect; therefore, we further combined GA and AA as one group to compare with the GG genotype. After adjusting for other covariates, the genotypes remained significantly associated with the ABI values ($p=0.027$). For the ABI tertiles, the risk GG genotype was more common in the lowest tertile (Table 3). The GG genotype had an OR of 2.87 (adjusted $p=0.018$) when compared with the middle ABI tertile, and OR of 2.92 (adjusted $p=0.015$) when compared to the highest ABI tertile.

Sex-specific analysis showed that the mean ABI was lowest (mean ABI=1.05) in females with the GG genotype, followed by females with the other two genotypes (mean ABI=1.09), and then by males with the GG genotype (mean ABI=1.11). Men with the GA or AA genotype had the highest ABI value (mean ABI=1.12). The difference of mean ABI between the highest and lowest risk subjects (i.e. between women with the GG genotype and men with the GA or AA genotype) was 0.07 ($p<0.0001$) (Fig. 1).

### Table 3. The relationship between SNP rs11066001 of BRAP and categorized ABI

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ABI (range)</th>
<th>GG N (%)</th>
<th>GA N (%)</th>
<th>AA N (%)</th>
<th>OR (95% CI)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (&lt;1.08)</td>
<td>24 (13.4)</td>
<td>75 (41.9)</td>
<td>80 (44.7)</td>
<td>2.87 (1.24-7.27)</td>
<td>0.018 $^a$</td>
<td></td>
</tr>
<tr>
<td>Middle (1.08-1.14)</td>
<td>9 (5.3)</td>
<td>78 (45.6)</td>
<td>84 (49.1)</td>
<td>2.92 (1.27-7.27)</td>
<td>0.015 $^a$</td>
<td></td>
</tr>
<tr>
<td>High (≥ 1.14)</td>
<td>9 (5.2)</td>
<td>63 (36.4)</td>
<td>101 (58.4)</td>
<td>2.92 (1.27-7.27)</td>
<td>0.015 $^a$</td>
<td></td>
</tr>
</tbody>
</table>

$^a$The OR and $p$ value were obtained by comparing middle ABI tertile vs. lowest ABI tertile or highest ABI tertile vs. lowest ABI tertile. Multiple logistic regression analysis was performed with adjustment for age, sex, BMI, smoking status, total cholesterol, triglyceride and diabetes.

$^p<0.05$

Abbreviations: ABI, ankle-brachial index; OR, odds ratio; 95% CI, 95% confidence interval

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![Fig. 1. Ankle-brachial index across groups with different risk levels (combined sex and risk genotype of SNP rs11066001 of BRAP). ABI value <0.9 is a diagnostic criterion for PAD and a dashed line indicates the cutoff point of ABI.](image-url)
Discussion

The estimated heritability of ABI ranged from 21% in the Framingham Offspring cohort to 48% in a twin study. BRAP has been identified as a susceptibility gene for several cardiovascular phenotypes. In the present study, we recruited a high-risk population based on a positive family history to test for BRAP genetic predisposition to ABI. We demonstrated that the GG genotype of SNP rs11066001 is significantly associated with a lower ABI value. Women with the GG genotype have a lower ABI value than men with the same genotype. Accordingly, women carrying this risk genotype may have a higher risk for PAD and other CVD. Our findings provide additional evidence to support the genetic effect of BRAP on a variety of cardiovascular phenotypes.

The value of ABI is based on systolic blood pressure measured at the ankle and arm. Arterial pressure increases with distance from the heart because of the impedance of smaller arteries; therefore, systolic blood pressure measured at the ankle is higher than that measured at the arm. Since ABI is derived from the ratio of ankle blood pressure divided by brachial blood pressure, the range of normal ABI values is generally between 0.9 and 1.3. Previous studies have suggested that an ABI value <0.9 could be used as a diagnostic criterion for PAD. This cutoff value is also recommended as a predictive index for other CVD. The mean ABI in our study population was 1.09 in women and 1.12 in men, similar to the report of 1.05 in women and 1.06 in men from another study conducted in Taiwan. From a multiple ethnic study, the MESA cohort showed that the mean ABI was lowest in African Americans while Chinese Americans and European Americans had similar means of approximately 1.03. The MESA cohort had a higher risk profile than our study population, such as a high prevalence of diabetes, smoking, dyslipidemia and older age, which can partially explain the lower mean of ABI values in the MESA cohort. Since our study population only had three subjects with ABI values <0.9 (i.e. only three subjects with PAD), this restricts the discussion of the relationship between BRAP and ABI. Previous studies have reported that patients with lower ABI values had a higher risk of MI or stroke regardless of using continuous ABI or the categorized ABI value. The negative correlation between lower ABI and cardiovascular risk was not doubted. Although the ABI values of most participants in our study were within the normal range, a negative relationship between lower ABI and cardiovascular diseases still existed in these high-risk subjects; therefore, the lowest tertile (ABI <1.08) may be used as an early warning sign.

Our data showed that the mean ABI was lower in women than men by 0.03, which is consistent with previous studies. The Genetic Epidemiology Network of Arteriopathy (GENOA) study and MESA study reported lower ABI values in women than men. In the National Health and Nutrition Examination Survey (NHANES) and ARIC reports, the prevalence of ABI <0.9 in women between ages 40 and 59 years was estimated to be about twice as high as in men. It has been suggested that lower ABI was associated with shorter height, leading to lower pulse amplification; therefore, sex and BMI were adjusted in our analyses and the sex-specific effect on ABI was also discussed in the present study.

Since the minor G allele has greater transcription efficacy than the common A allele, individuals with the GG genotype can have higher levels of BRAP protein and thus manifest more severe atherogenic phenotypes. Our previous findings have indicated that the genotype with high BRAP expression has a higher risk for MI, extent of coronary stenosis and carotid atherosclerosis. Our recent findings also show that BRAP can influence NF-κB nuclear translocation, leading to an increase of proinflammatory molecules (unpublished data). In addition, previous studies demonstrated that NF-κB can regulate a number of inflammatory genes, including interleukin-6 (IL-6), tumor necrosis factor (TNF), vascular cellular adhesion molecule-1 (VCAM-1), E-selectin, inducible nitric oxide synthase (iNOS), matrix metallo-proteinases (MMPs) and CRP. BRAP was originally identified as a cytoplasmic protein that recognizes the nuclear localization signal of the breast cancer suppressor protein, BRCA-1. In cancer research, BRAP was found to be an E3 ubiquitin ligase that associates with Ras and modulates mitogen activated protein (MAP) kinase signaling; therefore, BRAP may have distinct mechanisms in atherosclerosis and breast cancer.

The above inflammatory markers have been investigated in relation to ABI and PAD in large epidemiological studies. The G-174C IL-6 polymorphism had been documented to increase the development of PAD and incidence of CVD. The risk GG genotype of IL-6 can increase IL-6 release, which in turn increases the release of fibrinogen and CRP. From the Framingham Offspring Study, increased serum IL-6 and TNF receptor 2 (TNFR2) levels were associated with ABI decline and clinical PAD (intermittent claudication). The Edinburgh Artery Cohort demonstrated that a higher IL-6 level at the baseline was associated with
ABI decline after a 12-year follow-up. Furthermore, higher baseline CRP levels in the Edinburgh Artery Cohort were also associated with the risk of PAD. The Atherosclerosis Risk in Communities (ARIC) cohort study showed that CRP was significantly higher in subjects with PAD or patients with coronary heart disease; therefore, the inflammatory mechanism is likely to play a role in ABI, and the BRAP effect on ABI can be partially explained by such a mechanism.

Conclusion

In conclusion, we found that the GG genotype of SNP rs11066001 at BRAP was associated with lower ABI in a Taiwanese high-risk population. Our data indicated that the same genotype not only increases the risk for MI, coronary stenosis and carotid atherosclerosis, but also PAD.

Acknowledgments

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Conflict of Interest

The authors declare that they have no competing interests regarding the manuscript.

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