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Mediation analysis reveals a sex-dependent association between ABO gene variants and TG/HDL-C ratio that is suppressed by sE-selectin level

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ABSTRACT

Objective: Previous investigations have revealed an association between the *ABO* locus/blood group and total cholesterol and inflammatory biomarker levels. We aimed to test the statistical association of *ABO* locus variants with lipid profiles and levels of thirteen inflammatory markers in a Taiwanese population.
Methods and results: A sample population of 617 Taiwanese subjects was enrolled. Five *ABO* gene region polymorphisms were selected and genotyped. After adjusting for clinical covariates and inflammatory marker levels, the genetic-inferred ABO blood group genotypes were associated with sE-selectin level ($P = 3.5 \times 10^{-36}$). Significantly higher total and low-density lipoprotein cholesterol (LDL-C) levels were noted in individuals with blood group A ($P = 7.2 \times 10^{-4}$ and $P = 7.3 \times 10^{-4}$, respectively). Interestingly, after adjusting for sE-selectin level, significantly lower high-density lipoprotein cholesterol (HDL-C) level as well as higher triglyceride (TG) level and ratio of triglyceride to HDL-C (TG/HDL-C ratio) were noted in individuals with blood group A comparing to non-A individuals ($P = 0.009$, $P = 0.004$ and $P = 0.001$, respectively); these associations were also observed in the group A male subjects ($P = 0.027$, $P = 0.001$, and $P = 0.002$, respectively). Mediation analysis further revealed a suppression effect of sE-selectin level on the association between genetic-inferred ABO blood group genotypes and TG/HDL-C ratio in total participants ($P = 1.18 \times 10^{-6}$) and in males ($P = 5.99 \times 10^{-5}$).
Conclusion: Genetic variants at the *ABO* locus independently affect sE-selectin level in Taiwanese subjects, while the association of *ABO* locus variants with TG/HDL-C ratio is suppressed by sE-selectin level in Taiwanese males. These results provided further evidence for the mechanism in the association of ABO blood groups with atherosclerotic cardiovascular diseases.

1. Introduction

The *ABO* gene locus has been mapped to chromosome 9, and the molecular basis underlying alleles of the ABO histo-blood group has been characterized [1]. The ABO blood group antigens (A, B, and H determinants) have been shown to consist of complex carbohydrate molecules that are synthesized by the sequential action of various glycotransferases. Antigens of the ABO blood group system, traditionally regarded as red cell antigens, are actually expressed on a variety of human tissues, including epithelium, sensory neurons, platelets, and vascular endothelium [2]. In spite of the strong

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evidence for conservation of the ABO gene through evolution, there remains no known function for these carbohydrate molecules.

The association of ABO blood groups with the risk of atherosclerotic cardiovascular diseases, and venous thromboembolic disease have been widely recognized [3–5]. It has been suggested that non-O blood groups confer a higher risk of myocardial infarction, peripheral vascular disease, cerebral ischemia of arterial origin, and venous thromboembolism than does group O. These observations have led a number of authors to postulate a mechanism whereby some of these associations with ABO operate through biomarkers. There is growing evidence that the ABO histo-blood group is the major determinant of plasma coagulation protein levels, including factor VIII, von Willebrand factor (VWF), and thrombomodulin [6–8]. Recently, genome-wide association studies have revealed significant associations between genetic variants in the ABO blood group region and blood levels of various inflammatory markers, including soluble levels of E-selectin (sE-selectin), intercellular adhesive molecule 1 (sICAM1), and P-selectin (sP-selectin) [9–11]. Moreover, the ABO blood group has been shown to correlate with blood total cholesterol and low-density lipoprotein cholesterol (LDL-C) [12], but not with other lipid profiles. In this investigation, we tried to address the role of the *ABO* locus in inflammatory and lipid cardiovascular phenotypes in Taiwanese. Because of a significant association between serum lipid and inflammatory marker levels, mediation analysis was also performed between *ABO* genotypes and these biomarker levels.

2. Methods

2.1. Subjects

A total of 617 Han Chinese subjects (327 men with a mean age of 45.2 ± 10.5 years; 290 women with a mean age of 46.8 ± 10.1 years) were enrolled. They responded to a questionnaire on their medical history and lifestyle characteristics, and were recruited during routine health examinations between October 2003 and September 2005 at the Chang Gung Memorial Hospital. Fasting blood samples were obtained from each subject. All subjects provided informed consent. Exclusion criteria included a history of myocardial infarction, stroke, or transient ischemic attack, history of cancer, and current renal or liver disease. The clinical and biometrical features of the study population are summarized in Table 1. Current smokers were defined as those who smoked cigarettes regularly at the time of survey. The Ethics Committee of the Buddhist Tzu Chi General Hospital Taipei Branch approved the investigation.

2.2. Genomic DNA extraction and genotyping

Genomic DNA was extracted as previously reported [13]. Oligonucleotide primers were generated to amplify fragments of genomic DNA containing SNPs as reported on the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP). Genotyping for SNPs rs8176719, rs8176746, rs579129, and rs579459 was performed by polymerase chain reaction (PCR) with restriction enzyme digestion. In addition, a minisatellite, composed of one to five tandem repeats of the 43-bp consensus unit located on promoter of ABO gene was also genotyped by PCR. Genotyping data are shown in Supplementary Table 1.

2.3. Assays

Most markers were measured as previously reported [13]. Matrix metalloproteinase 1 (MMP1), monocyte chemotactic protein-1 (MCP1), sP-selectin, tumor necrosis factor-alpha receptor 2 (TNFR2), and interleukin-6 (IL6) were measured using commercially available ELISA kits from R&D Systems (Minneapolis, MN, USA). Total cholesterol and triglyceride (TG) concentrations were measured via automatic enzymatic colorimetry. High-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically after phosphotungsten/magnesium precipitation. Low-density lipoprotein cholesterol (LDL-C) levels were either calculated using the Friedewald formula or, in patients with triglyceride levels of >400 mg/dL, were measured enzymatically in a Hitachi 7600–210 autoanalyzer (Hitachi, Tokyo, Japan) by using commercially available kits (Daiichi Pure Chemicals, Tokyo, Japan).

Table 1
Clinical and biochemical characteristics of the study participants by gender.

	Total	Men	Women	*P* value
Number	617	327	290	
Age (years)	46.0 ± 10.4	45.2 ± 10.5	46.8 ± 10.1	0.044
Systolic BP (mmHg)	115.1 ± 17.6	113.8 ± 14.4	112.0 ± 17.8	0.203
Diastolic BP (mmHg)	76.0 ± 10.6	76.7 ± 9.8	73.1 ± 10.0	<0.001
Total cholesterol (mg/dL)	198.1 ± 36.6	200.1 ± 37.2	196.3 ± 36.1	0.205
HDL-cholesterol (mg/dL)	55.1 ± 14.2	49.7 ± 11.9	61.2 ± 14.3	<0.001
LDL-cholesterol (mg/dL)	115.6 ± 32.9	118.0 ± 34.0	113.3 ± 31.7	0.077
Triglyceride (mg/dL)	142.1 ± 117.6	171.1 ± 146.6	109.7 ± 60.6	<0.001
Body mass index (kg/m^2)	24.3 ± 3.5	25.0 ± 3.2	23.6 ± 3.7	<0.001
Current smokers (%)	19.3	33.0	3.8	<0.001
CRP (mg/L)	1.7 ± 6.2	1.9 ± 7.9	1.4 ± 3.1	0.122
SAA (μg/mL)	6.1 ± 15.4	7.0 ± 19.4	5.1 ± 8.9	0.124
Fibrinogen (mg/dL)	265.1 ± 70.1	262.9 ± 72.0	267.5 ± 68.0	0.411
IL6 (μg/L)	4.1 ± 7.5	4.2 ± 8.4	4.1 ± 6.4	0.868
sICAM1 (ng/mL)	241.7 ± 111.5	245.0 ± 111.0	238.1 ± 112.2	0.497
sVCAM1 (ng/mL)	491.5 ± 131.7	495.0 ± 148.3	487.6 ± 110.2	0.486
sE-selectin (ng/mL)	53.6 ± 26.3	60.6 ± 28.0	45.7 ± 21.8	<0.001
sP-selectin (ng/mL)	139.9 ± 117.0	154.8 ± 131.8	123.3 ± 95.4	<0.001
TNFR2 (pg/mL)	3277.0 ± 950.1	3337.4 ± 986.3	3209.3 ± 904.7	0.097
MCP-1 (pg/mL)	73.3 ± 58.3	78.1 ± 66.0	67.8 ± 47.9	0.028
MMP1 (pg/mL)	465.1 ± 1147.4	336.7 ± 542.4	609.0 ± 1558.1	0.624
MMP2 (ng/mL)	126.7 ± 40.9	123.5 ± 41.0	130.3 ± 40.4	0.014
MMP9 (ng/mL)	144.0 ± 111.7	155.7 ± 115.6	130.7 ± 105.7	0.006

BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SAA, serum amyloid A; IL6, interleukin 6; sICAM1, soluble intercellular adhesive molecule 1; sVCAM1, soluble vascular cell adhesive molecule 1; sE-selectin, soluble E-selectin; sP-selectin, soluble P-selectin; TNFR2, tumor necrosis factor-alpha receptor 2; MCP-1, Monocyte chemotactic protein-1; MMP2, matrix metalloproteinase 1; MMP2, matrix metalloproteinase 2; MMP9, matrix metalloproteinase 9.
Continuous variables are presented as mean ± SD. HD-C, LDL-C, Total cholesterol, Triglyceride, CRP, sICAM1, sE-selectin, sP-selectin, MMP1, MMP2, and MMP9 values were logarithmically transformed before statistical testing to meet the assumption of normal distributions; however, the untransformed data are shown.
BP levels and lipid variables were analyzed with the exclusion of subjects using antihypertensive drugs and/or lipid-lowering agents.

2.4. Statistical analysis

Statistical analysis was performed as we previously described [13]. The analysis of deviation from the Hardy–Weinberg equilibrium, estimation of linkage disequilibrium between polymorphisms, was performed using Golden Helix SVS Win32 7.3.1 software. To explore the mediation effects of sE-selectin level on the relation between genetic-inferred blood group A and lipid levels (such as LDL-C, HDL-C, and TG levels), a conceptual model was hypothesized for the test, and four criteria have been suggested to evaluate the mediation and suppression effect (Supplementary Fig. 1).