ABO blood group associations with markers of endothelial dysfunction in the Multi-Ethnic Study of Atherosclerosis

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ABSTRACT

Background and aims: ABO blood type is associated with cardiovascular disease, although the underlying mechanisms are presumed to be complex. While the relationship between non-O blood types and von Willebrand Factor (vWF) is well-established, associations with cellular adhesion molecules (CAMs) across diverse populations are understudied.

Methods: We genetically inferred ABO alleles for N = 6202 participants from the Multi-Ethnic Study of Atherosclerosis. Linear regression was used to evaluate associations between major ABO allele dosages and log-transformed measurements of vWF (N = 924), soluble E-selectin (sE-selectin, N = 925), soluble P-selectin (sP-selectin, N = 2392), and soluble ICAM-1 (sICAM-1, N = 2236) by race/ethnicity.

Results: For the selectins, the A1 allele was associated with significantly lower levels for all races/ethnicities, with each additional allele resulting in a 28—39% decrease in sE-selectin and 10—18% decrease in sP-selectin relative to Type O subjects. However, the A2 allele demonstrated effect heterogeneity across race/ethnicity for sE-selectin, with lower levels for non-Hispanic whites ($p = 0.0011$) but higher levels for Hispanics ($p = 0.0021$). We also identified elevated sP-selectin levels for B-allele carriers solely in Hispanic participants ($p = 1.0E-04$). ABO-by-race/ethnicity interactions were significant for both selectins ($p < 0.0125$). More modest associations were observed between A1 allele dosage and levels of sICAM-1, with ABO alleles explaining 0.8—1.1% of the total phenotypic variation within race/ethnicity. ABO associations with vWF activity were consistent across race/ethnicity, with B allele carriers corresponding to the highest vWF activity levels.

Conclusions: ABO blood type demonstrates complex associations with endothelial markers that are largely generalizable across diverse populations.

1. Introduction

The ABO histo-blood group is one of the major human blood antigen systems, classified by the presence of A and B antigens on the surface of red blood cells. These antigens are produced by glycosyltransferases encoded by the *ABO* gene, which modify terminal oligosaccharides of the H precursor antigen. In contrast to the A and B alleles of *ABO*, the O allele is enzymatically inactive due to the presence of functionally deleterious mutations, and homozygous O allele carriers (i.e., Type O) lack modified H antigens. ABO glycosyltransferases are also known to modify surface glycoproteins of platelets, vascular endothelium and epithelium, and other cell types [1].

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ABO blood type has been associated with phenotypic variation of cardiovascular disease (CVD) risk factors as well as susceptibility to CVD across multiple studies [2]. Increased risk of venous thromboembolism and stroke has consistently been observed for non-O blood types [3—6]. Associations of genetic variation at *ABO* have also been reported for myocardial infarction [7], atherosclerosis [8], and coronary heart disease [9], with variants tagging non-O alleles associated with increased disease risk. The association between ABO and stroke is likely mediated by the relationship between glycosyltransferase activity and plasma levels of the pro-coagulant von Willebrand Factor (vWF) [10,11]. However, a postulated mechanism relating ABO to atherosclerotic CVD is that ABO glycosyltransferases additionally impact circulation of cellular adhesion molecules (CAMs). CAMs are comprised of multiple protein families that are expressed on the vascular endothelium and recruit leukocytes in response to inflammatory stimuli, and their soluble forms present in blood are the product of shedding or proteolytic cleavage of the ectodomain. Soluble forms of CAMs E-selectin (sE-Selectin), P-selectin (sP-selectin), and ICAM-1 (sICAM-1) are biomarkers of inflammation, and increased circulation with one or more of these markers has been associated with coronary artery disease [12], myocardial infarction [13] and atherosclerosis [14—16].

Recent studies have indicated substantial racial/ethnic differences in endothelial markers among healthy individuals [17]. Hwang et al. [14] identified lower circulation of sICAM-1 in African Americans compared to subjects of European ancestry, while significant racial/ethnic differences in sICAM-1 concentrations were similarly observed between Asian, Hispanic, black, and non-Hispanic white subjects in the Women's Health Study [18]. Racial/ethnic differences have also been observed for sP-selectin [19], sE-selectin [17,20] and vWF [21,22]. Although circulating levels of CAMs are highly heritable [23,24], and genetic studies have consistently identified associations between soluble levels and the *ABO* locus [25—29], associations between ABO blood type and endothelial markers in diverse populations have not been extensively studied. Herein, we examine associations of circulating protein measurements of sE-selectin, sP-selectin, sICAM-1, as well as vWF with genetically inferred ABO blood group alleles in a large, multi-ethnic cohort: the Multi-Ethnic Study of Atherosclerosis (MESA).

2. Materials and methods

2.1. Study participants

The Multi-Ethnic Study of Atherosclerosis (MESA), described in greater detail elsewhere [30], enrolled N = 6814 participants aged 45—84 and without existing clinical CVD from 2000 to 2002. This study population included 38% non-Hispanic White American (EUR), 28% African American (AFA), 22% Hispanic American (HIS), and 12% Chinese American (CHN) participants. MESA participants were examined at six field centers located in Baltimore, MD; Chicago, IL; Forsyth County, NC; Los Angeles County, CA; northern Manhattan, NY; and Saint Paul, MN. At each visit, information on demographics, cardiovascular risk factors, past medical history and co-morbidities, social history, family history, and medications was collected through a combination of self-administered questionnaires and interview-administered questionnaires. Height was measured while participants were standing without shoes, heels together against a vertical mounted ruler. Body mass index (BMI) was calculated as weight (kg)/height^2 (m^2).

For this study, stored DNA was available for additional genotyping of key *ABO* polymorphisms on N = 6276 MESA participants who consented to genetic studies. MESA and its ancillary studies were approved by the Institutional Review Boards at participating centers and all participants gave written informed consent.

2.2. Protein measurements

Circulating levels of sE-selectin and vWF were measured at the baseline exam (2000—2002) in a random subset of 1000 participants, of which 997 have available genetic information. sE-selectin was measured in serum using a high sensitivity quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Parameter Human sE-Selectin Immunoassay; R&D Systems, Minneapolis, MN). The inter-assay CV of the assay ranged from 5.7 to 8.8% with a minimum detectable level of 0.1 ng/mL vWF activity was measured in citrated plasma by an immunoturbidimetric assay on the Sta-R analyzer (Diagnostica Stago, Parsippany, NJ) with an inter-assay CV of 4.5%. For circulating concentrations of sP-selectin and sICAM-1, a stratified random sample including 720 individuals for each of the four races/ethnicities represented in MESA at Exam 2 (2002—2004) was used (N = 2880), of which 2574 participants and 2441 participants had available plasma and serum, respectively. sP-selectin was measured in EDTA plasma on 2529 MESA participants by ELISA using the Human soluble P-selectin/CD62P Immunoassay kit (R&D Systems), with an inter-assay CV was 6.7%, and a minimum detectable level of 0.5 ng/mL. For sICAM-1, measurements were collected in serum on 2374 participants by ELISA using the Human sICAM-1 Instant ELISA (Bender MedSystems GmbH) with an inter-assay CV of 9.1% and a minimum detectable level of 2.17 ng/mL.

2.3. Genotyping and annotation

The genotype data consisted of three individual genotyping panels: the Illumina Exome BeadChip [31], the Illumina Cardio-MetaboChip [32], and the Illumina iSelect ITMAT/Broad/CARe (IBC) Chip [33]. Each of the three panels had quality control measures individually performed on the genotype data prior to merging them together using Plink v1.07 [34] under NCBI genome build 37. Population stratification was assessed using EIGENSTRAT [35] and the first three ancestry-informative principal components (PCs) were considered for covariate adjustment. Additional genotyping of key functional *ABO* genetic variants necessary for ABO blood type prediction was conducted using a custom Sequenom (San Diego, CA) panel that included 10 *ABO* variants as part of a 27 variant panel design, three of which (rs8176746, rs1053878, rs7853989) were previously assayed by the Illumina arrays. Quality control for the Sequenom genotype data was based upon sample call rate (>90%), genotype call rate (>85%), and Hardy-Weinberg equilibrium ($p > 0.05/40 = 0.00125$; evaluated separately by race/ethnicity and adjusted for multiple testing). Each plate included a CEPH trio for quality control purposes, along with a random selection of 73 samples with duplicates. Discordant duplicate samples (N = 2) and samples with discordant genotypes for the three previously genotyped SNPs (N = 7) were excluded from further analyses.

2.4. ABO blood type allele prediction

Genetic evaluation of ABO blood type was determined on the basis of all *ABO* coding variants in the union of the merged chip genotype data and the *ABO* variants interrogated on the Sequenom panel. To fully characterize all identifiable *ABO* alleles, we first applied *de novo* haplotyping methods using the R statistical software package *haplo.stats*, which employs an expectation-maximization type algorithm to estimate subject haplotype pairs (i.e., diplotypes) while accommodating missing data. This analysis produces a posterior probability for each unique diplotype for a