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No evidence for an association between ABO blood group and overall ischemic stroke or any of the major etiologic subtypes

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ABSTRACT

Introduction: The ABO blood group system is encoded by one gene, *ABO*. Previous studies have reported an association between blood group non-O (i.e. phenotype A, B or AB) and myocardial infarction. Studies on stroke and ABO are, however, more scarce. Therefore, we aimed to investigate whether ABO phenotype or genotype is associated with ischemic stroke and/or etiologic subtypes of ischemic stroke.
Materials and methods: The study was performed in the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS), which comprises 600 patients with ischemic stroke before the age of 70 years, and 600 matched controls. Patients were classified according to the TOAST criteria.
Results: There was no significant association between ABO phenotype (blood group O vs. non-O) and overall ischemic stroke (multivariable odds ratio of 0.9, 95% confidence interval 0.7-1.2). This was also true for blood group O vs. A and O vs. B. Furthermore, no association between *ABO* genotypes and ischemic stroke was detected. The ischemic stroke subtype analysis was confined to large-vessel disease, small-vessel disease, cardioembolic stroke and cryptogenic stroke. In this analysis, there was no significant association between any ischemic stroke subtype and ABO phenotype or genotype.
Conclusions: The findings in this study suggest that ABO phenotype or genotype does not have a major impact in the pathophysiology of ischemic stroke or any of the ischemic stroke subtypes.

Introduction

The ABO blood group system is encoded by one gene, *ABO*, which gives rise to different glycosyltransferases that add sugar residues to the H-antigen producing A or B antigens on the surface of red blood cells [1]. These antigens produce the four phenotypes: O, A, B and AB. Polymorphisms in *ABO* give rise to a variety of alleles, and the most common alleles among Caucasians are A^1, A^2, B, O^1 and O^2 [1].

Previous studies, including a meta-analysis, have shown that the non-O phenotype (i.e. A, B or AB) is associated with myocardial infarction (MI) and coronary artery disease (CAD) [2–4]. There are also two recent genome-wide association studies (GWAS), in which significant associations between single-nucleotide polymorphisms (SNPs) at the *ABO* locus and CAD, or MI in the presence of CAD, were identified [5,6]. Fewer studies have investigated ABO in stroke. Early studies reported a lack of association between ABO and stroke [7–10], whereas a more recent meta-analysis found a small but significant increased risk of stroke for the non-O phenotypes [3].

Against this background, we aimed to investigate whether ABO phenotype is associated with overall ischemic stroke and/or any of the ischemic stroke subtypes. In light of the recent GWAS finding of an association between the *ABO* locus and MI [6], we also examined whether we could find an association between *ABO* genotypes and ischemic stroke and/or any of the etiologic subtypes.

Materials and Methods

Study Population

Details of the Sahlgrenska Academy Study on Ischemic Stroke (SAHLSIS) have been described elsewhere [11]. Briefly, this case–control study includes 600 consecutively recruited patients with ischemic stroke before the age of 70 years. All patients were examined by a physician trained in stroke medicine and all underwent neuroimaging. Each patient was classified according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria into the ischemic stroke

Abbreviations: SAHLSIS, Sahlgrenska Academy Study on Ischemic Stroke; TOAST, Trial of Org 10172 in Acute Stroke Treatment; MI, myocardial infarction; CAD, coronary artery disease; GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; RFLP-PCR, restriction fragment length polymorphism polymerase chain reaction; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; IS, ischemic stroke; LVD, large-vessel disease; SVD, small-vessel disease; CE, cardioembolic stroke; Crypt, cryptogenic stroke; SD, standard deviation.

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etiologic categories: large-vessel disease (n = 73), small-vessel disease (n = 124), cardioembolic stroke (n = 98), cryptogenic stroke (n = 162), other determined cause of stroke including dissections (n = 51), and undetermined stroke (n = 92). Cryptogenic stroke was defined for cases in which no cause was identified despite an extensive investigation. The undetermined stroke group included patients for whom more than one cause was identified or for whom the evaluation was cursory. For each subject, a healthy community control, matched for age, sex and geographic area, was randomly selected from participants in a population-based health survey or the Swedish Population Register. All participants provided written informed consent prior to enrolment. For those participants who were unable to communicate, consent was obtained from the next-of-kin. This study was approved by the Ethics Committee of the University of Gothenburg.

ABO Phenotyping

Fresh whole blood was unavailable for traditional antibody-based ABO phenotyping. Therefore, a DNA-based approach was used: restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) [12]. In this method, DNA is PCR amplified, digested with restriction endonucleases, and separated by agarose gel electrophoresis to determine the RFLPs according to fragment length. This method determines 15 different genotypes, based on the five most common *ABO* alleles (A^1, A^2, B, O^1 and O^2). From these genotype data, individuals can be grouped according to phenotype: phenotype O includes genotype O^1O^1, O^1O^2, and O^2O^2; phenotype A includes A^1A^1, A^1A^2, A^1O^1, A^1O^2, A^2A^2, A^2O^1, and A^2O^2; phenotype B includes BB, BO^1, and BO^2; and phenotype AB includes A^1B and A^2B.

DNA was extracted from frozen whole blood with Maxi DNA isolation PLUS (Agowa, Berlin, Germany). Oligonucleotide primers (Applied Biosystems, Foster City, CA, USA) and protocols were used as previously described by Olsson et al. [12], with a few minor modifications. In brief, the PCR Master Mix was prepared using 10 ng/μl genomic DNA, 0.1 μM primer and 1 U Taq polymerase (AmpliTaq 360 DNA Polymerase, Applied Biosystems, Foster city, CA, USA), in a total volume of 11 μl. The GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA) was used for thermocycling with an initial temperature of 94 °C for 2 min, 10 cycles at 94 °C for 20 sec, 63 °C for 30 sec, and 72 °C for 1 min. This was followed by 30 cycles at 94 °C for 20 sec, 61 °C for 30 sec and finally 72 °C for 1 min. Two U of restriction endonucleases *Kpn1* and *HpaII* (New England Biolabs, Ibswich, MA, USA) were added in a digestion mix (5 μl). The cleaved PCR product was separated by electrophoresis using a 4% agarose gel (NuSieve GTG Agarose, Lonza, Rockland, USA). For each individual, the DNA fragment pattern was used to determine the *ABO* genotype.

ABO Genotyping

To capture the genetic variation at the *ABO* locus, genotype data from the CEU population in HapMap (release 22) was used to tag *ABO* with $r^2 = 0.8$ and minor allele frequency (MAF) > 0.1. Eight tagSNPs (rs512770, rs625593, rs630014, rs687621, rs7853989, rs8176731, rs8176682, and rs8176747) were selected. Genotyping was performed with TaqMan assays (Applied Biosystems, Foster City, CA, USA). The assay for rs8176747 was non-functional and could not be replaced. Genotyping was performed blinded to case/control status.

Statistical Analyses

ABO phenotype was analysed as a categorical variable (O, A, B, AB), or phenotype O vs. non-O. An additive model was used for analysis of tagSNPs. Associations between ABO phenotype, tagSNPs or haplotypes and overall ischemic stroke as well as ischemic stroke subtypes were investigated using uni- and multivariable conditional logistic regression, with adjustments for hypertension, diabetes mellitus, and smoking status. All statistical calculations were performed using IBM SPSS Statistics version 19 for Windows (SPSS Inc., Chicago, Illinois, USA) and HelixTree 6.3 (Golden Helix, Bozeman, MT, USA). The statistical significance level was 0.05 and *P*-values were two-tailed. For the tagSNPs, assuming a multiplicative genetic model, the odds ratios (ORs) that can be detected for overall ischemic stroke with 80% power are in the range of 1.25-1.41, depending on the MAF (0.43-0.11). Regarding ABO phenotype, ORs below 0.82 can be detected for overall ischemic stroke with 80% power for a protective effect of the O phenotype.

Results

ABO Phenotypes and Ischemic Stroke

Baseline characteristics of the participants in SAHLSIS are shown in Table 1. The genotyping success rate with RFLP-PCR was 100%. The distribution of the four most common ABO phenotypes, O, A, B and AB, is shown in Table 2. Since there were few individuals with blood group AB, no comparison between blood group O vs. AB was made. There was no significant association between ABO phenotype and overall ischemic stroke in the univariable regression analysis, for all three comparisons (i.e. O vs. non-O, O vs. A and O vs. B) (Table 2). Including vascular risk factors in the model did not change the results.

The ischemic stroke subtype analysis was confined to the four major etiologic subtypes, i.e. large-vessel disease, small-vessel disease, cardioembolic stroke, and cryptogenic stroke. None of these etiologic ischemic stroke subtypes were associated with ABO phenotype in the univariable regression analyses (Table 2). This was also true after adjustment for vascular risk factors, for all three comparisons (i.e. O vs. non-O, O vs. A or O vs. B), and for all subtypes. To increase the power in the subtype analysis, an unconditional regression analysis was also performed including the whole control population. Adjustment for age, sex, geographic area, hypertension, diabetes mellitus and smoking status was made in the multivariable analysis. In these analyses, similar results as in the uni- and multivariable conditional regression analysis were obtained (data not shown).

Table 1
Baseline characteristics of controls, overall ischemic stroke and the etiologic subtypes.

	Control (n = 600)	Overall IS (n = 600)	LVD (n = 73)	SVD (n = 124)	CE stroke (n = 98)	Crypt (n = 162)
Mean age, y (SD)	56 (10)	56 (10)	59 (8)	58 (7)	57 (10)	53 (12)
Male sex, n (%)	385 (64)	385 (64)	54 (74)	77 (62)	66 (67)	95 (59)
Hypertension, n (%)	224 (37)	354 (59)	44 (60)	89 (72)	50 (51)	87 (54)
Diabetes mellitus, n (%)	33 (6)	114 (19)	25 (34)	26 (21)	19 (19)	23 (14)
Current smoking, n (%)	109 (18)	233 (39)	39 (53)	54 (44)	34 (35)	60 (37)

IS indicates ischemic stroke; LVD, large-vessel disease; SVD, small-vessel disease; CE, cardioembolic stroke; Crypt, cryptogenic stroke; SD, standard deviation.