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Original Article

ABO blood group is a cardiovascular risk factor in patients with familial hypercholesterolemia

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BACKGROUND: The ABO blood group has been associated with cardiovascular disease (CVD) in observational studies. However, the effect of ABO blood group has never been studied in subjects affected by familial hypercholesterolemia (FH), a severe monogenic disease characterized by accelerated atherosclerotic plaque development.

OBJECTIVE: Our aim is to investigate the effect of the ABO blood group on CVD risk in FH patients.

METHODS: A total of 668 adult subjects with a heterozygous FH-causing mutation in the low density lipoprotein receptor (*LDLR*) gene were included in the present study. ABO blood group was determined using 2 functional single-nucleotide polymorphisms in the *ABO* gene (rs8176719 and rs8176746).

RESULTS: Total cholesterol was significantly higher in non-O subjects compared to carriers of the O group (9.48 vs 9.14 mmol/L, $P = .02$). We observed a greater proportion of subjects carrying the non-O groups (73.4%) in patients with CVD compared to subjects without CVD (63.3%). In a regression model corrected for cardiovascular risk factors, the non-O group was significantly associated with an increased prevalence of CVD (odds ratio = 2.14, 95% confidence interval = 1.25–3.65, $P = .005$). In average, patients in the non-O blood group experienced more CVD events (0.88 per individual) than those in the O group (0.60 per individual), $P = .008$.

CONCLUSION: Carrying a non-O blood group is associated with an independent twofold increased risk of CVD in FH patients. The ABO blood group represents a novel CVD risk factor in FH subjects that is often known by the patient and could be used to further stratify CVD risk in this population of patients.

Introduction

In 1901, Karl Landsteiner discovered the ABO system, which includes 4 principal blood groups, A, B, AB, and O.[1] ABO antigens are carbohydrate moieties expressed on the surface of red blood cells, as well as other cells and tissues, and represent the major determinants of blood transfusion compatibility. The A and B alleles encode for the glycosyltransferase enzymes N-acetylgalactosamine and D-galactose respectively, whereas carrying the O allele is associated with the absence of glycosyltransferase activity and the absence of A or B antigens on the surface of red cells.[2]

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The first association study between ABO system and cardiovascular disease (CVD) in a large study was published in 1962 by Bronte-Stewart et al., who observed a higher proportion of groups A and B and a reduced proportion of group O in patients with ischemic heart disease when compared with controls.[3] Since then, several observational studies have shown the association between the ABO system and CVD, as documented by a number of recent reviews and meta-analyses.[4–7]

Strong evidence linking the ABO system to CVD was obtained via large genome-wide association studies (GWASs). Indeed, 2 single-nucleotide polymorphisms (SNPs) of the *ABO* gene, rs579459 and rs514659, which are associated with the presence of the A (A1) and the O phenotype, respectively,[8–11] have both been linked to coronary artery disease (CAD) in large GWAS.[12–14] However, it is still not clear whether these SNPs are associated with CVD through their link with the ABO antigens or through an effect independent of the ABO system.[15]

Individuals carrying non-O blood groups have 25% higher plasma levels of von Willebrand factor (VWF) compared to group O.[16] Higher VWF is a risk factor for CVD by promoting both arterial and venous thrombosis.[17,18] Some studies also reported that ABO blood group might be associated with plasma lipid levels,[19] the non-O blood groups having higher levels of total cholesterol.[20,21]

Familial hypercholesterolemia (FH) is an autosomal codominant monogenetic disease affecting at least 1:250 people worldwide.[22–24] Causal mutations are located mainly in the low density lipoprotein (LDL) receptor (*LDLR*) gene and also in the apolipoprotein B (*APOB*) or the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes[25–27] and lead to extremely high levels of circulating low density lipoprotein cholesterol (≥95th percentile for age and sex).[22] This disease is associated with a drastic increase in the risk of premature atherosclerotic cardiovascular events, usually occurring between the age of 35 and 55 years.[28] Even if FH patients are already considered at very high risk of CVD due to the lifelong burden of low density lipoprotein cholesterol accumulation in blood, other genetic and clinical factors can significantly contribute to this cardiovascular risk.[29–33] We have recently shown that the combination of age, gender, high density lipoprotein cholesterol (HDl-C), hypertension, and smoking in a score (the Montreal-FH-SCORE) was very significantly associated with prevalent CVD events.[31]

Although the relationship between ABO blood antigens and the risk of CVD has already been studied in the general population, this association has never been investigated in patients with FH.

Material and methods

Study population and data collection

The present study followed a retrospective Mendelian randomization design. Heterozygous FH patients were recruited at the Nutrition, Metabolism and Atherosclerosis Clinic of the Institut de recherches cliniques de Montréal. Briefly, genetic testing was performed for the classical French-Canadian (F-C) *LDLR* mutations, among the Nutrition, Metabolism and Atherosclerosis Clinic patients in whom FH was suspected based on a Dutch Lipid Criteria score ≥ 3. These mutations included the deletion >15 kb of the promoter and exon 1, deletion >5 kb of exons 2 and 3, W66 G (exon 3), E207 K (exon 4), Y468X (exon 10), and C646Y (exon 14). Among the 1267 patients who were screened, a total of 725 mutation-positive FH individuals were identified. We further excluded 55 patients < 18 years old and 2 homozygous FH patients, and a total of 668 adult patients with genetically confirmed heterozygous FH were included in the analysis. Of these FH patients, 100% were Caucasians.

The primary outcome of this study was CVD events, defined as the presence of fatal or nonfatal coronary, cerebral or peripheral vascular disease (PVD). The specific cardiovascular events included in the present study were the following: angina, myocardial infarction, coronary angioplasty, coronary bypass surgery, claudication, peripheral angioplasty, peripheral arterial surgery, transient ischemic attack, stroke, and carotid endarterectomy.

The signature of a written informed consent, approved by the Institut de recherches cliniques de Montréal Ethics Committee in accordance with the Helsinki Declaration, was required to be included in the study.

Biochemical and DNA analysis

Details of the methods used for lipids, lipoproteins, and DNA analysis have been published elsewhere.[29] Briefly, samples were obtained after a 4-week washout from any lipid-lowering medication and after a 12-hour fast. Ultracentrifugation was used to determine lipoprotein concentrations according to the gold standard method,[34] and total cholesterol and triglycerides were measured using an enzymatic method (Abbott Bichromatic Analyzer Model 100; Abbott Laboratories, Pasadena, CA). Lipoprotein (a) (Lp [a]) and apolipoprotein B concentrations were assayed using an ELISA kit (Macra EIA Kit; Strategic Diagnostics Industries, Inc, Newark) and using electroimmunoassay (Behringwerke, Marburg, Germany), respectively.

Either a polymerase chain reaction–based method, a southern blot analysis, or a semiquantitative polymerase chain reaction assay were used to detect the presence of the F-C *LDLR* mutations included in this study.

ABO blood group determination

ABO blood group was determined from 2 known functional SNPs in the *ABO* gene (rs8176719 and rs8176746) as previously published.[35–37] The rs8176719 variant results in the O allele, whereas rs8176746 allows to distinguish the B allele from the A allele (see Supplemental Table 1). The Illumina HumanCoreExome-24, version