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ABO histo-blood group might modulate predisposition to Crohn's disease and affect disease behavior

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Abstract

Background and aims: *ABO* encodes a glycosyltranferase which determines the major human histo-blood group. The *FUT2* fucosyltransferase allows expression of ABO antigens on the gastrointestinal mucosa and in bodily secretions (secretor phenotype). A nonsense allele in *FUT2* represents a susceptibility variant for Crohn's disease, and both the secretor and ABO blood group status affect the composition of the gut microbiota. Thus, we evaluated if variants in *ABO* might represent good candidates as Crohn's disease susceptibility loci.
Methods: We recruited two case–control cohorts, from Italy (n = 1301) and Belgium (n = 2331). Subjects were genotyped for one SNP in *FUT2* and two variants in *ABO*.
Results: No effect on Crohn's disease risk was detected for *ABO* variants, whereas an association was observed between the *FUT2* polymorphism and Crohn's disease susceptibility in the Belgian sample, but not in the Italian cohort. The effect of histo-blood groups was evaluated using group O as the reference. Most non-O groups had odds ratios (ORs) higher than 1 in both cohorts, and combined analysis of the two samples indicated a predisposing effect for the A and B groups (OR = 1.17, 95% CI: 1.02–1.32 and OR = 1.33, 95% CI: 1.09–1.58, respectively). In Crohn's

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disease patients, the non-O blood group and the non-secretor status were associated with higher risk of developing a stricturing or penetrating disease.
Conclusions: ABO histo-blood group might confer susceptibility to Crohn's disease and modulate disease severity.

1. Introduction

The *ABO* gene encodes a polymorphic glycosyltranferase which transfers N-acetyl D-galactosamine (group A) or D-galactose (group B) to a common substrate (antigen H). *ABO* loss of function variants result in the O blood group. In humans an α (1,2)-fucosyltransferase encoded by *FUT2* (*Se* enzyme, Lewis blood group system) determines the expression of the H antigen in secretory glands and on the surface of epithelial cells. Individuals referred to as "secretors" have at least one functional *FUT2* allele and display H and ABO histo-blood group antigens on the gastrointestinal mucosa. Common *FUT2* null variants are present in many populations; in particular a frequent stop codon allele (se^{428}) is responsible for most non-secretor phenotypes in Europe.[1]

The secretor/non-secretor trait has been associated with several conditions such as development of duodenal ulcerations and predisposition to oral candidiasis, as well as susceptibility to different infections, including those caused by cholera, Norovirus, and *Helicobacter pylori* (reviewed in Ref. 2). These same pathogens have been shown to affect the host with variable severity depending on the ABO blood group status.[2] In fact, both pathogenic and commensal microorganisms exploit oligosaccharides on the gastrointestinal mucosa for adherence. For example some lactobacilli specifically bind A antigens[3] whereas *Lactobacillus plantarum* recognizes both A and B-oligosaccharides.[4] Thus, secretor and ABO blood group status affects both the predisposition to specific infections and the composition of the gut microbiota. For example bifidobacterial diversity and abundance are reduced in the intestine of non-secretor individuals,[5] whereas subjects carrying the B antigen show higher diversity of *Clostridium* species.[6]

Host–microbe interactions in the gut mucosa are thought to play a role in several human conditions, including Crohn's disease (CD), a chronic inflammatory disease of the gastrointestinal tract.[7] CD results from a combination of genetic and environmental risk factors, and recent evidence has indicated that the common non-secretor allele represents a risk factor for the diseases[8,9] in populations of European ancestry. Subsequent analyses indicated that the *FUT2* genotype explains a substantial portion of variability in microbiota composition between CD patients and healthy subjects.[10] Given the functional interaction between the *FUT2* and *ABO* gene products, as well as the role of both secretor status and blood group antigens in regulating the gut microbiota composition, variants in *ABO* represent potential candidates as genetic risk factors for CD. Thus, we assessed the role of *ABO* single nucleotide polymorphisms (SNPs) and histo-blood group in predisposing to CD and in affecting disease presentation.

2. Materials and methods

2.1. Subjects

Italian CD patients (333 males, 287 females) and controls (415 males, 266 females) were recruited by the IBD Unit of the Luigi Sacco Hospital in Milano, a third-level center for the management of inflammatory bowel disease (IBD) patients. The diagnosis of CD was based on international published criteria, according to clinical, endoscopic, histological and/or radiological data.[11] Controls were subjects who did not suffer from any pathology at the moment of recruitment. All patients and controls were unrelated Italians of European origin. Belgian CD patients (633 males, 889 females) and controls (382 males, 427 females) were recruited in the framework of the IBD genetics study that started in 1997 at the IBD unit of the University Hospital in Leuven, Belgium. Diagnosis of CD was based on standard clinical, endoscopic and histological criteria. Disease location and behavior were recorded according to the Montreal classification.[12] Taking into account that CD behavior and location vary over the course of the disease in a great proportion of patients,[13] only patients with a minimum of 10 years of follow-up since CD diagnosis were used for the analysis of disease behavior and location. The controls were all unrelated, and without a family history of IBD or other immune related disorders.

The ethical board of the UZ Leuven approved the study (approval number of Ethical Committee: B322201213950/S53684). All included participants gave informed consent. Samples and data were stored in a coded, anonymized database. DNA was extracted from whole venous blood and stored at −80 °C.

2.2. Genotyping and statistical analyses

Genotyping of rs492602 (*FUT2*), rs8176749 (*ABO*) and rs687289 (*ABO*) was performed by TaqMan probe assays (TaqMan SNP genotyping assay, Applied Biosystems, Foster City, CA, USA) using the allelic discrimination real-time PCR method. Genotyping rate was >0.97 for all SNPs.

Association analysis for single variants was performed using an additive model within a logistic regression framework using the PLINK software.[14] Meta-analysis was performed using a random effect model, as implemented in PLINK.[14] The association test between ABO histo-blood group and CD susceptibility was performed using Fisher's exact test with the O blood group as a reference. Therefore, odds ratios (ORs) and confidence intervals (CIs) are presented for each of the non-O groups and for all non-O groups together. As above, the combined odds ratios, confidence intervals

and p-values were obtained from a random-effect meta-analysis implemented in the "rmeta" R package.[15]

Multinomial logistic regression was performed using SPSS. Logistic regression models with covariates (as specified in the text) were implemented in R (http://cran.r-project.org/). Power analysis was performed using the Genetic Power Calculator.[16]

3. Results

To address the possible role of genetic variation in *ABO* in conferring susceptibility to CD, we recruited two case–control cohorts, from mainland Italy and from Belgium. All subjects were of European ancestry, and they were genotyped for one SNP in *FUT2* and two variants in *ABO*. In European populations rs492602 in *FUT2* is in full linkage disequilibrium ($r^2 = 1$ in HapMap CEU and TSI) with the stop codon polymorphism (rs601338, se^{428}) determining the common non-secretor status. The two *ABO* SNPs (rs8176749 and rs687289) were selected to allow inference of the three major haplotypes (O, A, and B) which in turn determine blood groups (A, AB, B and O).[17]

Power estimates indicated that, if each analyzed polymorphism/haplotype were to directly confer at least a 1.4-fold increase in the relative risk of CD, the case/control cohorts used in this study would be of sufficient size to have a power higher than 80% to detect a significant association at the 0.05 level assuming a recessive mode of inheritance both for the *FUT2* non-secretor allele and for the O group haplotype, and a dominant model for the non-O group haplotypes (considered together).

The two variants in *ABO* complied with Hardy–Weinberg equilibrium (HWE) in cases and controls in both the Italian and Belgian sample (Bonferroni-corrected p-values >0.5). Conversely, rs492602 in *FUT2* significantly deviated from HWE in Belgian CD patients (corrected p-value = 0.014), but not in controls or in Italian cases.

Logistic regression was used to compare allele and genotype frequencies in CD patients and healthy controls (HC) from both cohorts: no significant effect was detected for ABO variants in either cohort (Table 1). The *FUT2* polymorphism was significantly associated with CD susceptibility in the Belgian sample (Table 1). The G allele of rs492602 is in phase with the recessive non-secretor stop codon allele; thus, in line with previous reports,[8,9] the frequency of non-secretor individuals was higher among Belgian CD subjects (23.9%) compared to Belgian HC (18.3%); no such difference was evident in the Italian cohort (non-secretor frequency = 22.2% and 21.6% in CD patients and HC, respectively).

We next sought to verify whether any association could be detected between histo-blood groups and risk of CD. Thus, ABO haplotypes at rs8176749 and rs687289 were used to infer each individual's blood group (A and B groups co-dominant, O group recessive). A minority of subjects (less than 0.6% in both the Belgian and Italian cohorts) displayed rare haplotypes and were removed from the analyses as their blood group could not be inferred. Thus, after accounting for few failed genotypes in *ABO* or *FUT2* variants, blood group and secretor status could be determined for 1190 Italian (572 CD patients, 618 HC) and 2316 Belgian (1512 CD patients, 804 HC) subjects (Supp. Table 1). In both samples the A and O groups had similar frequency (slightly higher than 40%) and the latter was used as a reference for odds ratio (OR) calculation.

As shown in Fig. 1, the A and B groups had ORs >1 in both the Italian and the Belgian cohort; a combined analysis revealed a significant effect of these two groups on CD susceptibility. The AB status gave contrasting results in the two samples, possibly due to its low representation in the population. Overall, the combined analysis indicated that carrying a non-O blood group might increase the risk of developing CD (Fig. 1). Similar results were obtained when only secretor subjects were analyzed (Fig. 1), although the smaller sample size resulted in significant association in the combined samples for non-O subjects only. Non-secretors were not separately analyzed due to their small sample size.

We next assessed whether ABO histo-blood group affects disease presentation in CD patients. Because disease location and especially disease behavior tend to vary over time,[13] we included only patients with a follow-up of at least 10 years (i.e. 647 subjects from the Belgian sample). According to the Montreal classification,[12] disease location was defined as colonic, ileocolonic or ileal; a multinomial logistic regression that accounted for secretor status and age at diagnosis revealed no effect of histo-blood group (O vs non-O) on disease location; no effect was detected for secretor/non-secretor status, either.

Conversely, disease behavior was found to be affected by ABO status. Specifically, a logistic regression that accounted for secretor status, disease location and age at diagnosis revealed that O subjects are less likely to develop a stricturing (B2) or penetrating (B3) form than a non-complicated (B1) disease (OR = 0.70, 95% CI: 0.44–0.98, p = 0.038) (Fig. 2). Likewise, after accounting for O/non-O histo-blood group (as well as age at diagnosis and disease location), non-secretors were found to

Table 1 Association study for *ABO* and *FUT2* variants with CD in two case–control cohorts.

SNP (gene)	Minor allele	Italian cohort			Belgian cohort			Combined
		Genotype counts		p[a]	Genotype counts		p[a]	p[b] (corrected p[c])
		CD (n = 620)	HC (n = 681)		CD (n = 1522)	HC (n = 809)		
rs8176749 (*ABO*)	T	9/105/500	1/110/546	0.0868	11/211/1296	1/102/706	0.1371	0.0421 (0.1263)
rs687289 (*ABO*)	A	76/301/237	108/279/267	0.8042	179/721/620	102/341/366	0.2050	0.6111 (1)
rs492602 (*FUT2*)	G	137/321/158	142/342/174	0.8865	364/702/453	148/375/283	0.0006	0.1500 (0.4500)

[a] p-Values calculated using logistic regression using an additive model.
[b] Random-effect meta-analysis p-value.
[c] Bonferroni-corrected p-value.

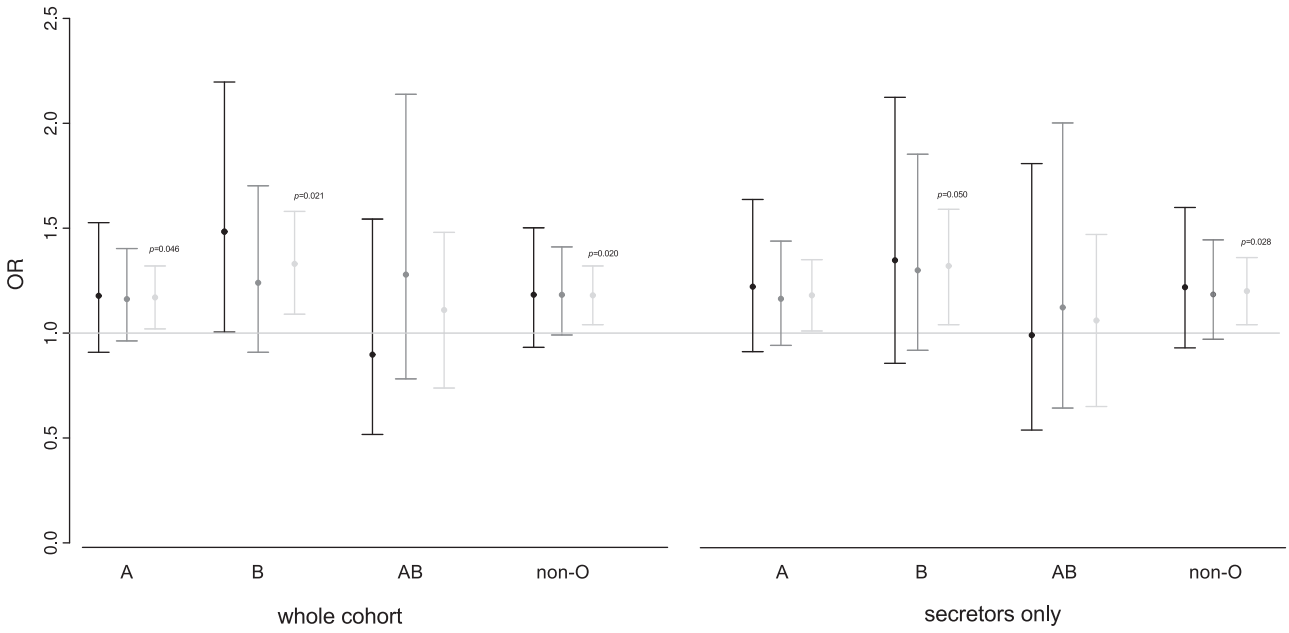

Figure 1 Odds ratios and 95% confidence intervals for the A, B, AB, and non-O blood groups relative to blood group O. Analyses were performed for the Italian (black) and Belgian (dark gray) cohorts and for the combined sample (light gray). Significant p-values are reported. Analysis for secretor subjects is also shown.

be at slightly higher risk of stricturing/penetrating behavior (OR: 1.51, 95% CI: 1.01–2.25, p = 0.046) (Fig. 2).

4. Discussion

In recent years, genome-wide association studies and meta-analyses have identified 140 susceptibility loci for CD.[18]

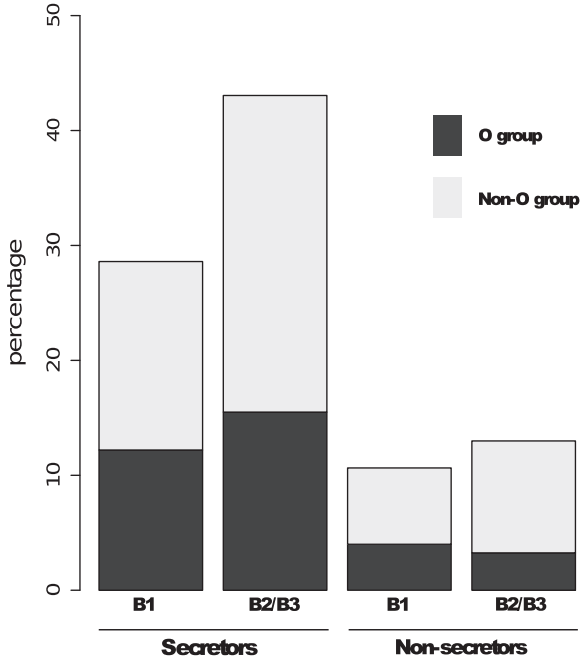

Figure 2 Distribution of disease behavior in 647 CD patients grouped according to O/non-O histo-blood group and secretor status.

Nonetheless, a major portion of genetic risk factors remains to be identified, as known variants explain less than 25% of disease heritability.[8,19] Some missing risk loci are likely to be accounted for by common variants with weak effect and by undetected interactions among genetic factors or between genes and environmental clues. Thus, analysis of genes that impinge on common biochemical pathways and have a potential role in CD pathogenesis might uncover novel associations. CD is thought to result from an abnormal immune response to commensal bacterial species, and the composition of the gut microbiota has been shown to differ between CD patients and healthy controls.[20–23] The identification of *FUT2* as a susceptibility locus for CD,[8,9] and the demonstration that the secretor/nonsecretor status affects the composition of the gut microbiota[5] have supported the hypothesis that the disease is caused by a combination/interaction of genetic and environmental effects. Indeed, it has recently been shown that CD disease status interacts with the *FUT2* genotype in shaping the composition of the gut microbiota.[10] The gene product of *FUT2* determines the expression of the precursor of ABO antigens on the intestinal mucosa and blood group status has also been shown to affect the composition of commensal microbial communities.[6] These observations make the common *ABO* variants and the resulting histo-blood groups very good candidates as modulators of CD susceptibility.

We analyzed variants in *ABO* and *FUT2* in two populations of European ancestry. The lack of association of single *ABO* polymorphisms is consistent with histo-blood group being specified by haplotypes that display both dominant/recessive and co-dominant behavior. Thus, when blood group status was analyzed we observed a tendency for non-O groups to predispose to CD in both cohorts, and a significant effect of A, B and non-O status in the combined sample was detected. Stratification on the basis of *FUT2* genotypes

yielded similar results in secretor subjects. In the Italian sample we found a similar frequency of non-secretor individuals in CD patients and healthy controls. A possible explanation for this finding is that the *FUT2* genotype interacts with environmental factors (e.g. specific commensal/pathogenic species) that differ across geographic locations; indeed, analysis of gut microbial communities has unveiled considerable differences among individuals living in different countries.[24]

By analyzing a limited number of subjects Miyoshi and coworkers have suggested that secretors and non-secretors might be differently prevalent among CD patients depending on the major site of inflammation (ileal, colonic or ileocolonic).[25] In their study the authors also reported that in CD patients and in *Il10*$^{-/-}$ mice, an experimental model of CD, the expression of the ABO and H-antigens is increased in the gut mucosa. Analysis of *Il10*$^{-/-}$ mice indicated that H-antigen mucosal over-expression precedes the onset of inflammatory symptoms, suggesting a role in promoting disease pathogenesis.[25] Data herein do not support a role for either ABO histo-blood group or secretor status in affecting disease location; conversely, in line with the report by Miyoshi and co-workers, our results indicate that non-O carriers are at higher risk of developing a severer disease compared to O-group subjects; the same observation holds for non-secretors, although the effect seems to be weaker. These data strengthen the potential role of non-O blood group status as a CD risk factor and suggest that genetic modulators of disease susceptibility also affect CD behavior, as previously shown for other CD risk variants.[26]

Nonetheless, given the relationship between the secretor phenotype and the ABO status, the observations whereby non-secretors and non-O carriers are at higher CD risk and present unfavorable disease behavior seem difficult to reconciliate. Thus, the precise role of H and ABO antigen expression in CD susceptibility remains to be clarified. In a recent work species belonging to the *Lactobacillus* genus were found to be particularly associated with healthy secretor subjects compared to CD patients and non-secretors.[10] The authors suggested that this finding is consistent with the probiotic effect of these bacteria and might derive from their expression of adhesins for blood group A and B antigens.[10,27] Again, this observation suggests a role for ABO group status in CD susceptibility, but a protective rather than predisposing role of A and B groups would be expected. Further analyses are clearly required to clarify these points. The complex interplay between genetic and environmental factors influencing microbial composition, as well as the role of chronic infection in CD susceptibility, is only beginning to be appreciated. Blood group antigens are exploited by several pathogenic and commensal bacterial and viral species for mucosal attachment. Indeed, it has been shown that polymorphisms in the *FUT2* and *ABO* genes are maintained in human populations by natural selection.[28–31] Thus, subjects with different blood group and secretor status are susceptible to some infections and resistant to others.[2] Recent evidence has indicated that chronic infections with bacterial species such as *Mycobacterium avium*[32] and *Clostridium difficile*[33] might underlay the pathogenesis of at least a portion of CD patients. Whether the susceptibility to these and other pathogens is modulated by the ABO and secretor/nonsecretor status remains to be evaluated, but might help explain the associations we report herein and, possibly, that previously described in *FUT2*. In summary, data herein suggest a role for non-O histo-blood groups in the susceptibility to CD and indicate that non-O group carriers and non-secretors are at high risk of developing a stricturing or penetrating disease.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2013.10.014.

Conflict of interest

The authors declare that they have no conflict of interest.

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