Mannose-binding lectin-2 genetic variation and stomach cancer risk

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Deficiency of the mannose-binding lectin (MBL) protein, an antigen-recognition molecule involved in systemic and mucosal innate immunity, is determined by variant alleles in MBL2 gene promoter and exon-1 regions. We conducted a population-based study on 305 stomach cancer cases and 427 controls in Warsaw, Poland, to determine whether MBL2 gene variants predispose to stomach cancer. Single nucleotide polymorphisms (SNPs) in MBL2 were determined by TaqManTM. The 5 tested MBL2 variants are in complete linkage disequilibrium and comprise 6 different haplotypes. The risk of stomach cancer was increased in subjects carrying the H/H promoter genotype (OR = 1.8, 95% CI 1.1–2.9; p = 0.020) relative to L/L carriers, after adjustment for age, gender, education and smoking. Carrying at least one D exon-1 allele was associated with nonsignificant excess risk (OR = 1.5, 95% CI 0.9–2.4; p = 0.09). In haplotype analysis, the HYD haplotype was associated with increased risk of stomach cancer when compared with HYA, the most common haplotype (OR = 1.9, 95% CI 1.1–3.2; p = 0.021). In diplotyping analysis, subjects carrying the YA/D haplotype combination showed the highest risk (OR = 3.0, 95% CI 1.5–5.9; p = 0.015), compared with YA/YA. Further analyses to examine the joint effect of MBL2 and IL-1B polymorphisms, previously shown to predispose to stomach cancer, indicated that the combination of at-risk IL-1B genotypes (CT or TT at location -511) and HYD MBL2 haplotype was associated with a 3.5-fold risk (OR = 3.5, 95% CI 1.6–7.6; p = 0.001). Our findings suggest that the codon 52 D MBL2 variant causing a cysteine > arginine replacement, but not B and C variants producing glycine substitutions, is specifically associated with gastric cancer risk.

Material and methods

Study design

The design of the population-based case-control study of stomach cancer has been described in detail previously. Briefly, Warsaw residents aged 21–79 years, who were newly diagnosed with stomach cancer (ICD-O 151 or ICD-O-2 C16) between 1994 and 1996, were identified by collaborating physicians in each of the 22 hospitals serving the study area. All diagnoses were pathologically confirmed. Controls were randomly selected among Warsaw residents from a computerized registry of all legal residents in Poland, the Polish Electronic System of Residence Evidence (PESEL), and were frequency-matched to cases by sex and age in 5-year groups. The system was updated monthly, and completeness of registration was estimated to be nearly 100%.

The study protocol was approved by the institutional review boards of the National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland, USA and M. Sklodowska-Curie Institute of Oncology, Warsaw, Poland. After written informed consent was obtained, detailed information on lifetime tobacco use, alcohol consumption, family history of stomach cancer, childhood living conditions, demographic background, history of acute lymphoblastic leukaemia appeared increased in children carrying defective MBL2 variants, suggesting modulation of an infectious cause that remains to be identified.

In previous investigations, we and other groups showed a significantly increased risk of stomach cancer and precancerous gastric lesions in subjects carrying proinflammatory gene polymorphisms of the IL-1 cluster. Between 2000 and mid-2005, 26 studies on the association of IL-1 polymorphisms and gastric cancer were published, of which 21 found significant positive associations, although the specific genotype differed somewhat between Caucasian and Asian populations.

We were, therefore, motivated to assess the relationship between other immune-response or inflammatory genes and the risk of stomach cancer. Herein, we report the findings for MBL2 variants and stomach cancer risk in a population-based case-control study in Warsaw, Poland.
selected medical conditions and medication use, lifetime occupational history and usual diet prior to 1990 was recorded during a personal interview. Among the 464 stomach cancer cases and 480 controls identified for the study, genomic DNA was obtained from 305 (65.7%) cases and 427 (90.1%) controls.

Genotyping assays

Genotyping of MBL2 single nucleotide polymorphisms (SNPs) was performed by TaqMan™ assays (Applied Biosystems, Foster City, CA) at the Core Genotyping Facility (CGF), National Cancer Institute, National Institutes of Health (NIH). Assays were validated and optimized as described in the SNP500 Cancer website (http://snp500cancer.nci.nih.gov). Assay-specific primer/probe concentrations and thermocycling conditions for the 5 tested MBL2 variants (rs5030737, rs1800450, rs1800451, rs110003125 and rs7096206) are also available on the website. For each genotype, as a laboratory internal quality control, 4 human DNA controls (Coriell DNA) as well as no template controls were run with study samples. Approximately 10% blind quality control samples from 40 individuals were interspersed with the study samples, showing greater than 99% concordance. Genotyping data for each tested SNP were successfully obtained for ≥95% of the subjects. Data on IL-1 genotypes previously described for this same study31 were evaluated in the present analysis to assess a possible interaction with the MBL2 variants.

Haplotype determination

SNP results were combined in haplotypes by assuming complete linkage disequilibrium between the tested loci, as previously demonstrated.7 MBL2, located on chromosome 10q11.1–q21, consists of 4 exons interrupted by 3 introns. The promoter polymorphisms tested in our study were C-550 G (rs11003125, known as H/L variant) and G-221 C (rs7096206, known as X/Y variant). The 3 tested polymorphisms in exon 1 were at codon 54 (G/GC to GAC) causing an arginine > histidine substitution,7,8 at codon 57 (CGT to TGT) causing an arginine > methionine substitution (rs5030737, also known as allele B), at codon 57 (GGA to GAA) causing a glycine > aspartic acid substitution (rs1800450, also known as allele C) and at codon 52 (CGT to TGT) causing an arginine > cysteine substitution (rs5030737, also known as allele D). Investigations conducted on large numbers of subjects in populations representing various genetic backgrounds have demonstrated that the 5 SNPs tested in our study are in linkage disequilibrium.8 Thus, every individual will express 2 of the only 6 possible haplotypes, indicated as HYA, LYA, LXA, LYB, LYC and HYD, using common nomenclature in which the first 2 letters stand for the 2 SNPs in promoter region, and the third letter indicates the combination of the 3 exon-1 SNPs using A (i.e., non-B, non-C, and non-D), B, C or D.6–8 Consistently, the HYA, LYA and LXA haplotypes were composed in our data by combinations of H-L and X-Y promoter polymorphisms with exon-1 variants are represented by a single-letter notation (B, C and D allele. The D/D genotype was found in only 1 case and 1 control. When subjects with one or both D alleles were combined, the OR was 1.5 (95% CI 0.9–2.4, p = 0.081). Exon-1 alleles at codon 54 and codon 57 were not associated with risk of stomach cancer.

Consistent with previous studies at the MBL2 gene loci,7,9 combination of the 5 tested SNPs resulted in 6 different haplotypes (Table III). The HYD haplotype was associated with increased risk of stomach cancer (OR = 1.9; 95% CI 1.1–3.2, p = 0.021), when compared with the HYA most common haplotype. The remaining haplotypes (LXA, LYA, LYA and LYC) were unrelated to risk (Table III). Consequently, when the LYB, LYC and HYD haplotypes, which include the B, C and D exon-1 variant alleles, were grouped together, no risk difference was seen compared with the remaining haplotypes (OR = 1.0; 95% CI 0.8–1.4; p = 0.808).

The results show the association between stomach cancer risk and MBL2 diplotypes by using the simplified diplotype scheme proposed by Garred et al.19 This scheme groups the HYA and LYA haplotypes in the same category (YA), and the haplotypes including exon-1 variants are represented by a single-letter notation (B, C or D). Compared to the most common diplotype (YA/YA), subjects carrying the YA/D diplotype (15 cases and 7 controls) showed the highest risk (OR = 3.0, 95% CI 1.2–7.1; p = 0.015). No changes in stomach cancer risk were observed for the remaining diplotypes.

We also examined the joint effect of MBL2 haplotypes and the -511 polymorphism of the IL-1β gene (Table IV), which has been

Results

Stomach cancer cases and controls were comparable with respect to distributions by age and gender (Table I). Cases tended to have lower education (p = 0.004) and higher proportion of current smokers (p < 0.001), as previously reported.17 The majority of stomach cancers were of the intestinal type, which was found in 206 cases (67.5%). The tumour rose in the distal portion of the stomach in 152 patients (73.1%).

Risk of stomach cancer was associated with the MBL2-550 promoter polymorphism, with ORs of 1.1 (95% CI 0.8–1.4) for the L/H genotype and 1.8 (95% CI 1.1–2.9, p = 0.020) for the L/H genotype, when compared with the most frequent L/L genotype (Table II). Genotypes of the -221 promoter locus (Y-X alleles) were not associated with stomach cancer risk. Among the exon-1 loci, risk was nonsignificantly higher for subjects carrying one (OR = 1.5, 95% CI 0.9–2.4) or both D alleles (OR = 1.6, 95% CI 0.9–2.7) compared with subjects carrying only an A allele. The D/D genotype was found in only 1 case and 1 control. When subjects with one or both D alleles were combined, the OR was 1.5 (95% CI 0.9–2.4, p = 0.081). Exon-1 alleles at codon 54 and codon 57 were not associated with risk of stomach cancer.

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Figure 1 shows the association between stomach cancer risk and MBL2 diplotypes by using the simplified diplotype scheme proposed by Garred et al.19 This scheme groups the HYA and LYA haplotypes in the same category (YA), and the haplotypes including exon-1 variants are represented by a single-letter notation (B, C or D). Compared to the most common diplotype (YA/YA), subjects carrying the YA/D diplotype (15 cases and 7 controls) showed the highest risk (OR = 3.0, 95% CI 1.2–7.1; p = 0.015). No changes in stomach cancer risk were observed for the remaining diplotypes.

We also examined the joint effect of MBL2 haplotypes and the -511 polymorphism of the IL-1β gene (Table IV), which has been
previously associated with stomach cancer risk in our study. \cite{BACCARELLI ET AL.} We found no statistical interaction (\(p = 0.964\)) between \textit{IL-1B} \(-511\) polymorphisms and \textit{MBL2} haplotypes. Regardless of \textit{MBL2} haplotype, carriers of CT or TT variants of \textit{IL-1B} \(-511\) were at increased risk of stomach cancer compared to those with the CC genotype. Likewise, regardless of \textit{IL-1B} \(-511\) genotype, an elevated risk was observed among subjects with the HYD \textit{MBL2} haplotype. The highest risk was observed for those with the HYD haplotype and at least 1 variant \textit{IL-1B} \(-511\) allele (OR = 3.5, 95% CI 1.6–7.6; \(p = 0.001\)), compared to those with the HYA haplotype and the \textit{IL-1B} \(-511\) CC genotype. When the -31 polymorphism of the \textit{IL-1B} gene was considered in the analysis together the \textit{MBL2} haplotypes, patterns of stomach cancer risk were similar to those observed for the joint effect of \textit{IL-1B} \(-511\) and \textit{MBL2} (data not shown).

When stomach cancer cases were classified according to tumour histology, the HYD haplotype was associated with increased relative odds for the Lauren’s intestinal type (OR = 2.2, 95% CI 1.2–3.8; \(p = 0.008\); based on 30 HYD and 138 HYA counts in intestinal type cases, and 28 HYD and 270 HYA counts in controls), but not for the diffuse type (OR = 0.4, 95% CI 0.8–1.7; \(p = 0.213\);
based on 2 HYD and 35 HYA counts in diffuse type cases, and 28 HYD and 270 HYA counts in controls). The association between MBL2 haplotypes and stomach cancer risk was similar for patients with cardia or distal stomach cancer (data not shown). In addition, there were no consistent patterns when results were stratified by a number of potential risk factors, including *H. pylori* status, smoking, family history of stomach cancer and intake of fruits and vegetables. Among control subjects, the HYD haplotype was associated, though not significantly, with *H. pylori* seropositivity (OR 2.0, 95% CI 0.5–7.2 relative to the HYA haplotype, *p* = 0.313; based on 25 and 3 HYD counts in *H. pylori*-positive and negative subjects, respectively; and 224 and 45 HYA counts in *H. pylori*-positive and negative subjects, respectively).

### TABLE III – RISK OF STOMACH CANCER ASSOCIATED WITH HAPLOTYPES OF THE MANNOSE-BINDING LECTIN-2 (MBL2) GENE

<table>
<thead>
<tr>
<th>SNP combination</th>
<th>MBL2 haplotype</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-L (rs11003125)</td>
<td>Y-X (rs7096206)</td>
<td>A-B (rs1800450)</td>
<td>A-C (rs1800451)</td>
<td>A-D (rs5030737)</td>
</tr>
<tr>
<td>G G G G C</td>
<td>HYA</td>
<td>205 (33.7%)</td>
<td>270 (31.6%)</td>
<td>1.0 Reference</td>
</tr>
<tr>
<td>C C G G C</td>
<td>LXA</td>
<td>144 (23.7%)</td>
<td>209 (24.5%)</td>
<td>0.9 (0.7–1.2)</td>
</tr>
<tr>
<td>C G G G C</td>
<td>LYA</td>
<td>123 (20.3%)</td>
<td>202 (23.7%)</td>
<td>0.8 (0.6–1.1)</td>
</tr>
<tr>
<td>C G A G C</td>
<td>LYB</td>
<td>89 (14.7%)</td>
<td>131 (15.3)</td>
<td>0.9 (0.7–1.3)</td>
</tr>
<tr>
<td>C G A A C</td>
<td>LYG</td>
<td>7 (1.2%)</td>
<td>14 (1.6)</td>
<td>0.6 (0.2–1.6)</td>
</tr>
<tr>
<td>G G G G T</td>
<td>HYD</td>
<td>39 (6.4%)</td>
<td>28 (3.3)</td>
<td>1.9 (1.1–3.2)</td>
</tr>
</tbody>
</table>

1 Haplotype counts are reported. Two haplotypes were derived from each subject’s SNP combination by assuming complete linkage disequilibrium between the tested loci (see Methods section).–2 Odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age, gender, education and smoking, using multivariable logistic regression.–3 *p* = 0.021 for difference vs. HYA haplotype.

### Odds Ratio

<table>
<thead>
<tr>
<th>MBL2 diplotype</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>YA/YA</td>
<td>1.0</td>
<td>Reference</td>
</tr>
<tr>
<td>YA/XA</td>
<td>0.7</td>
<td>0.15</td>
</tr>
<tr>
<td>XA/XA</td>
<td>0.5</td>
<td>0.9</td>
</tr>
<tr>
<td>YA/B</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>XA/B</td>
<td>0.7</td>
<td>1.3</td>
</tr>
<tr>
<td>YA/C</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>YA/D</td>
<td>1.2</td>
<td>3.0</td>
</tr>
<tr>
<td>XA/D</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>XA/C</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>0/0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Discussion**

To our knowledge, except for a report that related childhood acute lymphoblastic leukaemia to carrying any MBL2 variants, with no evaluation of the risk associated with individual haplotypes,12 this is the first study to examine whether MBL2 variants affect cancer risk. In our study of stomach cancer, haplotype analysis, which is preferentially used in MBL2 studies because of strong linkage disequilibrium among the MBL2 SNP alleles, showed that subjects with HYD MBL2 haplotype were at increased risk. Furthermore, the effect of MBL2 variants appeared to be independent of the effect of the *IL-1B*-511 variant genotype that we had previously linked to stomach cancer risk.13
The HYD MBL2 haplotype is characterized by an arginine–cysteine substitution that impairs MBL activity against several microbrial species by altering subunit oligomerization, leading to decreased MBL functional activity and serum levels, when compared with subjects carrying the HYA haplotype. One might speculate that HYD MBL2 haplotype may increase stomach cancer risk by altering immune defenses in the gastric mucosa and enhancing susceptibility to H. pylori infection. Although the LYB and LYC MBL2 haplotypes have also been associated with lower MBL serum concentrations and higher risk of infectious diseases, neither of these 2 haplotypes were associated with increased stomach cancer risk in our study.

MBL levels found in mucosal secretions have never been studied in relation to MBL2 haplotypes. A recent study by Bak-Romaniszyn et al. showed that MBL2 is expressed in gastric biopsies with higher levels in H. pylori-infected individuals. Disruption of serum MBL function associated with MBL2 exon-1 variants has been suggested to be produced by changes in MBL oligomerization patterns. Whether such alterations in MBL oligomerization also occur in the microenvironment of the gastric mucosa, characterized among other factors by much lower pH than that found in serum, is unknown. In addition, factors determining persistence of MBL protein in the stomach are quite likely to be different from those affecting MBL clearance in serum. It is, therefore, probable that different MBL2 haplotypes induce alterations in MBL function in the gastric mucosa that are dissimilar from the changes demonstrated in serum. However, lack of data on MBL concentrations and functional activity in the gastric fluid limited our capability to evaluate possible intermediate steps relating MBL2 haplotypes to gastric cancer.

Accumulating data in humans suggest that H. pylori-driven autoimmune processes may cause gastric atrophy, intestinal metaplasia and adenomatous dysplasia that are considered as precursors of intestinal-type carcinomas. In our study, the significant excess risk due to HYD MBL2 haplotype was confined to intestinal-type stomach cancer.

In vitro data have shown that addition of MBL protein to whole blood causes suppression of IL-1B production at high MBL concentration. H. pylori infection is known to upregulate IL-1B expression, a process enhanced in subjects carrying at-risk polymorphisms of the IL-1B gene cluster. MBL is considered to be an acute phase protein, and its level increases as much as 2–3 times the basal concentration after an inflammatory stimulus. Although no overall statistical interaction was observed between MBL2 and IL-1B polymorphisms, subjects with the combination of at-risk IL-1B-511 genotypes and HYD MBL2 haplotype had the highest risk of stomach cancer.

Studies conducted on the association between MBL2 haplotypes and disease risks have often relied on the 5 SNPs that were included in our investigation. However, several other polymorphisms have been identified in the gene that could be tested in future work on stomach cancer. In addition to the SNP at +4 (PQ), which appears to have the smallest influence on MBL serum levels among the promoter polymorphisms, recent work by Bernig et al. has demonstrated much higher complexity of the MBL2 gene and indicated that additional variants as well as markers of distinct haplotype blocks may contribute to circulating protein levels.

The results of the present study are strengthened by the fact that the investigation was population-based and had high participation rates. To assess potential selection bias, we compared selected demographic and lifestyle characteristics between subjects with and without genotype and haplotype data, and found no statistically significant differences. In our study, H. pylori status was assigned on the basis of serological tests, which may not accurately reflect past infection, thus limiting our ability to study the effect of MBL2 variants on H. pylori infection. Nonetheless, H. pylori seropositivity among control subjects showed a correlation with MBL2 HYD haplotype that, though nonsignificant, was consistent with the hypothesis that MBL2 genetic variation affected stomach can-
mbl2 haplotype and stomach cancer risk remained robust given Helicobacter pylori infection. Despite our relatively large sample size, the haplotype counts in some categories were small, particularly in stratified analyses, resulting in limited statistical power, so that our results should be interpreted with caution. However, when we formally evaluated the probability of false-positive findings by estimating the false-positive report probability (FPRP), using the methods described by Wacholder et al.,

the association between the HYD MBL2 haplotype and stomach cancer risk remained robust given prior probabilities of 10% (FPRP = 0.222) and 5% (FPRP = 0.375).

In summary, the present study suggests that genetic variation in the innate-immunity MBL2 gene may contribute to the etiology of stomach cancer. Although MBL2 function suggests that the increase in stomach cancer risk may be due to alterations in H. pylori-related immune and inflammatory responses, our data do not provide sufficient support to confirm this hypothesis. Our findings need to be substantiated by further research aimed at assessing the degree of MBL binding to H. pylori and the effects of MBL2 gene variants on the precancerous changes induced by H. pylori in the gastric mucosa.

References


30. Bernig T, Taylor JG, Foster CB, Staats B, Yeager M, Chanock SJ. Sequence analysis of the mannose-binding lectin (MBL) gene variants on the precancerous changes induced by H. pylori in the gastric mucosa.