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ARTICLE *in* INTERNATIONAL JOURNAL OF CANCER · OCTOBER 2006

Impact Factor: 5.09 · DOI: 10.1002/ijc.22075 · Source: PubMed

Int. J. Cancer: **119,** 1970–1975 (2006)

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 TaqMan™. 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 = 0.081). 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 diplotype analysis, subjects carrying the YA/D haplotype combination showed the highest risk (OR = 3.0, 95% CI 1.2–7.1; 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.
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Key words: stomach cancer; mannose-binding lectin; immunologic deficiency syndromes; haplotypes

Stomach cancer is the second leading cause of cancer-related death in the world, accounting for 8.6% of cancer diagnoses and 10.4% of cancer deaths.[1] Central and Eastern European populations, including Poland, have the highest incidence and mortality rates for this tumour among Caucasians.[1] Although *H. pylori* infection is the main risk factor, only a small proportion (<3%) of the infected individuals develop stomach cancer.[2,3] There is an evidence that genes involved in immune and inflammatory response may contribute to differences in susceptibility to *H. pylori*-related stomach cancer.[4,5]

The mannose-binding lectin (MBL) protein, coded by the *MBL2* human gene, has been recently identified as a key component in systemic and mucosal innate immunity.[6,7] MBL is able to bind to a range of microbes and subsequently kill them by activating the complement system and promoting complement-independent opsonophagocytosis.[8] MBL deficiency is considered as one of the most common human immunodeficiencies,[8] determined by variant alleles in *MBL2* exon-1 and promoter regions that disrupt MBL oligomerization and complement-activation capacity.[9] Defective *MBL2* variants have been associated with infectious and autoimmune diseases, such as sepsis in neutropenic patients, meningococcal disease, pneumonia, systemic lupus erythematosus, early-onset rheumatoid arthritis and celiac disease.[8,10,11] In addition, the risk of acute lymphoblastic leukaemia appeared increased in children carrying defective *MBL2* variants, suggesting modulation of an infective cause that remains to be identified.[12]

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.[5,13–15] 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 genotypes differed somewhat between Caucasian and Asian populations.[16]

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.

Material and methods

Study design

The design of the population-based case-control study of stomach cancer has been described in detail previously.[13,17] 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 Evidency (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

Abbreviations: 95% CI, 95% confidence interval; EM algorithm, expectation-maximization algorithm; *IL-1,* interleukin-1; FPRP, false-positive report probability; MBL, mannose-binding lectin protein; *MBL2,* mannose-binding lectin-2 gene; NIH, National Institutes of Health; OR, odds ratio; SNP, single nucleotide polymorphism.

Grant sponsor: NIH.

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Received 19 December 2005; Accepted 24 March 2006
DOI 10.1002/ijc.22075
Published online 23 May 2006 in Wiley InterScience (www.interscience.wiley.com).

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 (90.0%) 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 thermo-cycling conditions for the 5 tested *MBL2* variants (rs5030737, rs1800450, rs1800451, rs11003125 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 study[13] 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 (GGC to GAC) causing a glycine>aspartic acid substitution (*rs1800450*, also known as allele *B*); at codon 57 (GGA to GAA) causing a glycine > glutamic acid substitution (*rs1800451*, 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 number 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 regions with no variant alleles (A). The LYB, LYC and HYD haplotypes were made up of combinations of H-L and X-Y promoter polymorphisms with the exon-1 variant alleles B, C and D, respectively. The B and C variants are always found in combination with LY, while the D variant is linked to HY.[7] Because of missing SNP results, 68 and 75 haplotypes in cases and controls, respectively, could not be unequivocally assigned and were imputed by using best estimate haplotypes obtained from the PHASE 2.1 software, which uses the expectation-maximization (EM) algorithm for haplotype reconstruction. Results obtained after excluding the haplotypes reconstructed by PHASE did not significantly differ from those reported in the article.

Statistical analysis

Hardy–Weinberg equilibrium was confirmed for all loci, using the asymptotic Pearson's χ^2 test. The Fishers' exact test was used to assess the difference between cases and controls in the distribution of categorical variables.

Stomach cancer risk was estimated by odds ratios (ORs) and 95% confidence intervals (CIs), using unconditional multiple logistic regression models.[18] Stomach cancer risks associated with *MBL2* were computed based on SNP, haplotype and diplotype data. For each SNP, the most common genotype was used as reference category. In the analysis based on haplotypes, the HYA haplotype, which was the most common haplotype, was used as reference, also considering previous functional studies showing that HYA is associated with the highest MBL concentration in serum.[7,8] All ORs were adjusted for age, gender, education and smoking. Further adjustment for other potential confounding variables, including family history of cancer, pack-years of cigarette smoking, dietary intake (fresh fruits and vegetables, preserved vegetables, sausages and calories), history of gastro-esophageal reflux, use of ulcer medications and *H. pylori* seropositivity did not affect the risks meaningfully. Statistical interaction between *MBL2* haplotypes and *IL-1B* polymorphisms was tested by means of the likelihood-ratio test. We estimated the false-positive report probability (FPRP) for statistically significant observations, using the methods described by Wacholder *et al*.[19] We calculated the FPRPs for prior probabilities ranging from 50% to 0.1%. We considered FPRP = 0.50 as the FPRP noteworthiness value for our hypothesis, as suggested for novel findings regarding the association between a genetic variant or haplotype and risk of a common disease.[19]

All tests were two-sided. All analyses were conducted using the Stata 9.0 (Stata Corporation, College Station, TX) statistical package.

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 223 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 H/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 0.1–27.8) at codon 52, relative to subjects who did not carry any 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; 1.1–3.2, $p = 0.021$), when compared with the HYA most common haplotype. The remaining haplotypes (LXA, LYA, LYB 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; $p = 0.808$).

Figure 1 shows the association between stomach cancer risk and *MBL2* diplotypes by using the simplified diplotype scheme proposed by Garred *et al*.[7] 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-1B* gene (Table IV), which has been

TABLE I – DISTRIBUTION OF SELECTED VARIABLES IN STOMACH CANCER CASES AND CONTROLS

	Stomach cancer cases (n = 305)		Control Subjects (n = 427)		p-value[1]
	n	(%)	*n*	(%)	
Age (years)[2]					
<50	39	(12.8)	52	(12.2)	
50–59	56	(18.4)	75	(17.6)	
60–69	120	(39.3)	168	(39.3)	
≥70	90	(29.5)	132	(30.9)	0.972
Gender[2]					
Male	202	(66.2)	275	(64.4)	
Female	103	(33.8)	152	(35.6)	0.637
Education					
Less than high school	144	(47.2)	160	(37.5)	
High school or technical training	104	(34.1)	145	(34.0)	
Some college/college graduate	57	(18.7)	122	(28.6)	0.004
Smoking status					
Never	88	(28.8)	171	(40.0)	
Ex-smokers	91	(29.8)	137	(32.0)	
Current-smokers	126	(41.3)	119	(27.9)	<0.001
Laurén classification					
Intestinal	206	(67.5)	N/A		
Diffuse	50	(16.4)	N/A		
Indeterminate	32	(10.5)	N/A		
Unclassified/unknown	17	(5.6)	N/A		–
Site of tumour origin					
Cardia only	35	(11.5)	N/A		
Cardia/distal	36	(11.8)	N/A		
Distal only	223	(73.1)	N/A		
Unknown	11	(3.6)	N/A		–

N/A = Not Applicable.
[1]Fisher's exact test for distribution differences between stomach cancer cases and control subjects.
[2]Gastric cancer cases and control subjects were frequency-matched by age and gender.

TABLE II – RISK OF STOMACH CANCER BY MANNOSE-BINDING LECTIN-2 (*MBL2*) GENOTYPES

MBL2 Genotype	Common nomenclature	Stomach cancer cases	Control subjects	OR[1]	(95% CI)
Promoter Alleles					
-550 (rs11003125)					
CC	L/L	105 (35.8%)	170 (40.6%)[2]	1.0	Reference
CG	L/H	139 (47.5%)	205 (48.9%)	1.1	(0.8–1.4)
GG	H/H	49 (16.7%)	44 (10.5%)	1.8[2]	(1.1–2.9)
-221 (rs7096206)					
GG	Y/Y	169 (57.5%)	238 (57.7%)	1.0	Reference
GC	Y/X	109 (37.1%)	149 (26.2%)	1.0	(0.8–1.4)
CC	X/X	16 (5.4%)	25 (6.1%)	1.0	(0.5–1.9)
Exon-1 Alleles					
Codon 54 (rs1800450)					
GG	Non-B/Non-B	222 (74.2%)	307 (74.0%)	1.0	Reference
GA	Non-B/B	69 (23.1%)	95 (22.9%)	1.0	(0.7–1.4)
AA	B/B	8 (2.7%)	13 (3.1%)	1.0	(0.4–2.4)
Codon 57 (rs1800451)					
GG	Non-C/Non-C	283 (97.6%)	395 (96.6%)	1.0	Reference
GA	Non-C/C	7 (2.4%)	14 (3.4%)	0.7	(0.3–1.7)
AA	C/C	0 (0.0%)	0 (0.0%)	–	(–)
Codon 52 (rs5030737)					
CC	Non-D/Non-D	248 (85.3%)	358 (89.7%)	1.0	Reference
CT	Non-D/D	42 (14.4%)	40 (10.0%)	1.5	(0.9–2.4)
TT	D/D	1 (0.3%)	1 (0.3%)	1.6	(0.1–27.8)

[1]Odds ratios (ORs) and 95% confidence intervals (CIs) adjusted for age, gender, education and smoking, using multivariable logistic regression.–[2]p = 0.020 for difference *vs.* reference category.

previously associated with stomach cancer risk in our study.[13] We found no statistical interaction (p = 0.964) between *IL-1B*-511 polymorphisms and *MBL2* haplotypes. Regardless of *MBL2* haplotype, carriers of CT or TT variants of *IL-1B*-511 were at increased risk of stomach cancer compared to those with the CC genotype. Likewise, regardless of *IL-1B*-511 genotype, an elevated risk was observed among subjects with the HYD *MBL2* haplotype. The highest risk was observed for those with the HYD haplotype and at least 1 variant *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 *IL-1B*-511 CC genotype. When the -31 polymorphism of the *IL-1B* gene was considered in the analysis together the *MBL2* haplotypes, patterns of stomach cancer risk were similar to those observed for the joint effect of *IL-1B*-511 and *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;

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

SNP combination					*MBL2* haplotype	Cases[1]	Controls[1]	OR[2]	(95% CI)[2]
H-L (rs11003125)	*Y-X (rs7096206)*	*A-B (rs1800450)*	*A-C (rs1800451)*	*A-D (rs5030737)*					
G	G	G	G	C	HYA	205 (33.7%)	270 (31.6%)	1.0	Reference
C	C	G	G	C	LXA	144 (23.7%)	209 (24.5)	0.9	(0.7–1.2)
C	G	G	G	C	LYA	123 (20.3%)	202 (23.7)	0.8	(0.6–1.1)
C	G	A	G	C	LYB	89 (14.7%)	131 (15.3)	0.9	(0.7–1.3)
C	G	G	A	C	LYC	7 (1.2%)	14 (1.6)	0.6	(0.2–1.6)
G	G	G	G	T	HYD	39 (6.4%)	28 (3.3)	1.9[3]	(1.1–3.2)

[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.

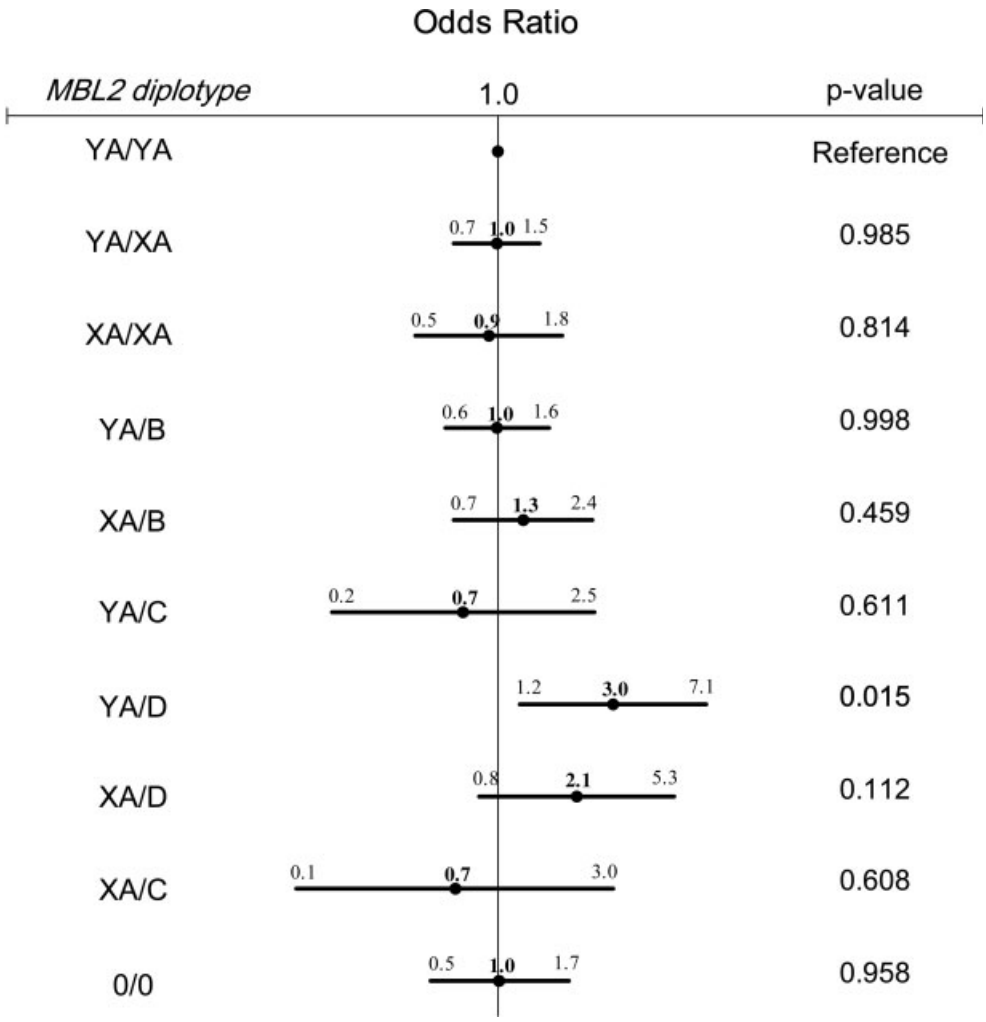

FIGURE 1 – Risk of stomach cancer by *MBL2* diplotype (YA/YA = reference diplotype). The 0/0 diplotype represents subjects carrying 2 exon-1 variant alleles. Odds ratios and 95% confidence intervals adjusted for age, gender, education and smoking.

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).

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]

TABLE IV – RISK OF STOMACH CANCER ASSOCIATED WITH *MANNOSE-BINDING LECTIN-2 (MBL2)* HAPLOTYPES AND *IL-1B* -511 GENOTYPES

SNP combination						Il-1B -511 Single nucleotide polymorphism							
						Homozygous consensus (CC)				Hetero or homozygous variant (CT or TT			
H-L (*rs1003125*)	*Y-X* (*rs7096206*)	*A-B* (*rs1800450*)	*A-C* (*rs1800451*)	*A-D* (*rs5030737*)	*MBL2* Haplotype	Cases[1]	Controls[1]	OR[2]	(95% CI)[2]	Cases[1]	Controls[1]	OR[2]	(95% CI)[2]
G	G	G	G	C	HYA	71 (31.6%)	129 (30.2%)	1.0	Reference	125 (33.9%)	138 (33.5%)	1.7	(1.1–2.4)
C	C	G	G	C	LXA	55 (24.4%)	113 (26.4%)	0.9	(0.6–1.5)	86 (23.3%)	91 (22.1%)	1.7	(1.1–2.6)
C	G	G	G	C	LYA	51 (22.7%)	98 (22.9%)	0.9	(0.6–1.5)	71 (19.3%)	102 (24.8%)	1.3	(0.8–2.0)
C	G	A	G	C	LYB	30 (13.3%)	65 (15.2%)	0.9	(0.5–1.6)	58 (15.8%)	62 (15.0%)	1.6	(1.0–2.6)
C	G	G	A	C	LYC	2 (0.9%)	7 (1.6%)	0.5	(0.1–2.7)	5 (1.4%)	7 (1.7%)	1.2	(0.4–3.9)
G	G	G	G	T	HYD	16 (7.1%)	16 (3.7%)	1.9	(0.9–4.1)	23 (6.3%)	12 (2.9%)	3.5	(1.6–7.6)

[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. $p = 0.964$, likelihood-ratio test for statistical interaction between *IL-1B*-511 polymorphism and *MBL2* haplotypes.

The HYD *MBL2* haplotype is characterized by an arginine–cysteine substitution that impairs MBL activity against several microbial species by altering subunit oligomerization, leading to decreased MBL functional activity and serum levels, when compared with subjects carrying the HYA haplotype.[6–9,20] 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,[8] 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.[21] Disruption of serum MBL function associated with *MBL2* exon-1 variants has been suggested to be produced by changes in MBL oligomerization patterns.[9] 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.[22–24] 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.[25] *H. pylori* infection is known to upregulate *IL-1B* expression,[26,27] a process enhanced in subjects carrying at-risk polymorphisms of the *IL-1B* gene cluster.[27] 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.[28,29] 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 (P/Q), 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 3′ haplotype blocks may contribute to circulating protein levels.[30,31]

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,[24,32] 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-

cer risk by modifying the severity or persistence of *H. 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*.,[19] 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.

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