

Lung Cancer Survival and Functional Polymorphisms in MBL2, an Innate-Immunity Gene

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- Background** The relationship among chronic inflammation, innate immunity, and cancer is well established. Mannose-binding lectin (MBL) is a key player in innate immunity. Five polymorphisms in the promoter and first exon of the MBL2 gene alter the expression and function of MBL in humans and are associated with inflammation-related disease susceptibility. These five polymorphisms create six well-characterized haplotypes that result in lower (i.e., LYB, LYC, HYD, and LXA) or higher (i.e., HYA and LYA) serum MBL concentrations. We investigated whether survival of patients with lung cancer was associated with these polymorphisms.
- Methods** We used a multivariable Cox proportional hazards model to study the association between MBL2 polymorphisms and their haplotypes and diplotypes in 558 white and 173 African American patients with non-small-cell lung cancer in the Baltimore, MD, area and lung cancer mortality. Smoking history and race were obtained from interviews, tumor stage was obtained from medical records, and cause of death was obtained from the National Death Index. All statistical tests were two-sided.
- Results** We found a statistically significant association between the X allele of the promoter Y/X polymorphism (which results in a lower serum MBL concentration) and improved lung cancer survival among white patients (risk ratio [RR] of death from lung cancer with X/X or X/Y genotype compared with Y/Y genotype = 0.61, 95% confidence interval [CI] = 0.46 to 0.81) but not among African American patients (RR = 1.11, 95% CI = 0.69 to 1.77). The associations among white patients were strongest in heavy smokers and were independent of stage. We also found a statistically significant interaction between the Y/X polymorphism and race for lung cancer survival ($P_{\text{interaction}} = .019$). The MBL2 LXA haplotype and XA/B diplotype, which are also associated with low serum MBL levels, were statistically significantly associated with improved lung cancer survival among white patients.
- Conclusion** The functional Y/X polymorphism of the innate-immunity gene MBL2 and MBL2 haplotypes and diplotypes appear to be associated with lung cancer survival among white patients.

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Lung cancer, predominantly non-small-cell lung cancer, is the leading cause of cancer mortality worldwide. There continues to be a wide variation in survival within any given stage, and the 5-year overall survival rates remain less than 20% (1,2). Familial aggregation and molecular epidemiologic studies have found that genetic factors are associated with lung cancer survival (3–5). Additional survival-associated genetic markers could improve the stratification of lung cancer patients into more precise risk groups, enhance prognosis models, and help understand the biology of the disease.

Genetic variations in innate-immunity genes may be associated with lung cancer mortality. Mannose-binding lectin (MBL) is encoded by the MBL2 gene and is a central component of the innate immune system. MBL binds to specific carbohydrate structures on the surface of microorganisms and activates both the lectin complement system and complement-independent opsonization by monocytes (6). Variations in MBL levels or activity are caused, in part, by two single-nucleotide polymorphisms (SNPs) in the promoter region, known as L/H and Y/X, and three SNPs in the first

exon, known as the D, B, and C variant alleles; the A allele for the SNPs in the first exon is the wild-type allele. We examined the SNPs L/H, position –618G>G; Y/X, position –289G>C; A/D, position Ex1–34C>T; A/B, position Ex1–27G>A; and A/C, position Ex1–18G>A. These SNPs are components of what is termed the secretor haplotype block and create six haplotypes—four of

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CONTEXT AND CAVEATS

Prior knowledge

The innate-immunity MBL2 gene encodes the mannose-binding lectin (MBL) protein, which activates the lectin pathway of the complement system, enhances opsonophagocytosis, and modulates the cytokine response to inflammation. Altered function or expression of MBL caused by polymorphisms within the secretor haplotype block of the MBL2 gene has been associated with increased susceptibility to inflammation-related disorders and certain cancers.

Study design

A prospective study to examine the association between five polymorphisms within the MBL2 secretor haplotype block and disease-specific survival among 558 white patients and 173 African American patients with non-small-cell lung cancer in the metropolitan area of Baltimore, MD.

Contribution

The functional MBL2 Y/X promoter polymorphism and the secretor haplotypes and diplotypes associated with low serum MBL concentrations were associated with improved lung cancer-specific survival among white patients but not among African American patients.

Implications

Genetic polymorphisms in genes in the innate immune response pathway that modulate inflammation could be a prognostic factor in lung cancer patients.

Limitations

This study had fewer African American patients than white patients and thus had reduced power to assess associations among African American patients. Stage and histotype information was not available on all patients. Clinical follow-up information was restricted to death certificate data.

which (i.e., LYB, LYC, HYD, and LXA) result in lower serum MBL concentrations and two of which (i.e., HYA and LYA) result in higher MBL concentrations (7). The haplotype pairs, or diplotypes, are described by a simplified scheme (7,8), in which the HYA and LYA haplotypes are in the same category (YA), the diplotypes that are exon 1 variants are noted by a single-letter notation (B, C or D), and the haplotype pairs containing homozygous or compound heterozygous variant alleles are represented by the letter O. From this scheme, 10 different diplotypes are distinguished.

Serum levels of MBL range more than 1000-fold in humans, and there is no consensus about the range of serum MBL concentrations that constitutes normal, healthy levels. Several studies (8–14) have reported associations between secretor haplotypes that result in low levels of serum MBL and susceptibility to repeated serious infections, autoimmune disorders, stomach cancer, and pediatric acute lymphoblastic leukemia. Low levels of serum MBL have also been associated with a decreased risk of certain mycobacterial infections (15), and high levels of serum MBL have been associated with inflammation-related disorders, such as childhood asthma, primary biliary cirrhosis, tissue damage from excessive complement activity, and reperfusion injury (16–20). Thus, both high and low serum MBL levels can be disadvantageous.

Chronic inflammation and innate immunity are associated with cancer development and progression, by generating DNA-damaging reactive oxygen and nitrogen species (21). In this study, we examined five MBL2 secretor haplotype polymorphisms from 558 white and 173 African American patients with lung cancer who were recruited from the metropolitan area of Baltimore, MD. We evaluated whether MBL2 polymorphisms were associated with lung cancer-specific survival.

Materials and Methods

Study Subjects

Patients with histologically confirmed non-small-cell lung cancer were prospectively recruited from the metropolitan area of Baltimore, MD, through two separate study protocols, a case-only series (22) and a case-control study (23,24). Patients from the case-only series were recruited from the following hospitals: University of Maryland, Baltimore Veteran's Administration, St Agnes Hospital, Northwest Hospital Center, Sinai Hospital, Mercy Medical Center, and Union Memorial Hospital. Three hundred nine patients with available tissue samples from surgeries that were performed between January 1, 1984, and December 31, 1999, were eligible; inclusion and exclusion criteria have been described previously (22). Suitable genomic DNA was available for 231 of the 309 case patients. Patients from the case-control study were residents of the Baltimore metropolitan area or any of the Maryland counties east of the Chesapeake Bay. Five hundred sixty-eight patients diagnosed between January 1, 1998, and December 31, 2004, were recruited; the inclusion and exclusion criteria have been described previously (23,24). Suitable genomic DNA was available for 500 of the 568 case patients. There were no statistically significant differences in current smoking status, stage, age, sex, or race between those who had suitable amounts and quality of DNA and those who did not for either protocol. Patients were combined for this study, for a total of 558 white and 173 African American patients. Written informed consent was obtained from all participants. This study was approved by the Institutional Review Boards of the participating institutions.

Genotyping

Genomic DNA was isolated from 500 buffy coat samples containing white blood cells from patients in the case-control study and from 181 frozen normal lung tissue samples and 50 buffy coat samples from patients in the case series. Five SNPs located within the secretor haplotype region of the MBL2 gene were genotyped at the National Cancer Institute Core Genotyping Facility by use of a Taqman assay (Applied Biosystems, Foster City, CA). The assay conditions for each genotype (L/H, rs11003125; Y/X, rs7096206; A/D, rs5030737; A/B, rs1800450; and A/C rs1800451) are detailed on the SNP500 Web site (<http://snp500cancer.nci.nih.gov>). The case, control, and 10% duplicate samples were randomly distributed for order of processing, and samples were assigned a unique identification number that lacked identifiers. Staff at the Core Genotyping Facility were blinded to the case, control, and duplicate statuses. The genotype concordance for each SNP was at least 99% among duplicates.

Statistical Analysis

Linkage disequilibrium of the secretor haplotype SNPs has been previously demonstrated (25). The secretor haplotype block was inferred separately for white and African American patients. Linkage disequilibrium was verified by use of the default modus for a haplotype block definition as implemented in the Haploview software (<http://www.broad.mit.edu/mpg/haploview/index.php>). Estimated haplotypes were obtained from Phase software, version 2.1 (<http://www.stat.washington.edu/stephens/software.html>), which uses a Bayesian method for haplotype reconstruction. The best-pairs diplotypes were estimated for each individual, and the diplotypes for each individual were split into individual haplotypes.

Patients were followed prospectively from the time of enrollment. For the case-control and case-only series studies, survival was calculated from the date of lung cancer diagnosis and the date of surgery, respectively, to the last reported search for death entries (December 31, 2004) in the National Death Index (<http://www.cdc.gov/nchs/ndi.htm>). The cumulative follow-up was 20 years (from date of first lung cancer diagnosis to date of the National Death Index search). We obtained death certificates for the deceased patients and censored all patients whose cause of death was not related to lung cancer. Deaths were attributed to lung cancer if the death certificate listed lung cancer as a cause or contributory factor of death or if any other solid-tumor cancer was listed as a cause of death within 2 years after diagnosis.

A multivariable Cox proportional hazards model was used to compute risk ratios (RRs) and 95% confidence intervals (CIs) of lung cancer-specific death by genotypes, haplotypes, and diplotypes. All analyses were stratified by race. The following covariates were included in the analyses: sex, age at diagnosis (as a continuous variable), tumor-node-metastasis stage (categorized as stages III-IV versus stages I-II), current smoking status (categorized as never and former smokers versus current smokers), and pack-years of smoking (as a continuous variable). Proportional hazards assumptions were verified by visual inspection of log-log plots and with a nonzero slope test of the Schoenfeld residuals (26). A Bonferroni-adjusted *P* value was calculated by multiplying the actual *P* value by the 10 analyses performed (i.e., five secretor SNPs and each race). A statistical test for interaction between MBL2 genotypes and covariates was performed by using a likelihood ratio test to calculate *P* values by comparing main effects models with main effects models plus an interaction term. This comparison was done by inclusion of a dichotomous indicator for the covariate and genotype (homozygous wild type versus heterozygous and homozygous variant). A power analysis to detect associations between the Y/X MBL2 SNP and lung cancer-specific survival was conducted with the Sample Size software, version 2.1.31 (27) (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>). We had 100% and 86% power to detect a twofold relative risk of death among the white patients and the African American patients, respectively, at an α value of .05, if we assumed a dominant effect for the variant allele. Smoking levels were categorized by using the 25th and 75th percentile pack-year values of the white patients as the cut points (i.e., 0.1 to <28.6 pack-years, 28.6 to 64.8 pack-years, and >64.8 pack-years) (28).

Calculations were performed by use of STATA version 9 software (STATA Corp, College Station, TX). A *P* value of less

than .05 was used as the criterion of statistical significance, and all statistical tests were two-sided.

Results

MBL2 Secretor Genotypes and Lung Cancer-Specific Survival

We investigated the relationship between five MBL2 polymorphisms and lung cancer-specific survival among 558 white and 173 African American patients. The relevant demographic and clinicopathologic features are given in Table 1. Among the 558 white patients, 244 (49.0%) were current smokers and 365 (67.3%) had stage I-II lung cancer. Among the 173 African American patients, 102 (58.9%) were current smokers and 89 (56.0%) had stage I-II lung cancer. The 20-year cumulative lung cancer-specific survival for white patients was 27.3% (95% CI = 15.0% to 41.3%) and for African American patients was 17.2% (95% CI = 7.1% to 30.9%). Allele frequencies of MBL2 genotypes are shown in Table 2. There was a statistically significant difference in allele frequencies between white patients and African American patients (two-sided chi-square test, for L/H, A/D, A/B, and A/C, $P < .001$, and for Y/X, $P = .02$).

The Y/X promoter SNP was statistically significantly associated with survival among white patients, as shown by a Kaplan-Meier analysis (Fig. 1) and multivariable analyses (Table 2). A total of 329 (61.7%) of the 533 genotyped white patients had the common Y/Y genotype, 180 (33.8%) had one variant allele (Y/X), and 24 (4.5%) had both variant alleles (X/X). Among white patients, a heterozygous or double-variant genotype, corresponding to lower levels of serum MBL, was associated with lower risk of death (RR = 0.61, 95% CI = 0.46 to 0.81, $P = .001$) than the Y/Y genotype (Table 2). In a univariate analysis, among white patients but not among African American patients, the X allele was also statistically significantly associated with improved survival compared with the Y/Y genotype (Table 3). None of the remaining four MBL2 SNPs were associated with survival in either multivariable (Table 2) or univariate (data not shown) analyses. The association between the Y/X MBL2 SNP and lung cancer-specific survival remained statistically significant after a Bonferroni correction ($P = .01$).

We found a statistically significant interaction between the Y/X SNP status and race for lung cancer-specific survival ($P_{\text{interaction}} = .019$). No association was found between the Y/X polymorphism and lung cancer-specific survival among the African American patients in a multivariable analysis (RR for lung cancer death in patients with at least one variant X allele compared with those with a Y/Y genotype = 1.11, 95% CI = 0.69 to 1.77) (Table 2).

Among white patients, the association between the X allele and lung cancer-specific survival was independent of the clinicopathologic factors: stage, age, sex, weight loss, histologic subtype (adenocarcinoma or squamous cell carcinoma), tumor grade, and chronic obstructive pulmonary disorder. We found no statistical interaction between the Y/X SNP and any of these factors, and in all stratified analyses, the 95% confidence intervals overlapped (data not shown). As expected, there was also no association between the Y/X SNP and chronic obstructive pulmonary disorder (chi-square test, $P = .69$).

We performed survival analyses for all causes of death. As shown in Supplementary Table 1 (available online), white patients

Table 1. Distribution of selected characteristics and clinical data for study patients with non–small-cell lung cancer*

Characteristic	White patients (N = 558)	African American patients (N = 173)
Age, y (mean ± SD)	66.4 ± 9.9	63.2 ± 9.2
Male	66.3 ± 9.8	64.0 ± 8.3
Female	66.6 ± 10.1	62.5 ± 10.1
Sex, No. (%)		
Male	317 (56.8)	88 (50.9)
Female	241 (43.2)	85 (49.1)
Smoking status, No. (%)†		
Never	36 (7.2)	11 (6.4)
Former	218 (43.8)	60 (34.7)
Current	244 (49.0)	102 (58.9)
Pack-years‡		
<28.6, No. of patients (<25th percentile)	138 (24.7)	66 (38.2)
28.6–64.8, No. of patients (25th–75th percentile)	276 (49.5)	75 (43.4)
>64.8, No. of patients (>75th percentile)	144 (25.8)	32 (18.5)
Tumor type, No.		
Adenocarcinoma	217	57
Squamous cell carcinoma	93	40
NSCLC§	172	61
Stage,† No. (%)		
I–II	365 (67.3)	89 (56.0)
III–IV	177 (32.7)	70 (44.0)
Follow-up		
Cumulative survival rate, % (95% CI)	27.3 (15.0 to 41.3)	17.2 (7.1 to 30.9)
Median follow-up, mo (95% CI)¶	45.0 (40.8 to 48.9)	43.9 (34.8 to 51.5)

* SD = standard deviation; NSCLC = non–small-cell lung cancer; CI = confidence interval.

† Patients with missing data were not included.

‡ Smokers were categorized by using the 25th and 75th percentile pack-year values of the white patients.

§ Histologic subtype data were not available for all NSCLC tumors.

|| The follow-up period was 20 years.

¶ Median survival months of patients who were alive at the end of follow-up.

who carried the X allele had better 5-year overall survival than those in the reference group who carried the Y/Y genotype (RR = 0.62, 95% CI = 0.47 to 0.81). We observed no association between any of the MBL2 polymorphisms and overall survival among the African American patients (data not shown).

When the analysis was stratified by the two study protocols from which the patients were recruited, white patients carrying at least one X allele had better lung cancer–specific survival than white patients carrying the wild-type Y/Y genotype in the case–control (RR = 0.65, 95% CI = 0.45 to 0.93) and case-series (RR = 0.56, 95% CI = 0.34 to 0.92) studies (Supplementary Table 2, available online). No association between any polymorphism and lung cancer–specific survival was observed in either study among the African American patients (Supplementary Table 2, available online).

Because smoking and MBL levels are both associated with inflammation and because smoking was recently associated with increased serum MBL levels (29), we hypothesized that smoking may modify the association between high MBL serum levels and lung cancer survival. The variant X allele was statistically significantly associated with improved survival among white patients who smoked more than 64.8 pack-years (the >75th percentile; RR = 0.33, 95% CI = 0.17 to 0.61, $P = .001$). When the smoking cut points were moved up and down by 5 pack-years, the results were similar (data not shown). Smoking did not modify the association between the X allele and lung cancer–specific survival among African American patients (Table 4).

Cause of Death Among Lung Cancer Patients

High and low serum MBL levels, respectively, have been associated with elevated inflammation during myocardial ischemia and reperfusion injury (17) and with serious infections in chemotherapy patients (30). We examined whether the association between the Y/X SNP and lung cancer–specific survival among white patients in our study was partially driven by cardiac- or sepsis-related causes of death. A total of 43 and 39 white patients, respectively, had a cardiac- or infection-related cause of death in addition to a lung cancer cause of death. The X allele was not associated with risk of cardiac-related (RR = 1.25, 95% CI = 0.55 to 2.92) or sepsis-related (RR = 0.94, 95% CI = 0.48 to 1.61) death, compared with the Y/Y genotype.

MBL2 Secretor Haplotypes or Diplotypes and Lung Cancer Survival

The six haplotypes that were estimated from the five secretor SNPs and examined in this study were the same as those analyzed in previous studies (7,8,31). The HYA haplotype was chosen as the referent because it is the most common among white patients and was previously used as the reference in association studies (6,8). The LXA haplotype, which causes reduced serum MBL levels (7), compared with the HYA haplotype, was associated with improved lung cancer–specific survival (RR = 0.70, 95% CI = 0.53 to 0.93, $P = .01$) and improved 5-year overall survival (RR = 0.71, 95% CI = 0.54 to 0.92) among white patients (Table 5; Supplementary Table 1, available online). There was no association between

Table 2. Association between secretor MBL2 polymorphisms and disease-specific survival*

Genotype	White patients			African American patients		
	No. (%)	RR of death† (95% CI)	P‡	No. (%)	RR of death† (95% CI)	P‡
Promoter SNPs						
L/H (mbl2_11; rs11003125)						
C/C (L/L)	215 (41.1)	1.0 (referent)		120 (71.4)	1.0 (referent)	
C/G (L/H)	238 (45.5)	0.94 (0.70 to 1.26)	.67	44 (26.2)	1.02 (0.62 to 1.69)	.93
G/G (H/H)	70 (13.4)	1.29 (0.86 to 1.92)	.22	4 (2.4)	2.27 (0.70 to 7.41)	.14
Y/X (mbl2_12; rs7096206)						
G/G (Y/Y)	329 (61.7)	1.0 (referent)		124 (73.4)	1.0 (referent)	
G/C (Y/X)	180 (33.8)	0.61 (0.46 to 0.82)	.001	41 (24.3)	1.02 (0.63 to 1.66)	.96
C/C (X/X)	24 (4.5)	0.62 (0.31 to 1.27)	.20	4 (2.3)	2.76 (0.81 to 9.40)	.11
G/C + C/C§	204 (38.3)	0.61 (0.46 to 0.81)	.001	45 (26.6)	1.11 (0.69 to 1.77)	.69
Exon 1 SNPs						
A/D (mbl2_03; rs5030737)						
C/C (non-D/non-D)	447 (85.0)	1.0 (referent)		160 (96.4)	1.0 (referent)	
C/T (non-D/D)	75 (14.2)	0.97 (0.68 to 1.40)	.89	6 (3.6)	0.48 (0.11 to 1.98)	.30
T/T (D/D)	4 (0.8)	3.32 (0.68 to 13.64)	.10	0	–	–
A/B (mbl2_01; rs1800450)						
G/G (non-B/non-B)	391 (75.5)	1.0 (referent)		152 (91.6)	1.0 (referent)	
G/A (non-B/B)	120 (23.2)	0.86 (0.63 to 1.17)	.34	13 (7.8)	1.63 (0.67 to 3.95)	.28
A/A (B/B)	7 (1.3)	1.91 (0.60 to 6.07)	.28	1 (0.6)	–	–
A/C (mbl2_02; rs1800451)						
G/G (non-C/non-C)	479 (97.0)	1.0 (referent)		91 (57.2)	1.0 (referent)	
G/A (non-C/C)	14 (2.8)	1.09 (0.44 to 2.67)	.18	58 (36.5)	0.84 (0.53 to 1.33)	.42
A/A (C/C)	1 (0.2)	–	–	10 (6.3)	1.09 (0.42 to 2.83)	.88

* RR = risk ratio; CI = confidence interval; SNP = single-nucleotide polymorphism; – = insufficient data.

† Risk ratio of lung cancer death was adjusted for sex, stage (III–IV versus I–II), age at diagnosis, current smoking status, and pack-years of smoking.

‡ A Cox proportional hazards test was used. All statistical tests were two-sided.

§ A dominant model was assumed.

survival and the LXA haplotype among African American patients (RR = 0.75, 95% CI = 0.47 to 1.19) (Table 5).

When the patients were stratified by study protocol, a statistically significant association between the LXA haplotype and lung cancer-specific survival was observed among the white patients in the case-control study, but not in the case-only series (Supplementary Table 2, available online), although the magnitudes of the associations were similar. When the white patients were stratified by smoking, the LXA haplotype was associated with improved survival among those who smoked more than 64.8 pack-years (RR = 0.34, 95% CI = 0.18 to 0.63, $P < .001$) (Table 5). Among African American patients who smoked between 28.6 and 64.8 pack-years, improved survival was associated with the LXA (RR = 0.33, 95% CI = 0.13 to 0.84, $P = .02$) and LYC (RR = 0.45, 95% CI = 0.20 to 0.97, $P = .04$) haplotypes, compared with the HYA haplotype (Table 5).

The haplotypes were dichotomized as previously described by Garred et al. (7) into high MBL (HYA and LYA) and low MBL (LXA, LYB, LYC, and HYD) haplotypes. Among white patients, the low MBL haplotypes were associated with better lung cancer-specific survival overall than high MBL haplotypes (RR = 0.78, 95% CI = 0.65 to 0.95, $P = .01$), and this association was even stronger in the subgroup of patients who had smoked more than 64.8 pack-years (RR = 0.48, 95% CI = 0.32 to 0.72, $P < .001$) (Table 5). Among African American patients who smoked between 28.6 and 64.8 pack-years, low MBL haplotypes were associated with improved lung cancer-specific survival (RR = 0.54, 95% CI = 0.33 to 0.88, $P = .01$) (Table 5). To determine whether the association

of the low MBL haplotypes with survival was driven mainly by the Y/X SNP, patients with the LXA haplotype were removed from the analysis. Among white patients, low MBL haplotypes (LYB, LYC, and HYD) were associated with better lung cancer-specific

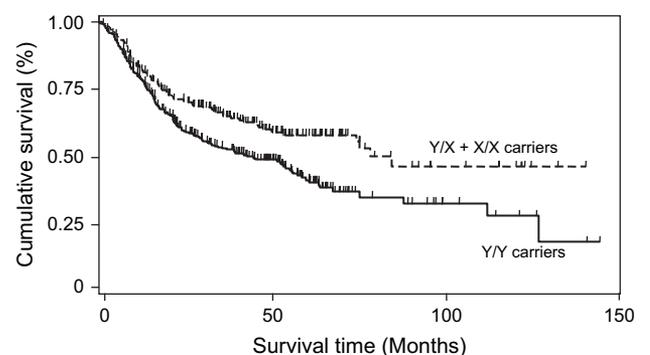


Fig. 1. MBL2 Y/X promoter single-nucleotide polymorphism (SNP) genotype in relation to lung cancer survival among white patients: Kaplan-Meier survival analysis. Cumulative disease-specific survival of white patients with lung cancer is presented by MBL2 Y/X SNP status (patients at risk at time zero = 533). Survival of patients who carried the variant X allele (patients at risk at time zero = 204) was statistically significantly better than that of patients with the common Y/Y genotype (patients at risk at time zero = 329; two-sided log-rank test: $P < .001$). The 5-year cumulative survival rate was 41.5% (95% CI = 35.0% to 47.9%) for patients with the Y/Y genotype (patients at risk at 5 years = 53) and 58.2% (CI = 50.0% to 65.4%) for patients with at least one X allele (patients at risk at 5 years = 43). At the end of the follow-up period (12 years), the cumulative survival rate was 18.9% (95% CI = 5.8% to 37.7%) for patients with the Y/Y genotype and 46.6% (CI = 32.2% to 58.0%) for patients with at least one X allele.

Table 3. Disease-specific survival of lung cancer patients in relation to clinicopathologic characteristics and the MBL2 Y/X polymorphism*

Variable	Category	Univariate analysis		Multivariable analysis	
		RR of death (95% CI)	P†	RR of death (95% CI)	P†
White patients					
Age	Continuous	1.00 (0.99 to 1.01)	.92	1.01 (1.00 to 1.02)	.19
Sex	Female vs male	0.88 (0.66 to 1.09)	.21	0.79 (0.60 to 1.04)	.09
Stage	Stages II–IV vs I	3.53 (2.70 to 4.61)	<.001	3.83 (2.89 to 5.07)	<.001
Smoking pack-years	Continuous	1.00 (0.99 to 1.00)	.30	1.00 (0.99 to 1.00)	.12
Y/X polymorphism	C/C + C/G vs G/G	0.66 (0.51 to 0.86)	.003	0.55 (0.42 to 0.73)	<.001
African American patients					
Age	Continuous	1.00 (0.98 to 1.03)	.74	1.02 (0.99 to 1.04)	.14
Sex	Female vs male	0.82 (0.55 to 1.21)	.31	0.70 (0.46 to 1.07)	.10
Stage	Stages II–IV vs I	4.32 (2.74 to 6.80)	<.001	4.71 (2.87 to 7.75)	<.001
Smoking pack-years	Continuous	0.99 (0.99 to 1.00)	.15	1.00 (0.99 to 1.01)	.88
Y/X polymorphism	C/C + C/G vs G/G	1.19 (0.77 to 1.85)	.43	1.01 (0.63 to 1.60)	.98

* RR = risk ratio; CI = confidence interval.

† A Cox proportional hazards test was used. All statistical tests were two-sided.

survival than high MBL haplotypes (HYA and LYA) (RR = 0.82, 95% CI = 0.73 to 0.93, $P = .002$). Thus, the association between the low MBL haplotypes and improved lung cancer-specific survival was not dependent on the Y/X SNP.

Diploypes were categorized by use of a common scheme (7,8), in which the HYA and LYA haplotypes, representing the highest MBL serum levels, were designated group Y and the exon 1 variants were designated by a single letter (A for wild type; D, B, or C for variants). Among white patients, the XA/B diploype was associated with better survival than the YA/YA diploype (RR = 0.30, 95% CI = 0.14 to 0.67, $P = .003$), consistent with the genotype and haploype data (Table 6). The YA/XA and XA/B diploypes were also statistically significantly associated with 5-year overall survival (Supplementary Table 1, available online). When the analyses were stratified by study protocol, the association between the XA/B diploype and lung cancer-specific survival remained statistically significant among white patients in the case-control study, but not among those in the case-only series (Supplementary Table 2, available online), although the magnitudes of the associations were similar.

We next dichotomized the patients into high (YA/YA, YA/XA, YA/B, YA/C, and YA/D) and low (XA/XA, XA/B, XA/C, XA/D, and O/O) serum MBL diploypes, according to serum MBL concentration, as described by Garred et al (7). Among white patients, low serum MBL diploypes, compared with high serum MBL diploypes, were associated with increased survival (RR = 0.63, 95% CI = 0.44 to 0.92, $P = .016$) (Table 6).

Discussion

Our investigation indicates that genetic variants in the innate-immunity gene MBL2 are associated with lung cancer-specific survival in white patients. The association was strongest among heavy smokers and was independent of other prognostic and demographic factors. The genetic variants are functional in that they alter serum MBL levels or oligomeric structures.

The X allele, which is associated with lower MBL levels, was statistically significantly associated with improved lung cancer-specific survival among white patients. In other words, the wild-type Y/Y genotype, and thus high MBL levels, was associated with

Table 4. Disease-specific survival in relationship to promoter Y/X single-nucleotide polymorphism genotypes and smoking*

Smoking level†	No. of patients		RR of death‡ (95% CI)	P§
	G/G	C/C + C/G		
White patients				
<28.6 pack-years	82	51	0.94 (0.54 to 1.65)	.84
28.6–64.8 pack-years	159	104	0.70 (0.47 to 1.03)	.07
>64.8 pack-years	88	49	0.33 (0.17 to 0.61)	.001
African American patients				
<28.6 pack-years	45	20	1.95 (0.94 to 4.05)	.07
28.6–64.8 pack-years	55	18	0.51 (0.22 to 1.18)	.11
>64.8 pack-years	24	7	0.92 (0.24 to 3.48)	.91

* RR = risk ratio; CI = confidence interval.

† Smoking levels were categorized by using the 25th and 75th percentile pack-year values of the white patient subjects (<25th percentile, 25th–75th percentile, and >75th percentile).

‡ Risk ratio of death of C/C (X/X) plus C/G (X/Y) versus G/G (Y/Y) genotypes was adjusted for sex, stage (III–IV/I–II), current smoking status, and age. A dominant model was assumed.

§ A multivariable Cox proportional hazards model was used. All statistical tests were two-sided.

Table 5. Association between secretor MBL2 secretor haplotypes and disease-specific survival by smoking amount*

Smoking status and haplotype†	White patients		African American patients	
	No. (%)	RR of death‡ (95% CI)	No. (%)	RR of death‡ (95% CI)
Overall				
GGCGG (HYA)	294 (28.6)	1.0 (referent)	46 (14.2)	1.0 (referent)
CGCGG (LYA)	280 (27.3)	1.14 (0.89 to 1.46)	135 (41.7)	0.85 (0.50 to 1.47)
CCCGG (LXA)	220 (21.4)	0.70 (0.53 to 0.93)	46 (14.2)	0.75 (0.47 to 1.19)
CGCAG (LYB)	135 (13.2)	0.94 (0.69 to 1.28)	15 (4.6)	0.98 (0.39 to 2.46)
CGCGA (LYC)	16 (1.6)	0.91 (0.37 to 2.24)	76 (23.4)	0.69 (0.42 to 1.14)
GGTGG (HYD)	81 (7.9)	1.03 (0.72 to 1.47)	6 (1.9)	0.40 (0.09 to 1.69)
Low vs high MBL§		0.78 (0.65 to 0.95)		0.93 (0.69 to 1.26)
<28.6 pack-years 				
GGCGG (HYA)	84 (32.5)	1.0 (referent)	17 (13.3)	1.0 (referent)
CGCGG (LYA)	68 (26.4)	1.11 (0.68 to 1.83)	52 (40.6)	1.00 (0.47 to 2.15)
CCCGG (LXA)	55 (21.3)	1.01 (0.59 to 1.74)	21 (16.4)	1.77 (0.77 to 4.02)
CGCAG (LYB)	32 (12.4)	1.32 (0.76 to 2.30)	7 (5.5)	1.52 (0.40 to 5.83)
CGCGA (LYC)	2 (0.8)	3.09 (0.69 to 13.93)	28 (21.9)	0.88 (0.39 to 1.99)
GGTGG (HYD)	17 (6.6)	0.80 (0.38 to 1.69)	3 (2.3)	1.81 (0.36 to 9.16)
Low vs high MBL§		1.04 (0.72 to 1.51)		1.23 (0.76 to 1.98)
28.6–64.8 pack-years 				
GGCGG (HYA)	136 (27.3)	1.0 (referent)	22 (15.9)	1.0 (referent)
CGCGG (LYA)	140 (28.1)	1.22 (0.86 to 1.73)	55 (39.8)	0.65 (0.13 to 0.84)
CCCGG (LXA)	111 (22.3)	0.81 (0.55 to 1.19)	19 (13.8)	0.33 (0.13 to 0.84)
CGCAG (LYB)	61 (12.3)	1.00 (0.64 to 1.58)	3 (2.2)	0.86 (0.11 to 6.83)
CGCGA (LYC)	7 (1.4)	0.40 (0.06 to 2.91)	36 (26.1)	0.45 (0.20 to 0.97)
GGTGG (HYD)	43 (8.6)	1.29 (0.78 to 2.12)	3 (2.2)	–
Low vs high MBL§		0.84 (0.64 to 1.10)		0.54 (0.33 to 0.88)
>64.8 pack-years 				
GGCGG (HYA)	74 (27.4)	1.0 (referent)	7 (12.1)	1.0 (referent)
CGCGG (LYA)	72 (26.7)	0.90 (0.56 to 1.45)	28 (48.3)	0.57 (0.16 to 1.97)
CCCGG (LXA)	54 (20.0)	0.34 (0.18 to 0.63)	6 (10.3)	0.57 (0.12 to 2.62)
CGCAG (LYB)	42 (15.5)	0.47 (0.25 to 0.89)	5 (8.6)	0.39 (0.04 to 4.04)
CGCGA (LYC)	7 (2.6)	0.71 (0.36 to 1.60)	12 (20.7)	0.62 (0.17 to 2.30)
GGTGG (HYD)	21 (7.8)	0.76 (0.36 to 1.60)	0	–
Low vs high MBL§		0.48 (0.32 to 0.72)		0.90 (0.42 to 1.92)

* RR = risk ratio; CI = confidence interval; MBL = mannose-binding lectin; – = insufficient data.

† Single-nucleotide polymorphisms are ordered in the haplotypes by location on the gene, as L/H, Y/X, A/D, A/B, and A/C.

‡ Risk ratio of death was adjusted for sex, stage (III–IV versus I–II), current smoking status, and age at diagnosis.

§ Haplotypes were categorized according to their association with low (LXA, LYB, LYC, and LYD) or high (HYA and LYA) serum MBL levels.

|| Smoking levels were categorized by using the 25th and 75th percentile pack-year values of the white patients (<25th percentile, 25th–75th percentile, and >75th percentile).

poorer survival than a genotype with at least one X allele. Consistent with the genotype data, the MBL2 secretor LXA haplotype and XA/B diplotypes, which result in low serum MBL levels, were associated with improved outcome among the white patients. In addition, the combined groups of haplotypes or diplotypes with low MBL levels were associated with better survival than the combined groups of haplotypes or diplotypes with high MBL levels, respectively.

The mechanism through which the MBL2 genetic variants are associated with lung cancer-specific survival is unclear. High levels of MBL, which result from certain polymorphisms within the MBL2 secretor haplotype, are associated with inflammation-related disorders (16–20). The MBL-mediated complement cascade leads to the activation of inflammatory cells and the liberation of proinflammatory factors, such as interleukins 1 β and 6 and tumor necrosis factor- α . Chronic inflammation and exposure to cytokines have been shown to potentiate tumor progression in animal tumor models (32,33) and to augment invasiveness of

human lung tumors (34). Mannose-binding lectin may also contribute to tumor aggressiveness by enhancing the DNA-damaging environment. Mannose-binding lectin, through interleukin 1 β and tumor necrosis factor- α , causes increased expression of the inducible form of nitric oxide synthase, which increases the production of DNA-damaging reactive nitrogen and oxygen species (35). Because smoking causes chronic inflammation of the lung, the combination of high MBL levels and heavy smoking would be expected to be associated with further decreased survival. Indeed, we found that the association between MBL2 variants related to high MBL levels and poor survival were strongest among patients who were heavy smokers. Furthermore, smoking was recently shown to be associated with increased MBL levels (29), which further supports the association between the MBL2 genetic variants and lung cancer-specific survival among the heavy smokers.

This study may provide insight into the continued disparity in lung cancer survival between African American and white patients.

Table 6. Association between MBL2 diplotypes and disease-specific survival*

Diplotype	White patients		African American patients	
	No. (%)	RR of death† (95% CI)	No. (%)	RR of death† (95% CI)
YA/YA	159 (31.0)	1.0 (referent)	46 (28.4)	1.0 (referent)
YA/XA	124 (24.2)	0.71 (0.49 to 1.04)	31 (19.1)	1.36 (0.73 to 2.53)
XA/XA	23 (4.5)	0.65 (0.46 to 1.39)	3 (1.9)	5.29 (1.10 to 25.51)
YA/B	78 (15.2)	1.01 (0.68 to 1.49)	7 (4.3)	1.82 (0.50 to 6.58)
XA/B	30 (5.8)	0.30 (0.14 to 0.67)	2 (1.2)	2.33 (0.29 to 18.71)
YA/C	8 (1.5)	1.38 (0.50 to 3.80)	47 (29.0)	1.10 (0.62 to 1.96)
YA/D	46 (9.0)	0.91 (0.56 to 1.48)	4 (2.5)	0.30 (0.04 to 2.28)
XA/D	17 (3.3)	0.58 (0.27 to 1.27)	0	–
XA/C	3 (0.6)	–	7 (4.3)	0.24 (0.05 to 1.03)
O/O‡	25 (4.9)	1.13 (0.61 to 2.08)	15 (9.3)	1.03 (0.40 to 2.62)
Low vs high§		0.63 (0.44 to 0.92)		0.67 (0.35 to 1.28)

* RR = risk ratio; CI = confidence interval; – = insufficient data.

† Risk ratio of death was adjusted for sex, stage (III–IV versus I–II), age at diagnosis, current smoking status, and pack-years of smoking.

‡ O/O indicates carriers of two exon 1 variants.

§ Diplotypes were categorized according to their association with low (XA/XA, XA/B, XA/C, XA/D, and O/O) or high (YA/YA, YA/XA, YA/B, YA/C, and YA/D) serum levels of mannose-binding lectin.

Low serum MBL levels are more prevalent among those of African descent (36), and the selection process directing MBL levels may also be affected by how patients respond to the disease. It has been suggested that, because low MBL levels are protective against opsonization of parasites and mycobacteria (36), which are more prevalent in African countries, there is selective pressure for decreased MBL levels. An interaction between the promoter Y/X SNP and race raises questions about the driving factor behind the racial differences. Additional components of the innate-immunity pathways could influence the aggressiveness of disease or response to treatment. One candidate, MBL serine protease 2 (MASP-2), which activates complement after forming a complex with MBL, also displays wide variability between interethnic/racial groups (36–38). Expression of MASP-2 in esophageal tumors was shown to be associated with aggressive tumor behavior (39). Thus, a comprehensive investigation of the relationship of MBL2 and MASP-2 to lung cancer-specific survival may lead to important insights about why African American and white patients with lung cancer have disparate survivals.

A limitation of this study was that only one of the five secretor haplotype SNPs was associated with survival, even though all five SNPs are considered to be functionally related to reduced serum MBL levels or structure. Changes in MBL levels are more dependent on the variant promoter than on structural alleles (7). Perhaps lung cancer survival is more dependent on total MBL serum levels than fully assembled MBL subunits. Functional promoter analyses have demonstrated that the Y/X SNP is much more sensitive to change of promoter activity than the L/H SNP (20), which suggests that the Y/X SNP might have a stronger impact on affecting disease related to abnormal serum MBL levels. A second limitation of the study was the low frequency of the variant allele for the Y/X SNP and the associated haplotypes and diplotypes, and so the results from the population examined in this study should be treated with caution until the associations can be validated in an independent study. Another limitation of the study was that there were fewer African American patients than white patients. The association between the low MBL LXA

haplotype and survival among African American patients who were also heavy smokers raises the possibility that MBL might also be associated with lung cancer survival among African American patients. Lastly, the study was limited to smoking status at the time of interview for the case-control study, and patients might have quit smoking between the time of diagnosis and the interview. Smoking status was considered to be current if the patient quit smoking within 6 months before the interview, and thus, the smoking status was accurate for the majority of patients. Because of this limitation, we were unable to stratify patients by smoking status and, instead, relied on total smoking amount. This result attenuated our ability to determine whether current and/or former smoking is statistically significantly associated with poor lung cancer survival among white patients who have MBL variants related to high MBL serum levels.

In summary, genetic variations in the innate-immunity gene MBL2 may be associated with lung cancer-specific survival. The improved survival associated with low MBL haplotypes indicated that, in a reduced inflammatory environment, the tumor may be less aggressive. The clinical meaning of a decreased innate immune response must be taken into consideration and the prognostic importance of modulation by smoking should be examined in more detail in future studies, as well as the role of MBL within the lung mucosa.

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