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Independent Effects of Genetic Variations in Mannose-Binding Lectin Influence the Course of HIV Disease: The Advantage of Heterozygosity for Coding Mutations

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Abstract

Background—The in vivo impact of mannose-binding lectin (MBL), a molecule involved in innate immunity, on the pathogenesis of human immunodeficiency virus (HIV)–1 infection and AIDS is unknown.

Methods—A total of 1102 HIV-positive and 2213 HIV-negative adult subjects were screened for polymorphisms in the coding and promoter regions of *MBL2*, the gene that encodes MBL.

Results—Variations in *MBL2* did not influence the risk of acquiring HIV-1. Heterozygosity for coding mutations (O allele) and homozygosity for the −221 promoter polymorphism (X allele) in *MBL2* were associated with a delay in and an accelerated rate of disease progression, respectively. *MBL2* variations influenced the rate of progression to AIDS-defining illnesses. In a multivariate model, the effects of *MBL2* variations were independent of several parameters known to influence disease progression, including steady-state viral load, baseline CD4+ T cell counts, and delayed-type hypersensitivity skin test responses, an in vivo marker of cell-mediated immunity. The effects of *MBL2* variations were most evident in those who possessed protective genotypes of CCR5 and a high copy number of *CCL3L1*, the most potent HIV-suppressive CCR5 ligand.

Conclusions—*MBL2* genotypes are independent determinants of HIV disease progression and heterozygosity for *MBL2* coding mutations confer disease-retarding effects. MBL-dependent immune responses may play a role in the pathogenesis of HIV infection.

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Introduction

There is incontrovertible evidence demonstrating that the observed intersubject variability in the course of HIV-1 disease is attributable, in part, to polymorphisms in genes that influence adaptive immune responses (e.g., *HLA*) and the entry of HIV-1 into cells [1]. For example, we found that polymorphisms in the HIV coreceptor, CCchemokine receptor 5 (CCR5), and the copy number of the gene encoding its potent HIV-suppressive ligand, CC chemokine ligand like-1 (CCL3L1) are major determinants of HIV-AIDS susceptibility [2–4]. Our recent studies suggest that variations in *CCL3L1* and *CCR5* that are categorized as low, moderate, and high *CCL3L1-CCR5* genetic risk groups might affect the pathogenesis of HIV disease by influencing both parameters that are dependent on viral entry (e.g., viral load) and parameters that are independent of viral entry (e.g., cell-mediated immunity [CMI]) [5]. By contrast, much less is known about the role of the innate immune responses in the pathogenesis of HIV disease. To address this gap in knowledge, in this study we determined whether variations in the gene encoding mannose-binding lectin (MBL), a major component of the innate immune response [6–9], influence the risk of HIV acquisition and HIV disease progression rates.

MBL is an evolutionarily conserved, circulating host-defense protein that acts as a broad-spectrum pattern-recognition molecule against a wide variety of infectious agents [7]. MBL mediates its effects by influencing complement activation [8], opsonization, and phagocytosis [9–11]. *MBL2*contains polymorphisms in the coding and promoter region [12]. The wildtype coding allele is designated the “A allele,” whereas the other polymorphisms in the coding sequence (B, C, and D alleles) are collectively designated the “O allele” (figure 1A and 1B) [12. The variations in the coding sequence affect the structural integrity of MBL, and are associated with a 2-fold to 100-fold reduction in circulating MBL levels [13]. Polymorphisms in the promoter region can also influence MBL levels. For example, the −221C polymorphism in the *MBL2* promoter (designated the “X allele”; figure 1A and 1B) is associated with dramatically lower levels of the structurally intact MBL protein [13]. Thus, variations in *MBL2* provide, in part, a genetic basis for the wide range of circulating MBL levels [13,14].

In several ethnic groups, there is a high prevalence of polymorphic alleles (e.g., B and C alleles) that result in low MBL levels, which suggests that a relative lack of MBL might be beneficial to the host under some circumstances [15–20]. This has led Garred et al. and others to hypothesize that these alleles might confer a selective advantage [15–20], such as an advantage against infection by intracellular pathogens [21]. An additional proposal is that the high frequencies of the *MBL2* polymorphisms that are observed in tropical regions may serve to reduce the detrimental effects of excessive complement activation [15, 22, 23]. Interestingly, although there is an extensive literature demonstrating that variations in *MBL2* might alter susceptibility to infectious and noninfectious diseases [7, 12, 24], only a single report has demonstrated a heterozygous advantage for possession of variations in the coding sequences [25].

It has been hypothesized that MBL deficiency might also play an important role in the context of coexisting immunodeficiency, such as that of HIV infection [26]. There is strong in vitro data linking MBL to the pathogenesis of HIV disease [26], and this is based on several observations, including: (1) purified MBL can bind to HIV-infected cell lines and directly inhibit HIV infection of lymphoblasts [27]; (2) MBL binds to and activates complement on gp120 [28]; (3)MBL binds to both CCR5-tropic and CXCR4-tropic primary HIV strains [29]; and (4) although HIV is relatively resistant to neutralization by MBL, the binding and opsonization of HIV by MBL may alter virus trafficking and viral-antigen presentation during HIV infection [30]. However, despite this extensive in vitro data linking

MBL to the pathogenesis of HIV disease, an in vivo link is less clear because the results of genetic epidemiological studies that have investigated the association between variations in *MBL2* and HIV-AIDS susceptibility have been inconclusive [14, 26, 31, 32]. Many of these associations were either weak or contradictory and/or were derived mostly from cohorts that involved small sample sizes.

In the present study, we capitalized on a large and well characterized cohort of HIV-1–positive (hereafter, "HIV^+") subjects of European and African descent to test the hypothesis that heterozygosity for polymorphisms in the coding region would result in delayed disease progression rates and possibly afford protection against specific AIDS-defining opportunistic infections. Given the phenotypic effects of the *MBL2* promoter X allele on reduction of the levels of the structurally intact MBL protein, in our analyses we also examined the effects of this allele on HIV disease. This was important because most of the prior studies that have examined the HIV disease–influencing effects of variations in *MBL2* have been restricted primarily to analyses of structural (coding) mutations, and thus have not accounted for the possible confounding effects of promoter polymorphisms that are in linkage disequilibrium with variations in the coding region [13]. Hence, we reasoned that accounting for this linkage pattern as well as distinguishing between the effects of polymorphisms that result in reduced levels of a structurally intact (promoter variants) versus an unstable protein (variants in coding sequence) might help to clarify the discordant findings regarding the role that variations in *MBL2* play in HIV disease. Additionally, we determined whether *MBL2* variants affected disease pathogenesis by impacting on the steady-state plasma viral load (VL), a strong predictor of disease progression [5, 33, 34], as well as delayed-type hypersensitivity (DTH) skin test reactivity, an in vivo marker of CMI [35, 36] that correlates strongly with T cell function in vitro [37, 38] and an independent prognosticator of HIV disease progression [5,37, 39, 40]. Finally, we assessed whether the disease-influencing effects of *MBL2* variants differed on the basis of the genetic context conveyed by CCL3L1-CCR5 genetic risk groups.

Patients, Materials, and Methods

Study population

Adult patients with HIV-1 infection who participated in the US Air Force portion of the Tri-Service AIDS Clinical Consortium Natural History Study contributed samples for this study. Wilford Hall Medical Center (WHMC) is the referral hospital for all US Air Force personnel who develop infection with HIV-1. Extensively detailed descriptions of this cohort have been published elsewhere [3, 5] and additional information is provided in the appendix (which is available only in the electronic edition of the journal).

Genotyping of MBL2 polymorphisms

Complete *MBL2* genotyping information was available for 1102 HIV-infected subjects and 2213 subjects not infected with HIV. Genotyping methods are described in the appendix (which is available only in the electronic edition of the journal).

CCL3L1-CCR5 genetic risk groups

Methods for determining the copy number of the *CCL3L1* and *CCR5* genotypes as well as their categorization into low, moderate, and high *CCL3L1-CCR5* genetic risk groups were as described elsewhere [2, 3, 5].

Delayed type hypersensitivity (DTH) skin test reactivity

The protocols for conducting the DTH skin tests in the HIV^+ WHMC cohort are as described elsewhere [5, 37, 39]. In brief, each patient, at enrollment and then prospectively,

received the standard Mantoux type of intradermal skin test to the following antigens: mumps, Trichophyton, Candida, and tetanus toxoid. Additional details about this parameter and the reasons for using the best DTH response recorded during the course of disease are as described elsewhere [5] and in the appendix (which is available only in the electronic edition of the journal).

Statistical analysis

The statistical procedures used in this paper are described in the appendix (which is available only in the electronic edition of the journal).

Results

Frequency distribution of promoter and coding region alleles

The distribution pattern of *MBL2* alleles in the HIV^+ WHMC cohort were consistent with those reported elsewhere [22, 41]. The frequencies of the promoter alleles Y (wild type) and X (polymorphic) and the wild-type coding allele A were relatively similar in European American, African American, and Hispanic American subjects (figure 1*A*–1*C*). By contrast, the frequencies of the polymorphic coding alleles B and C differed significantly among subjects of African and European descent (figure 1*C*). The polymorphic promoter X allele was in nearly complete linkage disequilibrium with the A allele (figure 1*D*) ($\chi^2 = 71.39$; $P < .001$). On the basis of this linkage pattern, 3 common haplotypes were identified, and in accord with the prevailing nomenclature for *MBL2* haplotypes [13], they were designated the YA, YO, and XA haplotypes. The allele frequencies of the coding and noncoding polymorphisms in *MBL2* among HIV^+ subjects did not deviate significantly from Hardy-Weinberg equilibrium.

Advantage of heterozygosity for MBL2 coding variants

Compared with homozygosity for the A allele (A/A genotype), heterozygosity for theAallele (i.e., A/O genotype) was associated with a significantly slower rate of disease progression (figure 2A–2C). Given the link between MBL deficiency and susceptibility to infectious diseases, we determined whether the A/O genotype afforded protection specifically against AIDS-defining opportunistic infections. In a stepwise logistic regression model, a reduced risk (odds) of developing of *Pneumocystis jiroveci* pneumonia and *Mycobacterium avium* complex was associated the possession of coding region heterozygosity (figure 2D). Additionally, the A/O genotype protected against a rapid rate of progression to P. jiroveci pneumonia and *M. avium* complex (figure 2*E* and 2*F*).

To determine the influence of coding variations on risk of acquiring HIV, we compared the genotype frequencies of *MBL2* polymorphisms in 1102 HIV-positive subjects and 2213 subjects not infected with HIV. Possession of the A/O genotype was associated with a trend for a 12% reduction in the risk of acquiring HIV (odds ratio [OR], 0.88 [95% CI, 0.76–1.03]; $P = .110$).

Detrimental effects of the X/X promoter genotype on disease

Compared to the Y/Y promoter genotype, possession of the X/X genotype was associated with a rapid rate of disease progression (figure 2*G*–2*I*). However, compared with the Y/Y genotype, possession of the X/X genotype was not associated with an increased risk of acquiring HIV (OR, 1.12 [95% CI, 0.77–1.64]; $P = .544$).

Effects of MBL2 haplotype pairs

Given the aforementioned results, we tested the following 2 hypotheses: (1) because both the A/A and X/X genotypes were each associated with disease-accelerating effects (figure 2*A* and 2*G*) and because the polymorphic promoter X allele is in nearly complete linkage with the wild-type coding A allele, the observed disease accelerating effects of the A/A and X/X genotypes are due mainly to the XA/XA haplotype pairs; and (2) based on the genotype phenotype relationships depicted in figure 2*A* and 2*G*, *MBL2* haplotype pairs that contain the detrimental X/X genotype and the protective A/O genotype will be at the polar ends of the susceptibility spectrum for disease progression rates. To test these hypotheses, we stratified the cohort into 4 *MBL2* genotypic groups (figure 3*A*). The findings shown in figure 3*B* support these 2 hypotheses. Thus, compared with possession of the A/O genotype (group 1, figure 3*A*), possession of the XA/XA genotype (group 4, figure 3*A*) was associated with a nearly 2-fold faster rate of disease progression (figure 3*B*)

Independent effects of MBL2 polymorphisms on HIV disease

The magnitude of the initial $CD4_+$ T cell loss, steady-state VL, and DTH skin test responses did not differ by *MBL2* genotypic group (figure 3*C*–3*E*). Furthermore, among subjects with a high baseline $CD4_+$ T cell count (figure 3*F*), low steady-state VL (figure 3*G*), and robust CMI as characterized by higher numbers of positive DTH skin test results (figure 3*H*), the *MBL2* genotypic groups remained predictors of variable rates of disease progression. Notably, as a general rule, the strength of the association between the *MBL2* genotypes and the disease progression rates observed in the cohort overall (figure 3B) was similar or greater in those subjects with favorable laboratory profiles and DTH responses (figure 3F–3H). For example, in the cohort overall, the hazard for progression to death for the XA/XA genotype was 2.29 (figure 3B), whereas it was 4.39 (95% CI, 1.84–10.5), 4.64 (95% CI, 1.73–12.5), and 2.79 (95% CI, 1.33–5.85) for those with a $CD4_+$ cell count ≥700 cells/mm^3, a VL <20,000 copies/mL, and ≥3 positive DTH skin test results, respectively (figure 3*F*–3*H*).

The aforementioned findings suggested that *MBL2* polymorphisms might have independent disease-influencing effects. To assess this possibility further, we determined whether *MBL2* genotypes were associated with independent disease-influencing effects after adjustment for parameters that have previously been shown to be independent determinants of disease progression, such as baseline and nadir $CD4_+$ T cell count, steady-state VL, *CCL3L1-CCR5* genetic risk groups, and DTH skin test reactivity [5]. Table 1 shows the results of a series of nested Cox proportional hazards models in which we adjusted either separately or in unison for these covariates. The findings indicated that the disease-accelerating effects associated with *MBL2* genotypic group 4 (XA/XA) were independent of these parameters (table 1; model 14) (*RH* = 1.75 [95% CI, 1.00–3.06]; *P* = .052).

Permissive *CCL3L1-CCR5* genetic environment for the effects of the XA/XA genotype

However, it was not known whether the independent disease-influencing effects of *MBL2* genotypes were distributed evenly across the low, moderate, and high *CCL3L1-CCR5* genetic risk groups or were restricted to a specific *CCL3L1-CCR5* genetic background. This was important to evaluate, as the identification of gene-gene interactions and gene modifiers is a principal challenge for the future understanding of the genetics of non-Mendelian diseases [42, 43]. In a recent study, we found that membership in a low *CCL3L1-CCR5* genetic risk group (figure 4A) was associated with high baseline $CD4_+$ T cell counts, low steady-state VL, better delayed-type hypersensitivity skin test responses, and slower rates of disease progression [3, 5].

In the present study, we found that the disease-accelerating effects associated with the XA/XA genotype were evident in laboratory and delayed-type hypersensitivity profiles that we had previously found to be associated with a low *CCL3L1-CCR5* genetic risk group (figure 3*F*–3*H*). This raised the possibility that the disease-influencing effects of *MBL2* genotypes might be restricted to subjects who were members of a low *CCL3L1-CCR5* genetic risk group. To test this possibility, we determined the effects of the *MBL2* genotypic groups in patients from low, moderate, and high *CCL3L1-CCR5* genetic risk groups (figure 4). Among subjects from a low *CCL3L1-CCR5* genetic risk group, those who also possessed the *MBL2* XA/XA genotype had a 5-fold higher risk of progressing rapidly to death, and this risk increased to 9-fold after adjusting for the baseline $CD4_+$ T cell count and steady-state VL (figure 4*B*). By contrast, a disease-accelerating effect for the *MBL2* XA/XA genotype was not evident in subjects from a moderate or high *CCL3L1-CCR5* genetic risk group (figure 4*B*). This suggested that evidence of the disease-influencing effects of *MBL2* genotypes may be restricted to a particular *CCL3L1-CCR5* genetic background (i.e., a low genetic risk group). Subjects in the WHMC cohort were followed up from the early stages of infection [5], and the data reported thus far were for all subjects, regardless of seroconversion status. However, the results were consistent even after adjusting for seroconversion status (table A1 in the appendix, which is available only in the electronic edition of the journal).

We next determined the association between the 4 *MBL2* genotypic groups and the rate of progression to AIDS-defining opportunistic illnesses. However, considering the aforementioned results (figure 4), the statistical analyses were restricted to subjects who were also members of a low *CCL3L1-CCR5* genetic risk group. In these analyses, the *MBL2* genotypic group 1 (i.e., subjects heterozygous for A/O) served as the reference category (*RH* = 1). Among the subjects who were members of a low *CCL3L1-CCR5* genetic risk group, when those with the A/O genotype were compared with subjects who had the other *MBL2* genotypic groups, we found the following. First, the *MBL2* genotypic groups 2 and 3 were associated with an increased risk of progressing rapidly to *Pneumocystis jiroveci* pneumonia and HIV-associated dementia, respectively (table A2 in the appendix, which is available only in the electronic edition of the journal). Second, although the relative hazards for progression to specific AIDS-defining illnesses were higher for subjects with the XA/XA genotype (genotypic group 4), compared with those with all other genotypes, they were highest for progression to Kaposi sarcoma and lymphoma (table A2 in the appendix, which is available only in the electronic edition of the journal).

Discussion

In the present study, we found that in the context of HIV infection, heterozygosity for polymorphisms that alter the structural integrity of MBL (i.e., the A/O genotype) is associated with disease-retarding effects (figure 2*A*–2*C*), whereas homozygosity for the promoter X allele—which results in a structurally intact protein but markedly reduced levels of MBL—is associated with disease-accelerating effects (figure 2*G*–2*I*). These results indicate an important role for *MBL2* in the pathogenesis of HIV disease and show that it is not only levels of MBL per se, but also the structural integrity of the MBL protein, that are critical determinants of the phenotypic outcomes associated with variations in *MBL2*. These results also provide support for the hypothesis that heterozygosity for variations in the coding sequence of MBL might provide an advantage against infectious diseases [15–20, 25].

The *MBL2* genotype-phenotype associations detected offer novel insights into the mechanisms that might mediate HIV disease. We found that variations in *MBL2* did not influence the classical determinants of HIV pathogenesis (figure 3*C*–3*E*) and that the effects

of these variants on HIV disease were evident among those who had immunologic profiles (high $CD4_+$ count and increased DTH reactivity), virologic profiles (low steady-state VL) and genetic profiles (low *CCL3L1-CCR5* genetic risk group) that favored a slow rate of disease progression. These findings indicated that *MBL2* genotypes might affect HIV pathogenesis by impacting on distinct pathways that contribute directly to the pathogenesis of specific AIDS-defining illnesses rather than influencing the level of viral replication or CMI as assessed by DTH skin test reactivity. This possibility was affirmed by the fact that the effects associated with *MBL2* genotypes remained after adjusting for other established independent determinants of disease progression (table 1).

The findings shown in figure 4 illustrate 2 points. First, it is important to consider the role of gene-gene interactions in the phenotypic effects associated with genetic variants. Second, the use of only AIDS or death as an end point might mask the effects of genotypes on specific AIDS-defining illnesses. Illustrating the first point, the effects of the XA/XA genotype on the rate of progression to death were most prominent in subjects who belonged to the low *CCL3L1-CCR5* genetic risk group. This indicated that specific *CCL3L1-CCR5* genotypes provided a permissive genetic environment in which the variants of another gene system, such as*MBL2*, can fully express their phenotypic effects. Illustrating the second point, even though membership in *MBL2* genotypic group 2 or 3 did not influence the overall rate of HIV disease progression among subjects who belonged to the low *CCL3L1-CCR5* genetic risk group, these genotypes were associated with more rapid rate of progression to specific AIDS-defining illnesses. The latter findings not only emphasize the range and complexity of the genotype-phenotype relationships but also the importance of accounting for the linkage pattern between polymorphisms in the promoter and coding regions of *MBL2*.

How might one explain these rather complex effects that MBL has on HIV-AIDS pathogenesis? Because MBL can act both by complement activation and direct opsonization, its net effect in HIV disease is likely to be the result of interplay between these 2 processes. We conjecture that the effect of reduced levels of structurally-impaired versus intact MBL on specific infectious versus noninfectious AIDS-defining illnesses might contribute to the genotype-phenotype relationships detected in this study (discussed in the “Supplementary Note” section of the appendix, which is available only in the electronic edition of the journal). Of interest, recent in vivo studies point to a role for MBL-dependent pathways in malignancy [44–46], and whether this underlies the association observed between the XA/XA genotype and lymphoma and Kaposi sarcoma will need validation in other large cohorts that involve subjects with these diseases. However, the failure to detect an association between *MBL2* variations and viral load suggests that the direct effects of MBL on HIV replication might be minimal.

Our results differ from those of previous studies that have also examined the association between variations in *MBL2* and HIV disease progression [14, 31, 32, 47]. There could be 2 reasons for this. First, these prior studies were limited by moderate or small sample sizes [14, 31, 32, 47]. Second, these studies did not account for the confounding from promoter polymorphisms. One limitation of the present study is that we only used the polymorphism at the −221 position in the promoter region (X allele). A polymorphism at position −550 has also been previously studied [48]. However, with respect to these additional polymorphisms, the following points are important to note: the polymorphism at the −221 position has a more dominant effect on the circulating levels of MBL [13], and it occurs only when there is a mutation at the −550 position. The polymorphic allele at −550 is designated the “L allele” [13], and these linkage patterns imply that the XA/XA genotype represents the LXA/LXA genotype. Consequently, the genotype-phenotype relationships associated with the XA/XA genotype studied here are also likely to capture the genotype-phenotype relationships

associated with the LXA/LXA genotype. Taken together, our findings provide novel insights into the genetic basis for intersubject differences in the rate of HIV disease progression, because they illustrate the importance of the immune response arm that is linked to MBL during HIV pathogenesis. The findings indicate that variations in the gene that encodes MBL affect HIV pathogenesis without directly impacting previously validated determinants of disease progression, including the extent of viral replication, and their effects might be targeted to specific AIDS-defining illnesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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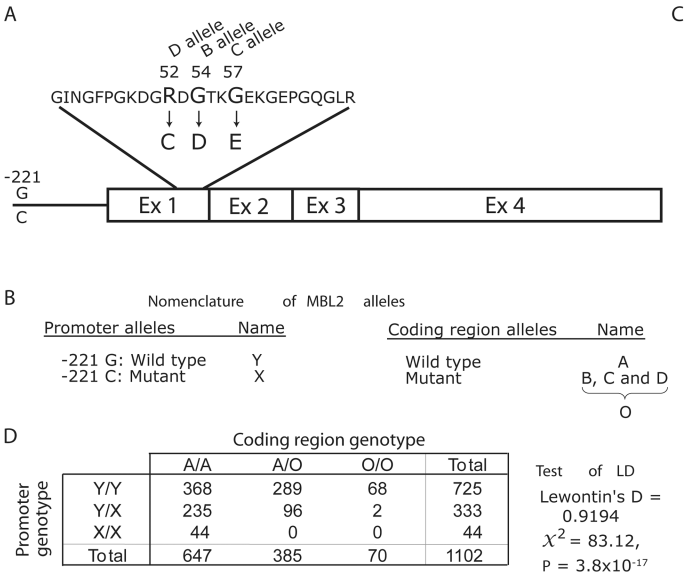

C

	European Americans (n=612)	African Americans (n=395)	Hispanic Americans (n=65)
Allele Frequency			
Promoter region alleles			
Y	0.79	0.84	0.85
X	0.21	0.16	0.15
Coding region allele			
A	0.76	0.73	0.80
B	0.14	0.03	0.14
C	0.02	0.21	0.04
D	0.08	0.03	0.02
Genotype Frequency			
Promoter region			
Y/Y	0.63	0.70	0.74
Y/X	0.32	0.28	0.23
X/X	0.05	0.02	0.03
Hardy-Weinberg χ^2	1.12	0.07	0.37
P	0.2893	0.7989	0.5433
Coding region			
A/A	0.60	0.54	0.62
A/O	0.33	0.38	0.36
O/O	0.07	0.08	0.02
Hardy-Weinberg χ^2	3.99	0.22	1.47
P	0.0457	0.6369	0.2246

Figure 1.

Gene structure of *MBL2* and distribution of the coding and promoter *MBL2* alleles in the HIV-positive (HIV^+) cohort. A, *MBL2*spans a 3.4-kb area on chromosome 10q11.2-q21 and comprises 4 exons. The positions of the 3 coding polymorphisms (B, C, and D alleles) and the single promoter (−221) polymorphism studied are shown (reference sequence numbers: codon 52: rs5030737, codon 54: rs1800451, codon 57: rs1800450, and promoter −221: rs7096206). The 3 coding polymorphisms result in nonsynonymous changes (indicated by arrows, e.g., R52C). The numbers at the top indicate the codon position where the polymorphism occurred. *B*, Nomenclature used for the *MBL2* alleles. The coding alleles B, C and D were analyzed as a single group (the "O allele") [13]. *C*, Allele and genotype frequency and Hardy-Weinberg equilibrium for the*MBL2* alleles and genotypes, by ethnic groups represented in the HIV^+ cohort. D, Linkage disequilibrium between the promoter and coding region mutations of the *MBL2* gene. Linkage disequilibrium was tested by using Lewontin's D′ and the χ^2 test of association. We assessed whether the polymorphisms in the promoter and coding region were in Hardy-Weinberg equilibrium by using the $\chi 2$ test with 1 degree of freedom. The linkage disequilibrium between promoter and coding polymorphisms was assessed by estimating the normalized disequilibrium parameter (Lewontin's D′). We detected an exception in only 4 individuals who had the XO haplotype (3 African Americans and 1 European American), which indicated that the XO haplotype is extremely infrequent and may represent a rare recombination event.

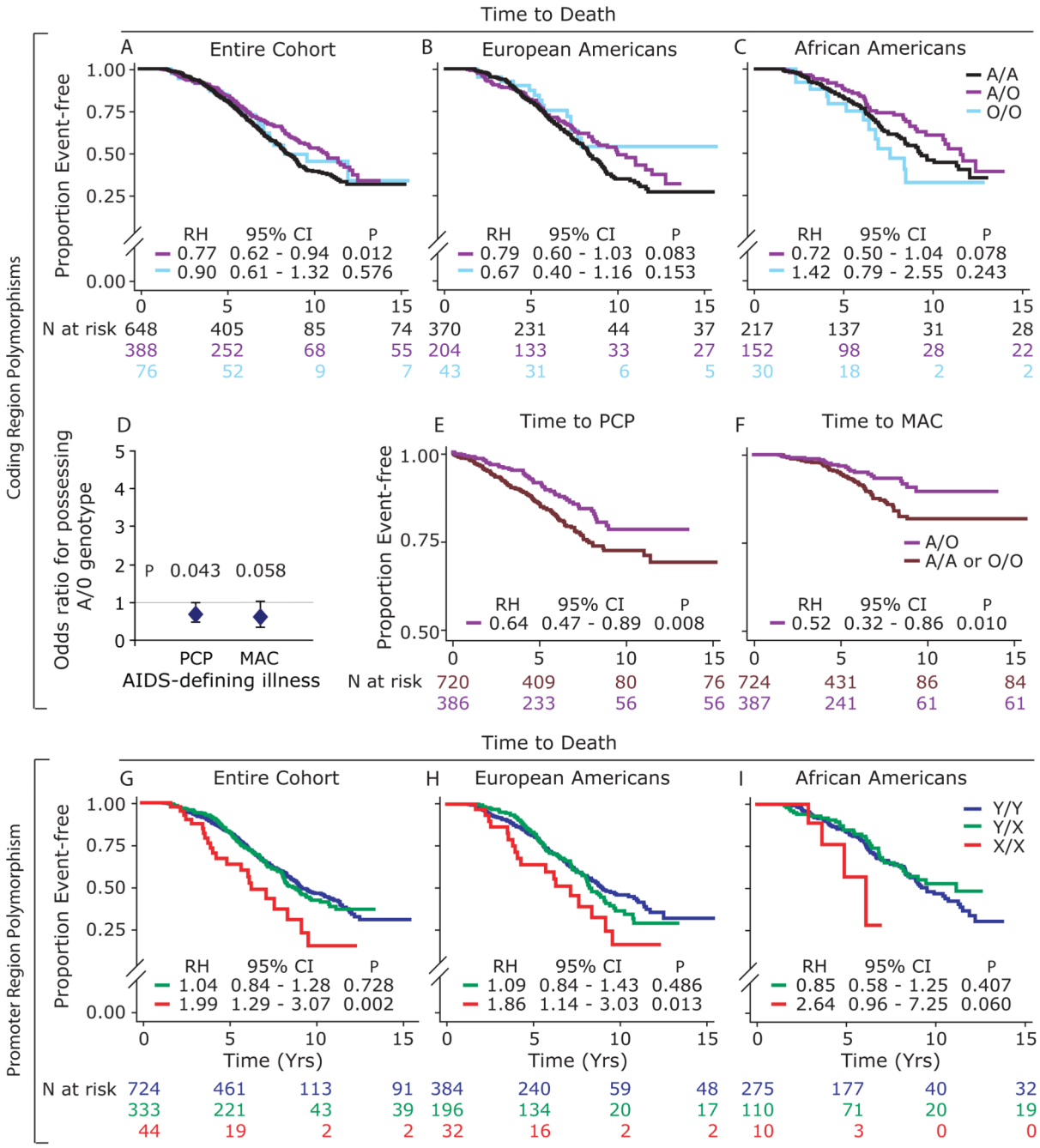

Figure 2.

Influence of polymorphisms in the coding and promoter sequences of *MBL2* on the rate of disease progression in the HIV$^+$ cohort. *A, B*, and *C*, Kaplan-Meier plots demonstrating the advantage of heterozygosity for coding region polymorphisms in HIV disease progression. The protective effect of heterozygosity with respect to progression to death was detected at the level of the entire cohort (A), as was well as in both the European *(B)* and African American *(C)* ethnic groups. “A” indicates wild-type allele and “O” indicates *MBL2* B, C, and D alleles combined; see figure 1 for definitions of X and Y. The reference category for statistical analysis was the A/A genotype. *D*, Influence of heterozygosity for coding region polymorphisms on the risk of developing specific AIDS-defining illnesses. The results are

based on the final model of a stepwise logistic regression analysis that accounted for the possibility of the association of coding-region heterozygosity with AIDS-defining illnesses (listed in table A2 in the appendix, which is available only in the electronic edition of the journal). In the full regression model, the possession of coding-region heterozygosity was used as the dependent variable and the AIDS-defining illnesses were used as independent variables that were all placed in a single model. Thus, the final stepwise regression model showed the statistically significant association between possession of coding-region heterozygosity and the AIDS-defining illnesses in a multivariate context, an approach that minimized the need for univariate associations and multiple comparisons. The stepwise regression approach used a backward elimination process with a probability criterion of $P < .1$. Significant or trending associations (depicted as odds ratio ± confidence intervals) that were close to the significance value of .05 are shown. Coding-region heterozygosity was associated with delayed progression to *Pneumocystis jiroveci* pneumonia (PCP) *(E)* and *Mycobacterium avium* complex (MAC) *(F)*. The association of coding-region heterozygosity with these 2 AIDS-defining illnesses is shown because of the multivariate results shown in D. The reference category in E and F was homozygosity for A/A or O/O. *G, H*, and *I*, Kaplan-Meier plots for progression to death based on possession of −221 promoter polymorphism in *MBL2*. The reference category for statistical analysis was the Y/Y genotype. Color-coded numbers under each plot indicate the number of subjects who were at risk at the time points specified on the abscissa. *P* significance value obtained by Cox proportional hazards regression. “Percentage event-free” refers to the percentage of the cohort who did not die (panels *A–C* and *G–I*) or who did not develop PCP *(E)* or MAC *(F)*. CI, confidence interval; RH, relative hazards.

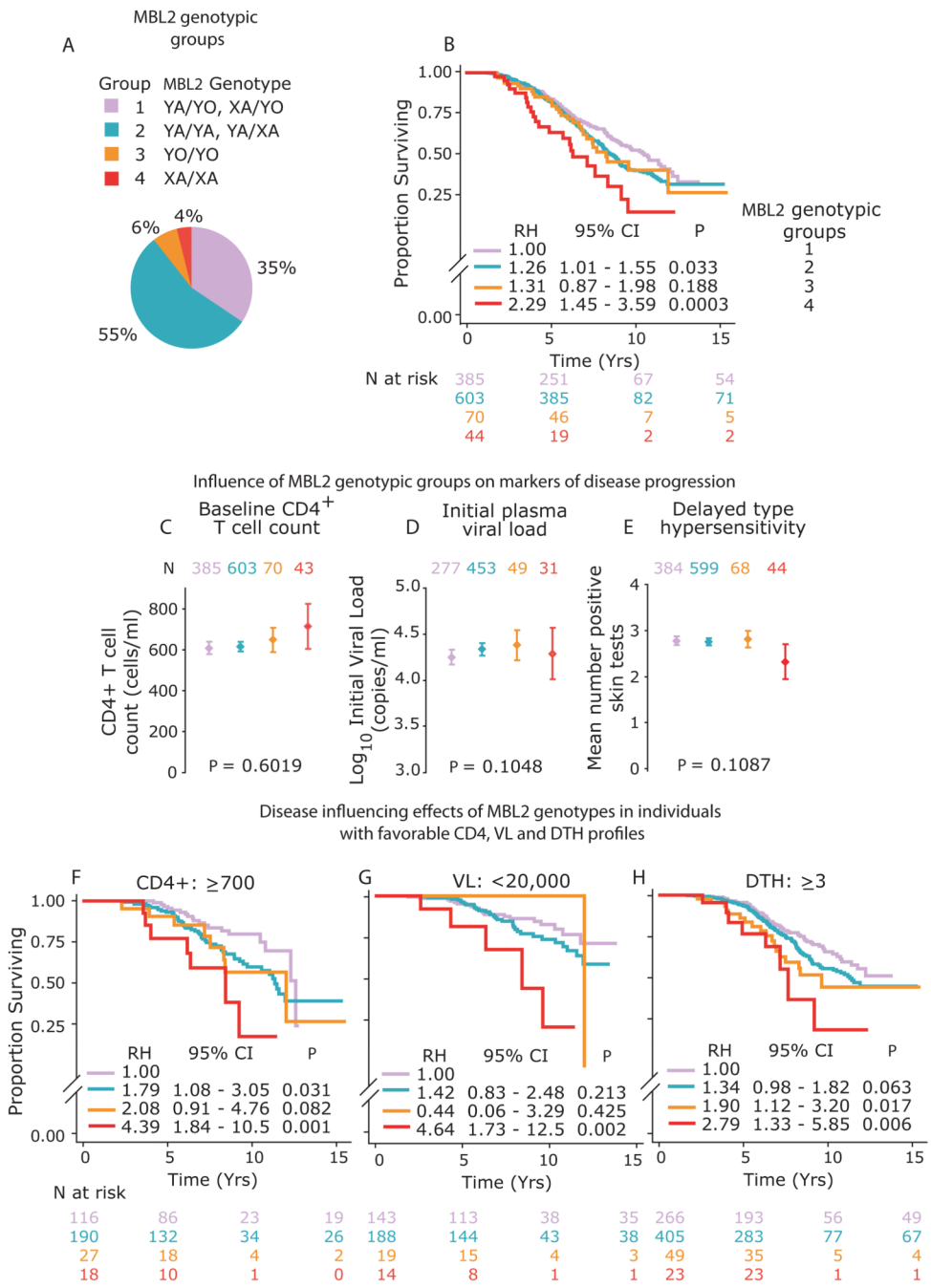

Figure 3.

Influence of *MBL2* genotypes on disease course in the HIV positive (HIV^{+}) cohort. A, Grouping of *MBL2* genotypes based on promoter and coding-region polymorphisms and distribution of the genotypes in the HIV^{+} cohort. *B*, Kaplan-Meier plots showing overall rate of progression to death in the cohort, by *MBL2*genotypic groups. *C, D*, and *E*, Influence of *MBL2* genotypes on the indicated determinants of HIV disease progression. Plots show the mean *(diamonds)* and 95% confidence intervals *(error bars)*. The overall difference of the medians across the genotypic groups was assessed by using the Kruskal-Wallis test. *F, G*, and*H*, Time to death in subjects with the indicated strata of baseline CD4$_{+}$ T cell count (CD4; cells/mm^{3}), initial viral load (VL; $\log_{10}$copies/mL) and delayed-type hypersensitivity

responses (DTH; no. of positive skin test results). The *MBL2* genotypic groups stratified subjects with a high baseline $CD4_+$ T cell count (*F*, ≥700 cells/mm^3), a low initial viral load (*G*, <20,000 copies/mL), and robust DTH responses (≥3 positive skin test results). In panels *B, F, G*, and *H*, possession of coding-region heterozygosity was considered the reference category (relative hazard [RH] = 1). Color-coded numbers under each plot indicate the number of subjects who were at risk at the time points specified on the abscissa. *P* value obtained by Cox proportional hazards regression. 95% CI, 95% confidence interval.

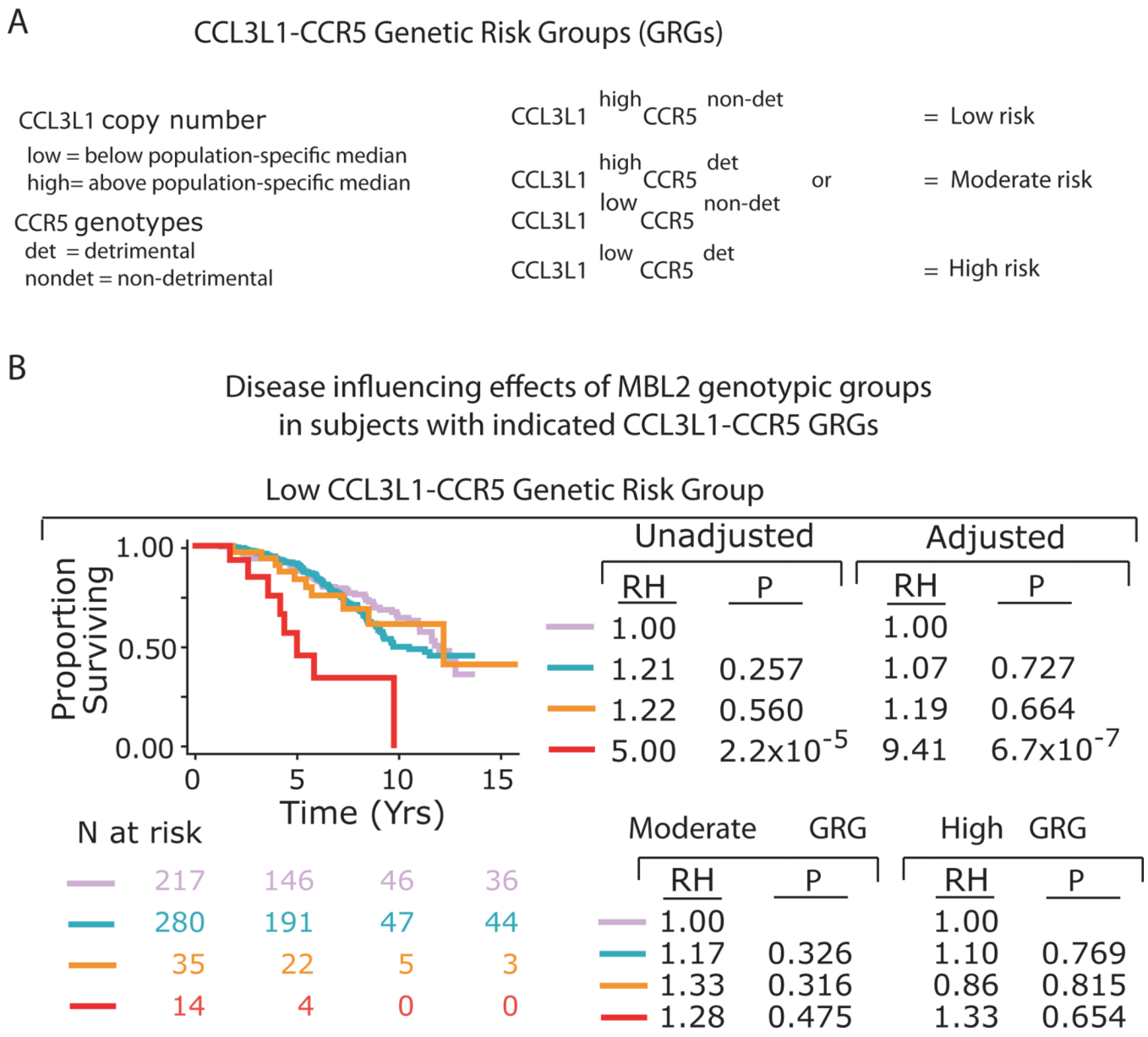

Figure 4.

Disease-influencing effects of *MBL2* genotypes on the background of the *CCL3L1-CCR5* genetic risk groups. *A*, Classification schema for the *CCL3L1-CCR5* genetic risk groups (GRG). *B*, Association between *MBL2* genotypic groups and HIV disease progression in subjects from the low, moderate, and high*CCL3L1-CCR5* genetic risk groups. Color-coding for the *MBL2*genotypic groups is as shown in figure 3A. In panel *B*, the relative hazard (RH) was also adjusted for the baseline $CD4_+$ cell count and steady-state viral load. Color-coded numbers under the Kaplan-Meier plot indicate the number of subjects who were at risk at the time points specified on the abscissa.

Table 1

Multivariate Cox proportional hazards regression analyses to assess the independent disease-influencing effects of the *MBL2*polymorphisms in the HIV-positive cohort.

Model	Covariate	N	Group 2 RH (95% CI) *P*	Group 3 RH (95% CI) *P*	Group 4 RH (95% CI) *P*
1	None	1102	1.26 (1.02 – 1.55) 0.033	1.31 (0.87 – 1.98) 0.188	2.28 (1.45 – 3.59) <0.001
2	bCD4+	1102	1.29 (1.05 – 1.60) 0.017	1.54 (1.03 – 2.32) 0.038	2.72 (1.73 – 4.29) <0.001
3	VL	810	1.21 (0.94 – 1.56) 0.131	1.09 (0.65 – 1.82) 0.756	1.74 (1.00 – 3.01) 0.048
4	T	1102	1.12 (0.91 – 1.38) 0.291	1.21 (0.81 – 1.82) 0.356	2.12 (1.35 – 3.34) 0.001
5	nCD4	1102	1.23 (1.00 – 1.52) 0.054	1.26 (0.84 – 1.89) 0.265	2.22 (1.41 – 3.85) 0.001
6	DTH	1095	1.25 (1.01 – 1.54) 0.040	1.43 (0.94 – 2.16) 0.091	1.76 (1.12 – 2.76) 0.015
7	%CD4+	1083	1.29 (1.04 – 1.59) 0.021	1.50 (1.00 – 2.26) 0.052	2.43 (1.54 – 3.82) <0.001
8	GRG	1090	1.18 (0.96 – 1.46) 0.122	1.25 (0.83 – 1.88) 0.278	1.87 (1.17 – 2.98) 0.008
9	bCD4+, VL	810	1.18 (0.92 – 1.51) 0.204	1.13 (0.67 – 1.89) 0.655	1.90 (1.09 – 3.30) 0.022
10	bCD4+, VL, T	810	1.06 (0.83 – 1.37) 0.626	1.11 (0.66 – 1.87) 0.693	1.95 (1.12 – 3.38) 0.018
11	bCD4+, VL, T, nCD4+	810	1.11 (0.86 – 1.43) 0.431	1.19 (0.71 – 2.00) 0.517	1.98 (1.14 – 3.44) 0.015
12	bCD4+, VL, T, nCD4+, DTH	807	1.03 (0.80 – 1.33) 0.820	1.18 (0.70 – 1.99) 0.533	1.96 (1.13 – 3.40) 0.016
13	bCD4+, VL, T, N, DTH. %CD4+	795	1.11 (0.86 – 1.44) 0.422	1.15 (0.68 – 1.94) 0.596	2.06 (1.19 – 3.57) 0.010
14	bCD4+, VL, T, N, DTH. %CD4, GRG	788	1.09 (0.84 – 1.41) 0.532	1.09 (0.64 – 1.84) 0.756	1.75 (1.00 – 3.06) 0.052

NOTE. Results are for multivariate Cox proportional hazards modeling of time to death based on the MBL2 genotypic groups before adjustment (model 1) and after adjustment for the indicated covariate(s), either individually (models 2–8) or in unison (models 9–14). The reference category was subjects in MBL2 genotypic group 1 (A/O heterozygyosity). %CD4+, percentage of CD4+ T cells; bCD4+, baseline CD4+ T cell count (cells/mm3); CI, confidence interval; DTH, delayed-type hypersensitivity skin test reactivity; GRG, CCL3L1-CCR5 genetic risk group; n, no. of study subjects; nCD4+, nadir CD4+ T cell count; T, cohort membership during therapy era; RH, relative hazard; VL, steady-state plasma viral load (log10 copies/mL). T, DTH, and CCL3L1-CCR5 GRG (low, moderate, and high) are as described elsewhere [5].