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An age-dependent association of mannose-binding lectin-2 genetic variants on HIV-1-related disease in children

Kumud K. Singh, PhD[a], **Alexis Lieser, MD**[b], **Ping K. Ruan, PhD**[c], **Terry Fenton, EdD**[c], and **Stephen A. Spector, MD**[a]

[a]Department of Pediatrics, Division of Infectious Diseases, University of California, San Diego, La Jolla, California

[b]University of California Irvine Medical Center, Orange, California

[c]Center for Biostatistics in AIDS Research, Harvard School of Public Health, Boston, Mass

Abstract

Background—Mannose-binding lectin (MBL) is part of the lectin pathway of complement activation against various pathogens; however, its role in innate immune responses against HIV-1 infection in children is unknown.

Objective—This study evaluated the effects of mannose-binding lectin-2 *(MBL2)* alleles on HIV-1 disease progression and central nervous system (CNS) impairment in children.

Methods—A cohort of 1037 HIV-1-infected children enrolled in Pediatrics AIDS Clinical Trial Group protocols P152 and P300 before the availability of effective antiretroviral therapy was genotyped for *MBL2* and evaluated for disease progression.

Results—Children with the homozygous variant *MBL2-O/O* genotype were more likely to experience rapid disease progression and CNS impairment than those with the wild-type *AA* genotype. The effects were predominantly observed in children younger than 2 years. In unadjusted Cox proportional hazards models, children younger than 2 years with *MBL2-O/O* experienced more rapid disease progression (*O/O* vs *AA*: relative hazard [RH], 1.54; 95% CI, 1.07-2.22; $P = .02$; *O/O* vs *A/O*: RH, 2.28; 95% CI, 1.09-4.79; $P = .029$). Similarly, children with *MBL2-O/O* were more likely to experience rapid progression to CNS impairment (*O/O* vs *A/A*: RH, 2.78; 95% CI, 1.06-2.69, $P = .027$; *O/O* vs *A/O*: RH, 1.69; 95% CI, 1.07-7.21; $P = .035$). The effects remained significant after adjustment for $CD4^+$ lymphocyte count, plasma HIV-1 RNA, and other genotypes.

Conclusions—*MBL2-O/O* genotypes, which result in lower expression of MBL, are associated with more rapid HIV-1-related disease progression, including CNS impairment, predominantly in children younger than 2 years. These data suggest that *MBL2* variants are associated with altered HIV-1 disease progression, particularly in young children.

Reprint requests: Stephen A. Spector, MD, Department of Pediatrics, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0672. saspector@ucsd.edu; or Kumud K. Singh, PhD, Department of Pediatrics, University of California, San Diego, 9500 Gilman Dr, La Jolla, CA 92093-0672. kusingh@ucsd.edu..

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Clinical implications: HIV-1-infected children who have *MBL2* genetic variants that result in lower MBL levels have a significantly greater risk for HIV-related complications, including cognitive impairment, particularly young children.

Keywords

Mannose binding lectin-2; polymorphisms; HIV-1 disease; central nervous system impairment; children

Innate immunity comprises antigen-nonspecific defense mechanisms that a host uses immediately or within hours after exposure to a broad spectrum of antigens to eliminate microbes and prevent infection. Unlike adaptive immunity, innate immunity is designed to recognize a few highly conserved structures present in many different microorganisms. Mannose-binding lectin protein (MBL), also called mannose-binding protein, encoded by the mannose binding lectin-2 *(MBL2)* gene, is an important determinant of the innate immune response during an infection.1-4 MBL is an acute-phase protein that is synthesized by the liver and released into the blood-stream, where it binds to the mannose residues present on some bacteria, yeast, viruses, and parasites. Binding activates the lectin complement pathway and production of C3b protein through MBL-associated serine proteases,5 which results in opsonization of pathogens, chemotaxis, activation of leukocytes, and direct killing of pathogens.

The *MBL2* gene encodes 32-kd subunits that oligomerize to form 96-kd MBL structural units or “monomers.” The “monomers” then further associate to form high-molecular-weight MBL oligomers.4 Only the high-molecular-weight oligomer structure is capable of activating complement. *MBL2* variants at the following nucleotide positions affect MBL levels: 2 single nucleotide polymorphisms at promoter positions -550-G/C (*H/L* variant) and -221-G/C (*X/Y* variant), one in the 5′ untranslated region +4-C/T (*P/Q* variant) and 3 genetic variants at codons 52, 54, and 57 in exon 1 at nucleotide positions 223-C/T (Arg52Cys, *A/D* allele), 230-G/A (Gly54Asp, *A/B* allele), and 239-G/A (Gly57Glu, *A/C* allele), respectively. *MBL2* exon 1 variants result in single amino acid changes affecting oligomerization of MBL. Homozygous wild-type *(A/A)* sera contain predominantly fully functional MBL, whereas homozygous mutant sera (any combination of *B*, *C*, or *D* alleles) contain mostly low-molecular-weight MBL. Sera containing heterozygous variant alleles (*A/O*, *O* = *B*, *C*, or *D*) contain both high-molecular-weight and low-molecular-weight MBL, with the ratio determined by the promoter type on the normal haplotype (*A* allele).

MBL deficiency was initially recognized as an opsonic defect in children with frequent unexplained infections and has been linked to increased severity and incidence of complications for several inherited immunodeficiency and autoimmune diseases.6-11 MBL deficiency has also been associated with increased HIV-1 vertical transmission.12 Recently, an age-dependent association of *MBL2* variants on the susceptibility to acute respiratory tract infection and meningococcal disease was reported.8,13

The current study examined the distribution of *MBL2* genotypes/haplotypes and their effect on HIV-1-related disease progression and central nervous system (CNS) impairment in children. We hypothesized that the presence of genetic variants of *MBL2* resulting in production of lower or nonfunctional protein would be associated with susceptibility to HIV infection and disease progression in children. We further hypothesized that younger children with *MBL2* variants would experience more rapid HIV-1 disease than older children. Our findings support an age-dependent association of *MBL2* variants with HIV-1-related disease in children.

METHODS

Patient populations

Two randomized, double-blind, multicenter Pediatric AIDS Clinical Trial Group (PACTG) protocols, P15214 and P30015, for seroprevalent children with symptomatic HIV-1 infection were designed to compare the effects of mono-therapy with either zidovudine or didanosine and combination therapy with zidovudine plus didanosine and to compare the effects of zidovudine plus lamivudine with didanosine monotherapy and zidovudine plus didanosine combination therapy, respectively. Children from both the studies, PACTG 152 (n = 448) and PACTG 300 (n = 589), were screened for *MBL2* exon 1, untranslated region, and promoter region polymorphisms, retrospectively.

Details of characteristics of children, eligibility criteria, study end points, disease progression, and neuropsychological tests used have been described earlier.14-16 The neuropsychological and neurodevelopmental tests administered were the Bayley Scales of Infant Development (3-30 months old), the McCarthy Scales of Children's Abilities (31 months to 6 years old), and the Wechsler Intelligence Scale for Children: Revised (WISC-R, for 6-15 years, 11 months old) or the Wechsler Adult Intelligence Scale: Revised (WAIS-R; for >16 years of age). The Mental Developmental Index of Bayley scales, the General Cognitive Index of the McCarthy scales, and the full-scale IQ of the WISC-R or WAIS-R were used to assess cognitive function. The standard score for each test was 100, and the SD was 16 points for the Bayley Mental Developmental Index and the McCarthy General Cognitive Index and 15 points for the WISC-R and WAIS-R. Children were considered to have significant developmental delay if they had a standardized score of 70 or less or (almost equal to) 2 SDs less than the normal test score of 100. Informed consent was obtained from study participants. This study followed the human experimentation guidelines of the US Department of Health and Human Services and the University of California, San Diego review board.

Genotyping

MBL2 genotyping was done by means of real-time PCR with melting curve analysis (LightCycler; Roche, Indianapolis, Ind), as described earlier.17 The studied genotypes included 2 single nucleotide polymorphisms at promoter positions -550-G/C (*H/L* variant) and -221-G/C (*X/Y* variant), one in the 5′ untranslated region +4-C/T (*P/Q* variant) and 3 genetic variants at codons 52, 54, and 57 in exon 1 at nucleotide positions 223-C/T (Arg52Cys, *A/D* allele), 230-G/A (Gly54Asp, *A/B* allele), and 239-G/A (Gly57Glu, *A/C* allele), respectively.

Statistical analyses

The primary end points for the analyses were either time to progression to first clinical HIV-1-related disease end point or death, which constituted the progression-free survival (PFS). The disease progression included weight-growth failure, ≥2 opportunistic infections, malignancy, and/or a new abnormality of the CNS (eg, neurologic deterioration, a decrease in neurocognitive test scores, and/or brain growth failure). The CNS impairment end point, a subset of PFS, was defined as time from entry on the study to the deterioration in brain growth, psychologic function, and/or neurologic status.14-16 Table I provides a summary of end points by age group. The cross-tabulations of *MBL2* genotypes/haplotypes by race/ethnicity and age groups were used to evaluate the genotype and allele frequencies.

Analyses of time to PFS and CNS impairment end points were performed by using Kaplan-Meier methods, and proportional hazards models were used to investigate the effects of genotype variants on PFS and CNS end points in univariate and multivariate analyses that

included baseline covariates ($CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex), as well as other host genotypes previously found to alter HIV-related disease. We also controlled for potential effects of treatment and treatment failure by including the change in $CD4^+$ lymphocyte counts and plasma HIV-1 viral load during follow-up as time-varying covariates in the Cox proportional hazard model. Based on the hypothesis that younger children with *MBL2* variants might have a different experience with regard to disease progression from the older children and because the median age of the children in this cohort was 2.3 years, we performed subgroup analysis using age less than 2 years and ≥2 years as the cutoff points to provide a balanced number of children in each age group. The interaction term between age and *MBL2* genotype was also included in the model. Although the results were as hypothesized, with the effects of genotype on outcome most evident within the younger age group, the interaction term between age groups and *MBL2* genotypes that were included in the model was not statistically significant. The log-rank test was used for comparisons of survival curves for different genotypes. All *P* values are 2-sided.

RESULTS

Characteristics of children

Of the 1037 children included in this analysis, 568 (55%) were girls and 469 (45%) were boys. The children evaluated were 60.3% non-Hispanic black, 26.1% Hispanic, and 13.6% non-Hispanic white. Ages ranged from 42 days to 18 years (<2 years, 46%; >2-5 years, 26%; 5-10 years, 19%; and >10 years, 9%). The median age was 2.3 years (10th and 90th percentiles, 0.45 and 9.5 years). Of the 1037 children, 300 (29%) were followed for less than 12 months, 450 (43%) for 12 to 23 months, 197 (19%) for 24 to 35 months, and 90 (9%) for more than 35 months. A total of 226 (22%), of whom 151 (67%) were less than 2 years old, were identified as having HIV-1-related disease progression during the course of the 2 studies. Characteristics of the children by 2 age groups have been summarized in Table I.

Distribution of *MBL2* genotypes and haplotypes

MBL2-O allele frequency was 0.27 in non-Hispanic blacks, 0.25 in Hispanics, and 0.21 in non-Hispanic whites. The prevalence of the homozygous wild type *(A/A)* was similar across all races/ethnicities (see Table E1 in the Online Repository at www.jacionline.org). The heterozygous *A/B* genotype was less common and the heterozygous *A/C* genotype was more frequent in the non-Hispanic blacks when compared with either Hispanics or non-Hispanic whites ($P < .001$). The non-Hispanic black children had the highest *C* allele frequency (non-Hispanic blacks, 0.20; Hispanics, 0.07; and non-Hispanic whites, 0.05). Of note, the homozygous mutant *C/C* genotype was found exclusively in the non-Hispanic blacks. The incidence of *L/L* and *Q/Q* genotypes was more frequent in the non-Hispanic blacks. The *P/P* genotype was less common in the non-Hispanic blacks compared with Hispanics or non-Hispanic whites ($P < .001$). The homozygous *Y/Y* genotype was the most common, whereas *X/X* and *X/Y* genotypes were less frequent in all races/ethnicities. Haplotype *LYPA* was more common in non-Hispanic blacks compared with Hispanics or non-Hispanic whites, whereas haplotype *HYPA* was less common in non-Hispanic blacks. The distribution of *MBL2* variants did not differ significantly by age groups of less than 2 or more than 2 years (see Table E2 in the Online Repository at www.jacionline.org).

Associations between disease status at study entry and *MBL2* polymorphisms

At study entry, none of the *MBL2* genotypes or haplotypes was significantly associated with $CD4^+$ lymphocyte count, $CD4^+$ lymphocyte percentage, plasma HIV-1 RNA, weight for age *z* score (WAZ), and sex and cognitive score (data not shown). Additionally, there were differences in baseline characteristics in children younger than 2 years compared with those

in children ≥2 years (Table I). Of note, the children younger than 2 years had statistically significant lower baseline WAZ scores.

MBL2 genotypes and HIV-1-related disease progression and CNS impairment in all children

In the whole cohort the children with the *MBL2* variant genotypes showed trends toward more rapid disease progression; the results, however, were not statistically significant (Table II). These associations followed the same trends in multivariate analyses, controlling for baseline $CD4^+$ lymphocytes, $\log_{10}$ HIV-1 RNA, treatments, race, sex, or other genotypes (*CCR5-wt/Δ32*, *CCR5-59029*, *CX3CR1-249-V/I*, *-280-T/M*, or *stromal cell-derived factor-1*). However, children with the homozygous variant *MBL2-O/O* genotype experienced more rapid CNS impairment than those with the wild-type *A/A* genotype (relative hazard $[RH]_{O/O \text{ vs } A/A}$, 1.46; 95% CI, 0.98-2.16; $P = .054$) and those with the heterozygous *A/O* genotype ($RH_{O/O \text{ vs } A/O}$, 2.24; 95% CI, 1.00-5.01; $P = .045$; see Table III). In addition, children with the *O/O* genotype showed more rapid CNS impairment than those with either the *A/A* or *A/O* genotypes ($RH_{O/O \text{ vs } (A/O+A/A)}$, 2.15; 95% CI, 1.00-4.64; $P = .045$). To address the issue of potential bias by race/ethnicity, we controlled for race/ethnicity in a multivariate model to assess the association between disease progression/CNS end points and the *MBL2-O/O* genotype. The baseline WAZ score was also controlled for the multivariate analyses. The results showed that the significant association of the *MBL2-O/O* group and CNS disease progression remained. No other *MBL2* variants in the promoter or untranslated regions affected HIV-1 disease in children.

MBL2 haplotypes and HIV-1-related disease progression and CNS impairment in all children

MBL2 haplotypes were defined as described earlier (eg, the *MBL2*HYPA* haplotype has *-550C>G*, *-221C>G*, *4T>C*, *223T>C*, *230A>G*, and *239A>G* variants).18 For combined *A/O* and *H/L* genotypes, children with combined heterozygous and homozygous variants (*AHOL +OLOL*) showed trends toward more rapid disease progression compared with those with the wild-type *AHAH* haplotype ($P = .084$). Furthermore, the combined *AHOL+OLOL* haplotype was associated with more rapid CNS disease progression than was seen in those with the wild-type *AHAH* haplotype ($P = .023$). Children with the *LYPA* haplotype exhibited a higher RH for CNS impairment compared with the combined group with *LYPA* or *LXPO* haplotypes (RH, 3.39; $P = .053$). There were no other significant associations of haplotypes with HIV-1 disease in children.

HIV-1 disease progression and CNS impairment for children younger than 2 versus ≥ 2 years with *MBL2* variants

Children younger than 2 years have immature adaptive immune responses and are known to rely heavily on innate immunity. We examined the association of *MBL2* variants on HIV-1-related disease for children younger than 2 and ≥2 years (Tables IV and V and Figs 1 and 2).

For the analyses of time to disease progression comparing *MBL2* genotypes, children younger than 2 years experienced more rapid disease progression (*O/O* vs *A/O*: RH, 2.28; 95% CI, 1.09-4.79; $P = .029$; *O/O* vs *A/A*: RH, 1.54; 95% CI, 1.07-2.22; $P = .02$; *O/O* vs *A/O+A/A*: RH, 2.33; 95% CI, 1.14-4.76; $P = .02$; Table IV). However, no statistically significant associations were observed in the analyses for children ≥2 years (*O/O* vs *A/O*: RH, 0.83; 95% CI, 0.25-2.70; $P = .75$; *O/O* vs *A/A*: RH, 1.05; 95% CI, 0.58-1.90; $P = .87$; *O/O* vs *A/O+A/A*: RH, 0.97; 95% CI, 0.30-3.07; $P = .95$; Table IV).

The analyses of time to CNS impairment comparing *MBL2* genotypes yielded similar results. Children younger than 2 years with *O/O* genotypes versus *A/O* genotypes

demonstrated more rapid development of cognitive impairment ($RH_{OO\ vs\ AO}$, 2.78; 95% CI, 1.07-7.21; $P = .035$), whereas this association was again not significant for children ≥2 years ($RH_{OO\ vs\ OA}$, 1.93; 95% CI, 0.41-9.01; $P = .40$). Similarly, for a comparison between *O/O* and *A/A* genotypes, children younger than 2 years experienced more rapid CNS impairment ($RH_{OO\ vs\ AA}$, 1.69; 95% CI, 1.06-2.69; $P = .027$), whereas children ≥2 years did not ($RH_{OO\ vs\ AA}$, 1.33; 95% CI, 0.63-2.81; $P = .45$). A comparison between *O/O* and *A/O+A/A* genotypes for the children younger than 2 years also showed a difference in CNS impairment (*O/O* vs *A/O+A/A*: RH, 2.82; 95% CI, 1.14-7.00; $P = .03$). The comparison for the children ≥2 years did not show significant effects (see Table V). The associations of *MBL2* genotypes and disease progression or CNS impairment when comparing *MBL2-O/O* versus *A/O*, *A/A*, or *A/O+A/A* remained significant in children younger than 2 years after controlling for the effects of *H/L*, *P/Q*, and *X/Y* alleles. Additionally, children younger than 2 years with *P/Q* or *Q/Q* genotypes underwent more rapid CNS impairment compared with those with the wild-type *P/P* genotype ($RH_{P/Q\ vs\ P/P}$, 1.73; 95% CI, 1.03-2.91; $P = .037$; $RH_{Q/Q\ vs\ P/P}$, 1.40; 95% CI, 1.01-1.93; $P = 0.042$).

DISCUSSION

To our knowledge, this is the first comprehensive study of the distribution of *MBL2* alleles and their association with HIV-1 disease in children. The homozygous variant *MBL2-O/O* and *L/L* promoter genotype, associated with production of nonfunctional MBL, were more common in non-Hispanic black children compared with Hispanic and non-Hispanic white children, suggesting this population might have even a higher risk of HIV-1 infection. The low frequency of the *X/X* promoter genotype, associated with low MBL expression, suggests that there might be a related altered susceptibility to infection in the US population, regardless of race/ethnicity. It is important to note, however, that we found no differences in the *MBL2* polymorphisms studied based on race/ethnicity on disease progression or development of CNS impairment. We found common *MBL2* haplotypes in the studied cohort; however, an earlier reported rare *HXPA* haplotype was also observed.19 At baseline, no effects of *MBL2* polymorphisms on HIV-1 disease were observed. The hypothesis that reduced MBL expression and function can compromise immune responses is also supported by the data demonstrating that decreased expression of MBL on lymphocytes is associated with disease progression in HIV-infected adults.20

The presence of the *MBL2-O/O* genotype, which results in lower expression of MBL protein and impaired innate immunity, was associated with more rapid development of HIV-1-related CNS impairment in children. Similar to earlier studies of the association of *MBL2* in HIV-1-infected adults,12,20,21 we observed an overall trend toward a more rapid disease progression in children with variant *MBL2* genotypes; however, these results were not statistically significant for the whole cohort. Most strikingly, we observed significant associations between *MBL2* variants and disease progression, including cognitive impairment in children younger than 2 years but little evidence of such an effect in older children. Thus older children did not behave as did adults in their susceptibility to more frequent infections. This finding in children older than 2 years likely reflects the high susceptibility that children have to infectious complications associated with advanced HIV-1 disease or AIDS when effective antiretroviral therapy is unavailable. Similar to our study in HIV-1-infected younger children, others have also reported age-related association of *MBL2* variants with acute respiratory tract infections and meningococcal disease.8,13 Our results demonstrate that children younger than 2 years who have *MBL2* genetic variants that result in lower MBL levels have a significantly greater risk for disease progression and CNS impairment. The findings in children younger than 2 years support a major role of MBL in the immune defense of young children when the adaptive immune system is still immature and passively acquired maternal antibodies are exhausted.22 The presence of an *MBL2-O/O*

variant allele in an HIV-1-infected child suggests a higher risk of disease progression and CNS impairment and could be useful as an indicator of antiretroviral therapy in a child not receiving treatment.

The MBL carbohydrate recognition domain has been shown to interact with the HIV-1 gp120/gp41 complex,23 and dendritic cell-specific intercellular adhesion molecule-grabbing noninte-grin-mediated transinfection of T cells is inhibited by MBL.24 The presence of the *MBL2-O/O* genotype, which results in lower expression of MBL protein and impaired innate immunity, might lead to impaired viral recognition and increased infection and inflammation associated with HIV-1-related CNS impairment in children. Additionally, modulatory effects of MBL on cytokine responses have been reported.25 Enhanced TNF-α responses were observed when PBMCs from healthy control subjects and HIV-infected patients were stimulated with MBL and costimulated with HIV-1 gp120 or mannan. Thus the presence of a variant allele causing lower expression and function of MBL protein might potentially impair both innate and adaptive immune responses during HIV-1 neuropathogenesis. Deficiency of MBL related to impaired innate and adaptive immune responses could be an important determinant of susceptibility to neurologic diseases. Lower levels of MBL have been reported previously to be associated with meningococcal disease26,27 and Alzheimer's disease.28

Although the role of *MBL2* genetic variants in susceptibility of several diseases is well established, a recent evolutionary analysis of *MBL2* alleles suggested that the patterns of *MBL2* variation were compatible with neutral evolution, as opposed to negative, positive, or balanced natural selection.29 Accordingly, the high worldwide frequencies of *MBL2* alleles associated with the production of little or no protein appear to have evolved from human migration and genetic drift and not survival pressure.

To our knowledge, this is the first study to examine the effect of genetic variants of *MBL2* on CNS impairment in HIV-1-infected children. When compared with adults, children have more rapid HIV-1 disease progression, higher absolute numbers of $CD4^+$ lymphocytes, higher viral loads, and more neurocognitive impairment. Thus the effect of *MBL2* polymorphisms on HIV-1 infection in children could be fundamentally different from that seen in adults. Although our cohort was a seroprevalent population, the timing of infection for this perinatally infected cohort is known, it does not suffer from widely disparate timing and routes of infection that might be present in adult cohorts, and antiretroviral therapy was well documented. Children in our cohort received 1 or 2 nucleoside reverse transcriptase inhibitors (NRTIs), which were standardized for each participant. Additionally, the high number of clinical end points suggests that the effects of mono-NRTI or dual-NRTI therapy were limited. We also extended the analysis of the associations of *MBL2* and disease progression to evaluate whether the associations varied between the study treatments by including interaction terms in the model. However, no significant variations were observed.

In summary, our findings indicate that the presence of *MBL2* variants is associated with more rapid HIV-1 disease progression and CNS impairment in children in an age-dependent manner, with the greatest effect observed in children younger than 2 years.

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Abbreviations used

CNS	Central nervous system
MBL	Mannose-binding lectin
MBL2	Mannose binding lectin-2
NRTI	Nucleoside reverse transcriptase inhibitor
PACTG	Pediatric AIDS Clinical Trial Group
PFS	Progression-free survival
RH	Relative hazard
WAIS-R	Wechsler Adult Intelligence Scale: Revised
WISC-R	Wechsler Intelligence Scale for Children: Revised

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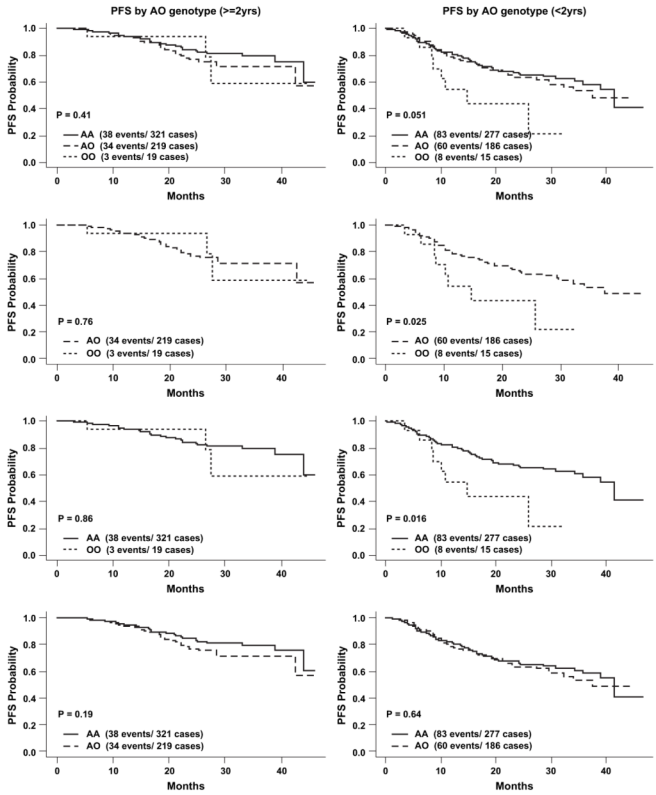

FIG 1.

Association of *MBL2* exon 1 genotypes on PFS by age (≥2 vs <2 years).

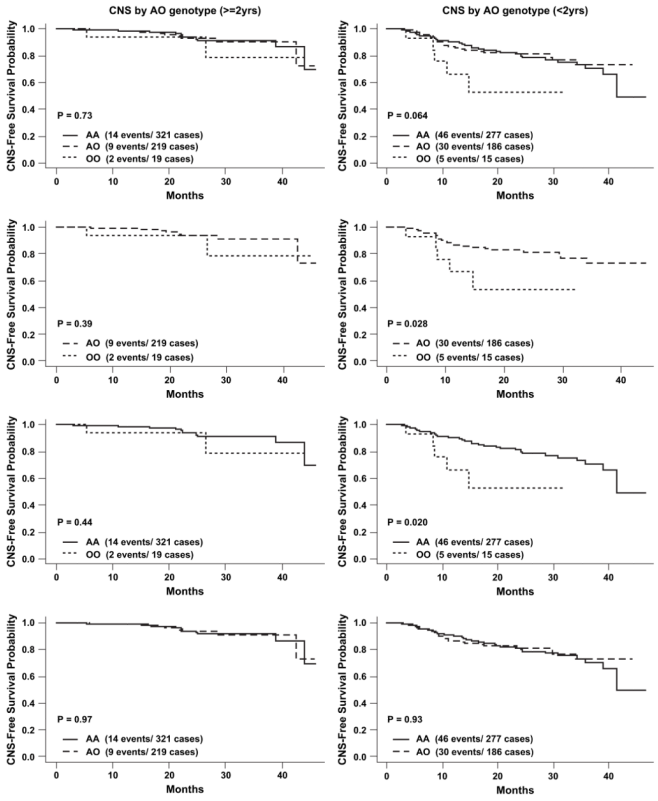

FIG 2.

Association of *MBL2* exon 1 genotypes on CNS PFS by age (≥2 vs <2 years).

TABLE I

Characteristics of children by age groups

	Total (n = 1037)	≥2 y (n = 559)	<2 y (n = 478)	*P* value
Sex				
Male	469 (45%)	249 (45%)	220 (46%)	.6614
Female	568 (55%)	310 (55%)	258 (54%)	
Race/ethnicity				
Black	625 (60%)	332 (59%)	293 (61%)	.4025
Hispanic	271 (26%)	155 (28%)	116 (24%)	
White	141 (14%)	72 (13%)	69 (14%)	
Follow-up time				
<12 mo	300 (29%)	149 (27%)	151 (32%)	.3084
≥12-23 mo	450 (43%)	254 (45%)	196 (41%)	
>23-35 mo	197 (19%)	109 (19%)	88 (18%)	
>35 mo	90 (9%)	47 (8%)	43 (9%)	
PFS events				
No	811 (78%)	484 (87%)	327 (68%)	<.0001
Yes	226 (22%)	75 (13%)	151 (32%)	
Disease progression end point				
No event	811 (78%)	484 (87%)	327 (68%)	
death	25 (2%)	1 (0%)	24 (5%)	
≥2 Opportunistic infections	7 (1%)	4 (1%)	3 (1%)	
Weight growth failure	75 (7%)	41 (7%)	34 (7%)	
Malignancy	1 (0%)	0 (0%)	1 (0%)	
CDC clinical disease category C	12 (1%)	4 (1%)	8 (2%)	
All CNS disease progression events:	106 (10%)	25 (4%)	81 (17%)	
a. Deterioration of brain growth	65 (6%)	21 (4%)	44 (9%)	
b. Deterioration of neurocognitive function	12 (1%)	1 (0%)	11 (2%)	
c. Deterioration of neurologic status	29 (3%)	3 (1%)	26 (5%)	
Baseline characteristics by age groups				
Baseline CD4 (%)				
N	1,029	557	472	<.001

	Total (n = 1037)	≥2 y (n = 559)	<2 y (n = 478)	*P* value
Median	24	22.45	26.98	
Baseline CD4 count/mm^3				
N	1,029	557	472	NA*
Median	774.98	596	1269.50	
Baseline $\log_{10}$ RNA copies/mL				
N	861	467	394	<.001
Median	5.12	4.73	5.64	
Baseline cognitive score				
N	979	525	454	.274
Median	83	83	83	
Baseline WAZ score				
N	1037	559	478	<.001
Median	-0.69	-0.29	-1.11	

*Not calculated because younger children generally have higher absolute CD4 counts than older children.

TABLE II

Association of *MBL2* genotypes on progression-free survival in children: Whole cohort

***MBL2* genotypes compared**	**RH (95% CI), *P* value**
O/O vs *A/O*[*]	1.45 (0.78-2.72), .24
Adjusted[†]	1.90 (0.94-3.85), .07
Adjusted[‡]	1.49 (0.78-2.84), .22
Adjusted[§]	1.46 (0.77-2.76), .25
O/O vs *A/A*[*]	1.29 (0.95-1.76), .10
Adjusted[†]	1.37 (0.97-1.94), .07
Adjusted[‡]	1.22 (0.89-1.67), .21
Adjusted[§]	1.34 (0.97-1.84), .078
O/O vs *A/O+A/A*[*]	1.57 (0.86-2.88), .14
Adjusted[†]	1.91 (0.97-3.76), .06
Adjusted[‡]	1.49 (0.80-2.75), .20
Adjusted[§]	1.62 (0.87-3.00), .13

[*] Unadjusted.

[†] Adjusted for $CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex.

[‡] Adjusted for *CCR5-wt/Δ32*, *-59029-G/A*, *CX3CR1-249-V/I*, *-280-T/M*, and *SDF-1-180-G/A*.

[§] Adjusted for *MBL2-H/L*, *MBL2-P/Q*, and *MBL2-X/Y*.

TABLE III

Association of *MBL2* genotypes on CNS progression-free survival in children: Whole cohort

***MBL2* genotypes compared**	**RH (95% CI), *P* value**
O/O vs *A/O*[*]	2.24 (1.00-5.01), .045
Adjusted[†]	2.77 (1.13-6.79), .03
Adjusted[‡]	2.51 (1.09-5.81), .03
Adjusted[§]	2.37 (1.02-5.50), .04
O/O vs *A/A*[*]	1.46 (0.98-2.16), .054
Adjusted[†]	1.63 (1.06-2.51), .03
Adjusted[‡]	1.38 (0.92-2.06), .12
Adjusted[§]	1.58 (1.04-2.41), .032
O/O vs *A/O+A/A*[*]	2.15 (1.00-4.64), .04
Adjusted[†]	2.74 (1.19-6.33), .02
Adjusted[‡]	2.04 (0.94-4.46), .07
Adjusted[§]	2.44 (1.10-5.39), .027

[*] Unadjusted.

[†] Adjusted for $CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex.

[‡] Adjusted for *CCR5-wt/Δ32*, *-59029-G/A*, *CX3CR1-249-V/I*, *-280-T/M*, and *SDF-1-180-G/A*.

[§] Adjusted for *MBL2-H/L*, *MBL2-P/Q*, and *MBL2-X/Y*.

TABLE IV

Association of *MBL2* genotypes on progression-free survival in children: Age (≥2 vs <2 y)

***MBL2* genotypes Compared**	**Age ≥2 y: RH (95% CI), *P* value**	**Age <2 y: RH (95% CI), *P* value**
O/O vs *A/O*[*]	0.83 (0.25-2.70), .75	2.28 (1.09, 4.79) p=0.029
Adjusted[†]	3.73 (0.71-19.6), .12	2.21 (0.94-5.15), .067
Adjusted[‡]	0.91 (0.26-3.16), .88	2.07 (0.95-4.49), .066
Adjusted[§]	0.88 (0.26-2.95), .84	2.27 (1.05-4.88), .036
O/O vs *A/A*[*]	1.05 (0.58-1.90), .87	1.54 (1.07-2.22), .02
Adjusted[†]	1.00 (0.46-2.17), .99	1.38 (0.92-2.08), .12
Adjusted[‡]	1.02 (0.56-1.88), .94	1.53 (1.04-2.24), .03
Adjusted[§]	1.11 (0.60-2.03), .74	1.53 (1.04-2.25), .029
O/O vs *A/O+A/A*[*]	0.97 (0.30-3.07), .95	2.33 (1.14-4.76), .02
Adjusted[†]	1.55 (0.35-6.83), .56	2.14 (0.98-4.69), .058
Adjusted[‡]	0.87 (0.27-2.87), .82	2.19 (1.05-4.55), .036
Adjusted[§]	1.04 (0.32-3.35), .95	2.33 (1.12-4.83), .024

[*] Unadjusted.

[†] Adjusted for $CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex.

[‡] Adjusted for *CCR5-wt/Δ32*, *-59029-G/A*, *CX3CR1-249-V/I*, *-280-T/M*, and *SDF-1-180-G/A*.

[§] Adjusted for *MBL2-H/L*, *MBL2-P/Q*, and *MBL2-X/Y*.

TABLE V

Association of *MBL2* genotypes on CNS progression-free survival in children: Age (≥2 vs <2 y)

***MBL2* genotypes compared**	**Age ≥2 y: RH (95% CI), *P* value**	**Age <2 y: RH (95% CI), *P* value**
O/O vs *A/O*[*]	1.93 (0.41-9.01), .40	2.78 (1.07-7.21), .035
Adjusted[†]	4.72 (0.43-52.1), .20	3.56 (1.24-10.3), .019
Adjusted[‡]	3.71 (0.67-20.4),.13	3.08 (1.11-8.54), .031
Adjusted[§]	2.25 (0.44-11.6), .33	3.11 (1.14-8.47), .027
O/O vs *A/A*[*]	1.33 (0.63-2.81), .45	1.69 (1.06-2.69), .027
Adjusted[†]	1.00 (0.35-2.86), 1.00	1.75 (1.06-2.90), .028
Adjusted[‡]	1.46 (0.68-3.13), .33	1.65 (1.01-2.69), .045
Adjusted[§]	1.85 (0.82-4.15), .14	1.67 (1.02-2.73), .042
O/O vs *A/O+A/A*[*]	1.78 (0.42-7.62), .44	2.82 (1.14-7.00), .03
Adjusted[†]	1.30 (0.17-10.28), .80	3.38 (1.31-8.74), .012
Adjusted[‡]	2.25 (0.50-10.1), .29	2.81 (1.10-7.16), .031
Adjusted[§]	2.64 (0.58-12.0), .21	3.05 (1.19-7.77), .020

[*] Unadjusted.

[†] Adjusted for $CD4^+$ lymphocyte count, HIV-1 RNA, treatments, race, and sex.

[‡] Adjusted for *CCR5-wt/Δ32*, *-59029-G/A*, *CX3CR1-249-V/I*, *-280-T/M*, and *SDF-1-180-G/A*.

[§] Adjusted for *MBL2-H/L*, *MBL2-P/Q*, and *MBL2-X/Y*.

TABLE E1

Distribution of *MBL2* genotypes and haplotypes by race/ethnicity

		Race/ethnicity			
	Total (n = 1037)	**Black (n = 625)**	**Hispanic (n = 271)**	**White (n = 141)**	***P* value**
Exon 1 genotype					
A/A	570 (55%)	335 (54%)	148 (55%)	87 (62%)	<.0001
A/B	137 (13%)	45 (7%)	62 (23%)	30 (21%)	
A/C	223 (22%)	182 (29%)	33 (12%)	8 (6%)	
A/D	45 (4%)	18 (3%)	16 (6%)	11 (8%)	
O/O	62 (6%)	45 (7%)	12 (4%)	5 (4%)	
Promoter region genotype					
Y/Y	762 (73%)	472 (76%)	192 (71%)	98 (70%)	.4474
X/Y	248 (24%)	139 (22%)	71 (26%)	38 (27%)	
X/X	27 (3%)	14 (2%)	8 (3%)	5 (4%)	
Promoter region genotype					
H/H	60 (6%)	20 (3%)	24 (9%)	16 (11%)	<.0001
H/L	359 (35%)	171 (27%)	124 (46%)	64 (45%)	
L/L	618 (60%)	434 (69%)	123 (45%)	61 (43%)	
Untranslated region genotype					
P/P	362 (35%)	159 (25%)	139 (51%)	64 (45%)	<.0001
P/Q	492 (47%)	317 (51%)	110 (41%)	65 (46%)	
Q/Q	183 (18%)	149 (24%)	22 (8%)	12 (9%)	
Haplotypes					
HYPA	74 (7%)	25 (4%)	30 (11%)	19 (13%)	<.0001
HXPA	328 (32%)	156 (25%)	112 (41%)	60 (43%)	
LYPA	199 (19%)	127 (20%)	48 (18%)	24 (17%)	
LXPA	221 (21%)	153 (24%)	47 (17%)	21 (15%)	
Others	215 (21%)	164 (26%)	34 (13%)	17 (12%)	

TABLE E2

Distribution of *MBL2* genotypes and haplotypes by age

		Age groups		
	Total (n = 1037)	**≥2 y (n = 559)**	**<2 y (n = 478)**	***P* value**
Exon 1 genotype				
O/O	34 (3%)	19 (3%)	15 (3%)	.9650
A/O	405 (39%)	219 (39%)	186 (39%)	
A/A	598 (58%)	321 (57%)	277 (58%)	
Promoter region genotype				
Y/Y	762 (73%)	416 (74%)	346 (72%)	.0394
X/Y	248 (24%)	135 (24%)	113 (24%)	
X/X	27 (3%)	8 (1%)	19 (4%)	
Promoter region genotype				
H/H	60 (6%)	35 (6%)	25 (5%)	.6483
H/L	359 (35%)	188 (34%)	171 (36%)	
L/L	618 (60%)	336 (60%)	282 (59%)	
Untranslated region genotype				
P/P	362 (35%)	184 (33%)	178 (37%)	0.2501
P/Q	492 (47%)	278 (50%)	214 (45%)	
Q/Q	183 (18%)	97 (17%)	86 (18%)	

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