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From genotype to structure: MBL2 exon 1 and promoter variants associated with cytomegalovirus seropositivity in acute lymphoblastic leukemia from the Brazilian Amazon

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Abstract

Background

Variants in the *MBL2* gene influence serum levels and structure of mannose-binding lectin (MBL) and may increase infection risk in immunocompromised individuals. We evaluated *MBL2* exon 1 and promoter variants in relation to cytomegalovirus (CMV) and Epstein–Barr virus (EBV) seropositivity in patients with acute lymphoblastic leukemia (ALL) from Amazonas, Brazil, and modelled the structural impact of the exon 1 variants.

Methods and results

107 patients with ALL and 76 controls were genotyped; CMV and EBV seropositivity was determined by ELISA, and exon 1 variants were analysed *in silico* with AlphaFold and DUET. CMV IgG associations were estimated by group using Firth logistic regression adjusted for age and sex. The exon 1 O allele was associated with higher CMV seropositivity in ALL (aOR 5.12, 95% CI 1.24–20.53; p = 0.025), whereas in controls the − 221 X allele (aOR 0.09, 95% CI 0.014–0.462; p = 0.003) and XY genotype (aOR 0.09; p = 0.003) were associated with lower seropositivity. No variant was associated with EBV, and expression-based haplotypes showed no association with either virus. Modelling predicted that the exon 1 substitutions destabilise the MBL monomer, consistent with impaired oligomerisation of the collagen-like region.

Conclusions

Against a background of high CMV seroprevalence, *MBL2* variants showed context-dependent associations with CMV seropositivity. Structural modelling provides a plausible rationale for these exploratory associations, which require confirmation in larger cohorts.

1. INTRODUCTION

Mannose-binding lectin (MBL) is a key protein that activates the lectin pathway of the complement system, an essential component of innate immunity (Sarma and Ward, 2010). As a member of the collectin family, MBL contains carbohydrate recognition domains (CRDs) in its C-terminal region, enabling it to bind to pathogens (Worthley et al., 2005). Upon recognition, MBL can directly mediate pathogen uptake or activate serine protease complexes (MASP-1 and MASP-2), triggering a cascade of enzymatic reactions that ultimately lead to pathogen elimination (Turner, 1998; Garred et al., 2016).

MBL is encoded by the *MBL2* gene, located on the long arm of human chromosome 10 (10q11.1-q21) (Sastry et al., 1989; Ip et al., 2009; Kalia et al., 2016). This gene contains several single-nucleotide polymorphisms (SNPs), including those at codons 52, 54, and 57 of exon 1 (designated as alleles D, B,

and C, respectively) (Lipscombe et al., 1992; Madsen et al., 1994; Sumiya et al., 1991; Adamek et al., 2013), as well as SNPs in the promoter region at positions − 550 (H/L alleles) and − 221 (X/Y alleles) (Madsen et al., 1995).

Structural variants in exon 1 result in amino acid substitutions in MBL: at codon 52 (CGT→TGT), arginine is replaced by cysteine (Madsen et al., 1994); at codon 54 (GGC→GAC), glycine is replaced by aspartic acid (Sumiya et al., 1991); and at codon 57 (GGA→GAA), glycine is replaced by glutamic acid (Lipscombe et al., 1992). These structural variants are collectively designated as the "O" allele, while the wild-type allele is designated as "A" (Gadjeva et al., 2004).

MBL2 promoter variants can influence circulating MBL concentrations, while exon 1 substitutions in the collagen-like region can impair the assembly of functional MBL oligomers (Ip et al., 2009; Skattum et al., 2011; Auriti et al., 2017). Associations between these variants and infectious complications have been reported in hematological malignancies, although the direction and magnitude of these associations vary across populations and clinical settings (Mullighan et al., 2002; Pana et al., 2014; Moreto et al., 2014).

ALL is a malignant neoplasm characterized by the abnormal proliferation of lymphoid lineage precursor cells (Iacobucci and Mullighan, 2017). The uncontrolled proliferation of immature cells causes an accumulation of lymphoblasts in the peripheral blood and bone marrow, leading to the replacement of healthy cellular elements (Zubair et al., 2018). Patients with ALL are at increased risk for infections, particularly opportunistic infections, due to immunosuppression resulting from the neoplasm itself or from chemotherapy regimens (Busca, 2012; Buus-Gehrig et al., 2020; Handous et al., 2020). The increased risk of severe infections in leukemia patients undergoing allogeneic hematopoietic stem cell transplantation has previously been associated with *MBL2* gene variants(Mullighan et al., 2002; Worthley et al., 2005; Puente et al., 2019).

Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) infections are aggravating factors for patients with ALL and are associated with clinical complications that can reduce survival in patients with acute leukemia (Loutfy et al., 2017). These viruses produce host-like immunomodulators that interfere with immune responses against viral infections and malignant cells, establish latency in host cells, and reactivate when the host is immunocompromised (Moschovi et al., 2016; Silva et al., 2020). In addition, viral reactivation has been associated with adverse clinical outcomes in patients with hematologic diseases undergoing hematopoietic stem cell transplantation (Ding et al., 2021; Li et al., 2022), underscoring the clinical importance of CMV and EBV infections in immunocompromised patients.

These observations provide a rationale for examining *MBL2* variation in ALL, recognizing that associations with viral IgG indicate prior exposure rather than susceptibility to active infection. We therefore investigated *MBL2* exon 1 and promoter variants in relation to CMV and EBV seropositivity in patients with ALL and controls from Amazonas, Brazil. Additionally, because the exon 1 variants are located in the collagen-like region that governs MBL oligomerisation, we used *in silico* structural

modelling to assess whether these substitutions could plausibly affect the monomer structure, offering a mechanistic perspective for interpreting any observed genetic associations.

2. MATERIALS AND METHODS

2.1 Population

This retrospective observational study analyzed stored peripheral blood samples from Manaus, Amazonas, Brazil. The ALL group comprised 107 patients with a confirmed diagnosis of ALL (39 female and 68 male participants, aged 2–63 years) treated at the Amazonas State Hematology and Hemotherapy Hospital Foundation (FHEMOAM). The control group comprised 76 individuals without a reported history of hematological malignancy (51 female and 25 male participants, aged 7–73 years) residing in the same metropolitan region. Median age was 10 years (IQR 6–18.5) in the ALL group and 19 years (IQR 14–33) in controls. Patients and controls were analysed separately, consistent with their distinct immune contexts and group-specific hypotheses. Although MBL2 genotype is constitutional and independent of age and sex, the groups differed in age and sex composition, and CMV seropositivity is known to vary with these factors. Therefore, each association was additionally adjusted for age and sex as a robustness check, using Firth penalised logistic regression to accommodate the sparse seronegative cells. While Hardy-Weinberg equilibrium supports the internal consistency of genotyping within each group, it does not establish comparability between groups or rule out the possibility of population stratification.. For each participant, a 5-mL peripheral blood sample was collected into EDTA. Samples were processed and stored at − 80°C in the Virology and Immunology Laboratory of the National Institute for Amazonian Research (INPA) until analysis.

This study was approved by the Research Ethics Committee of the Amazonas Hematology and Hemotherapy Foundation (FHEMOAM) (CAAE 25710819.0.0000.0009; approval no. 5.081.493). All procedures were conducted in accordance with the Declaration of Helsinki and Brazilian National Health Council Resolution 466/2012. Written informed consent was obtained from all participants; for participants under 18 years of age, written informed consent was provided by a parent or legal guardian, together with the participant's assent where applicable.

2.2 Genotyping variants in exon 1

Exon 1 *MBL*A, MBL*B*, and *MBL*C* alleles were detected by polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. PCR was performed in a final volume of 50 µL containing 25 µL of master mix (0.05 U/µL Taq DNA polymerase, reaction buffer, 3 mM $MgCl_2$, 0.4 mM each dNTPs, dye, 2 mM gel loading buffer, and stabilizers), 3 µL of extracted DNA (300 ng/µL), 18 µL of ultrapure H_2O and 2 µL of primers (Table 1).

PCR was performed according to Pontes et al. (2005), generating a 349 bp amplicon. Alleles were identified by RFLP, BshNI was used to identify *MBL*A* by cleaving the amplicon into 260 bp and 89 bp

fragments, whereas *MBL*B* was identified by the absence of enzymatic digestion (349 bp). MboII was used to identify *MBL*C* by generating 279 bp and 70 bp fragments.

The *MBL*D* allele was identified by nested PCR performed in a final volume of 25 µL containing 3 µL of DNA (100 ng/ µL), 12.5 µL of master mix (0.05 U/µL Taq DNA polymerase, reaction buffer, 3 mM $MgCl_2$, 0.4 mM each dNTP, dye, 2 mM gel loading buffer, and stabilizers), 5.5 µL of ultrapure H_2O, and 2 µL of the primer pairs for round 1 (sspMBP1a and 52-D) and round 2 (sspMPB1a and 52-ABC).Because presence-based semi-nested PCR is insufficient to determine MBL*D homozygosity, exon 1 was analysed using the biallelic A/O system (A/A, A/O, and O/O), with B, C, and D variants reported at the allele level (Steffensen et al., 2000). Each participant's A/O genotype was derived deterministically from the observed exon 1 calls (A/A to A/A; A/B and A/D to A/O; B/B and B/D to O/O).No assay pattern yielded an ambiguous assignment, so A/O classification was non-differential between groups. Primer sequences are provided in Table 1

Thermal cycling consisted of an initial cycle at 94°C for 2 min, followed by 30 cycles of 94°C for 20 s, 62°C for 20 s, and 72°C for 30 s, with a final extension step at 72°C for 5 min. The amplified products were analyzed by electrophoresis (100 V, 45 min) on a 2% agarose gel using 1× TAE buffer stained with Safe Dye Nucleic Acid Stain (Cellco).

2.3 Genotyping of promoter region variants

Polymorphisms in the − 221 (X/Y) and − 550 (H/L) promoter regions were genotyped by real-time PCR (qPCR) using the TaqMan system (Applied Biosystems® TaqMan Genotyping Assays). The qPCR was performed in a final volume of 25 µL containing 12.5 µL of master mix (PCR polymerase, dNTPs, reaction buffer with KCl, $(NH_4)_2SO_4$, $MgCl_2$, and stabilizers), 0.5 µL of each probe (2 µM), 0.5 µL of each region-specific primer (10 µM), 1 µL of DNA (100 ng/µL), and 9.5 µL of nuclease-free water. Thermal cycling consisted of an initial cycle at 50°C for 2 min, followed by 1 cycle at 95°C for 10 min and 40 cycles of 95°C for 15 s and 60°C for 15 s. Region-specific primers and probes were used as previously described (Vasconcelos et al., 2011) (Table 1).

Table 1
Primers and probes used to detect polymorphisms in the *MBL2* gene.

Regions	Primers	Nucleotide sequences
	mblE01	**5'-AGTCGACCCAGATTGTAGGACAGAG-3'**
Exon 1 (codons 54 and 57; alleles A, B, and C)	mblE02	5'AGGATCCAGGCAGTTTCCTCTGGAAGG-3'
	sspMBP1a	5'-CTGCACCCAGAT TGTAGGACAGAG-3'
Exon 1 (codon 52; allele D)	52-D	5'-TCTCCCTTGGTGCCATCACA-3'
	52-ABC	5'- TCTCCCTTGGTGCCATCACG-3'
Promoter (– 550)	*Primer forward*	5´-CCAACGTAGTAAGAAATTTCCAGAGA-3´
	Primer reverse	5´-CAACCCAGCCCAGAATTAACTG-3´
	Probes VIC	5'-VIC-CCTGTCTAAAACACC-MGB-3
	Probes FAM	5'-FAM-AGCCTGTGTAAAAC-MGB-3
Promoter (– 221)	*Primer forward*	5´- GCACGGTCCCATTTGTTCTCA-3´
	Primer reverse	5´-GCGTTGCTGCTGGAAGACTATAAA-3´
	Probes VIC	5'-VIC-CATGCTTTCGGTGGCAG-MGB-3'
	Probes FAM	5'-FAM-CATGCTTTCCGTGGCAG-MGB-3'

2.4 Haplotypes

The *MBL2* gene genotypes were grouped for haplotype classification into functional groups of serum MBL expression. The haplotypes HYO/HYO, LYO/LYO, HYO/LYO, HYO/LXA, LXA/LYO, LYO/LXO, LXA/LXO, LXO/LXO, and HYO/LXO were considered to have low MBL concentration levels; the haplotypes HYA/HYO, HYA/LXA, HYA/LYO, LYA/LXA, and LYA/LYO were considered to have intermediate levels; and the haplotypes LYA/LYA, HYA/HYA, and HYA/LYA were considered to have high MBL concentration levels. The classification was performed as previously described in the literature (Figueiredo et al., 2016; Garred et al., 2003; Vasconcelos et al., 2011)Diplotypes were assigned deterministically from the unphased promoter and exon 1 genotypes; where more than one phase configuration was compatible with a multilocus genotype, the assignment was treated as putative and flagged as such in Supplementary Table S1.

2.5 Determination of CMV and EBV Seropositivity

Anti-CMV and anti-EBV IgM and IgG antibodies were assessed using commercial enzyme-linked immunosorbent assays (EUROIMMUN Medizinische Labordiagnostika AG), according to the

manufacturer's instructions. Microplates were coated with CMV antigens derived from AD169-infected MRC-5 cell lysates or EBV viral capsid antigens. Optical density was read at 450 nm. The reported interpretation thresholds were a ratio < 0.8 for negative results, 0.8 to < 1.1 for borderline results, and ≥ 1.1 for positive results. Analyses used the recorded serological classifications. IgG seropositivity was interpreted as serological evidence of previous exposure, not as evidence of active viral replication or clinical disease. IgM results were described separately and were not used to diagnose reactivation or reinfection.

2.6 *In silico* structural analysis of exon 1 variants

The exon 1 substitutions at codons 52, 54 and 57 were examined in an exploratory in silico analysis of MBL monomer stability. The partial experimental structure of human MBL (PDB 1HUP) contains the neck and carbohydrate-recognition domain (CRD) but lacks the N-terminal collagen-like region containing the three variant sites. A wild-type (WT) monomer was modeled with AlphaFold (Abramson et al., 2024). WT and variant sequences were aligned in MEGA 11 (Tamura et al., 2021) to check substitution positions. Among the five ranked models, the top-ranked model was selected with reference to per-residue confidence (pLDDT), predicted aligned error (PAE), and multiple-sequence-alignment coverage. The p.Gly57Glu substitution was included as a literature-defined variant; the corresponding C allele was not detected in this sample.

Local confidence analysis (pLDDT) of the selected model indicated high confidence in the C-terminal region, encompassing the neck and CRD (amino acids 110–248), with values ≥ 90. In contrast, pLDDT values for the N-terminal region (amino acids 1–100), which includes residues 52, 54, and 57, were lower (< 80), indicating relatively lower confidence in the structural prediction for this portion of the model.

The predicted model was superimposed on the experimental structure (PDB 1HUP) in PyMOL over the common neck + CRD region (residues 107–248), and structural agreement was quantified by root-mean-square deviation (RMSD). The effect of each substitution on monomer stability was predicted using DUET (Pires et al., 2014), which integrates two complementary methods (mCSM and SDM) and reports the predicted change in folding free energy (ΔΔG) and three-dimensional structural models of the variant monomers. Values less than zero indicate a destabilizing effect, whereas values greater than zero indicate a stabilizing effect.

2.7 Data analysis

Two-sided Fisher's exact tests were used for comparisons of allele and genotype frequencies and exploratory serological associations. CMV IgG associations were assessed separately in the ALL and control groups using Firth penalized logistic regression adjusted for age as a continuous variable and sex. Each allele-carrier model compared participants with at least one copy of the allele with noncarriers. Separate pairwise genotype models compared X/Y with Y/Y, H/L with H/H, and L/L with H/H, excluding other genotypes from each comparison. Odds ratios (ORs), 95% profile penalized-likelihood confidence intervals, and penalized likelihood-ratio p-values were reported. IgM-positive observations were summarized descriptively because of their small number.

The original analyses were performed using GraphPad Prism 8 and R. Hardy–Weinberg equilibrium was assessed within each group; Table 2 reports Pearson chi-square p-values for the promoter loci and the collapsed A/O grouping. Exact biallelic tests were additionally used to inspect individual exon 1 variant calls. As a post hoc sensitivity assessment, Benjamini–Hochberg adjustment was applied to the 20 CMV comparisons, using unrounded p-values.

Structural modeling of the wild-type (WT) MBL protein was performed using the AlphaFold tool (Abramson et al. 2024). Monomer stability analysis was conducted using the DUET server (Pires et al. 2014), and predicted structures were visualized with the Protein Imager (Tomasello et al. 2021)

3. RESULTS

3.1 Allele and genotype frequencies

No significant differences were observed in the allelic and genotypic frequency distributions of *MBL2* gene variants between patients and healthy controls ($p > 0.05$). All three loci were in Hardy-Weinberg (HW) equilibrium in both ALL patients and controls (all $p > 0.05$, exact test): promoter − 221 (X/Y), $p = 0.97$ in patients and $p = 0.75$ in controls; promoter − 550 (H/L), $p = 0.10$ and $p = 0.97$; and exon 1 (A/O), $p = 0.78$ and $p = 0.14$. These results support the internal consistency of genotyping (Table 2).

In the − 221 region of the promoter, the Y allele was the most frequent in both groups, with frequencies of 0.90 in patients with ALL and 0.87 in controls (Table 2). In the − 550 region of the promoter, the L allele had the highest frequency in both patients with ALL (0.59) and the control group (0.62) (Table 2). The homozygous Y/Y genotype (− 221 region) was the most frequently detected in both groups, with frequencies of 0.81 in patients and 0.75 in controls. In the − 550 region, the heterozygous H/L genotype showed the highest frequency, with 0.56 in patients and 0.47 in controls, followed by the L/L genotype, with frequencies of 0.30 and 0.38, respectively (Table 2).

In exon 1, the frequency of the variant allele O was 0.62 in patients with ALL and controls. In contrast, the wild-type allele A (0.38) was detected at a lower frequency in both groups (Table 2). The heterozygous A/O genotype was the most frequent, with frequencies of 0.49 in patients with ALL and 0.55 in controls, while the wild-type A/A genotype was the least frequent in ALL (0.15) and in controls (0.11). The O/O genotype was 0.38 in the patient group and 0.34 in controls (Table 2).

Table 2
Allele and genotype frequencies in the *MBL2* gene.

Variants	**Patients ALL N (F*)**	**HW**	**Control group N (F*)**	**p****	**HW**
Promoter (-221)					
Alleles		0.97			0.75
X	21 (0.10)		20 (0.15)	0.41	
Y	193 (0.90)		132 (0.87)	0.41	
Genotypes					
X/X	1 (0.01)		1 (0.01)	1.00	
Y/Y	87 (0.81)		57 (0.75)	0.37	
X/Y	19 (0.17)		18 (0.24)	0.4	
Promoter (-550)					
Alleles		0.10			0.97
H	88 (0.41)		58 (0.38)	0.59	
L	126 (0.59)		94 (0.62)	0.59	
Genotypes					
H/H	14 (0.13)		11 (0.14)	0.82	
L/L	33 (0.30)		29 (0.38)	0.27	
H/L	60 (0.56)		36 (0.47)	0.18	
Exon 1 (Alleles A and O)		0.78			0.14
A	81 (0.38)		58 (0.38)	1.00	
O	133 (0.62)		94 (0.62)	1.00	
Genotypes					
A/A	16 (0.15)		8 (0.11)	0.65	
A/O	49 (0.46)		42 (0.55)	0.38	
O/O	42 (0.39)		26 (0.34)	0.64	

**F = frequency ** Fisher's exact test (p < 0.05 was considered statistically significant)*

The stratified frequency of the variant alleles *MBL*B*, *MBL*C*, and *MBL*D* in exon 1 revealed similar frequencies for the *MBL*B* and *MBL*D* alleles in patients with ALL and controls, with frequencies of 0.31 and 0.30 for *MBL*B* and 0.31 and 0.33 for *MBL*D*, respectively. The *MBL*C* allele was not detected in either group (Table 3).

The heterozygous B/D genotype was the most frequent in both groups, with frequencies of 0.32 in patients and 0.33 in controls, followed by the A/D genotype, which frequencies of 0.30 and 0.33, respectively. The heterozygous A/B genotype showed frequencies of 0.16 in patients and 0.21 in controls, whereas the homozygous B/B genotype showed the lowest frequencies, with 0.07 in patients and 0.03 in controls (Table 3).

Table 3
Allele and genotype frequencies of alleles A, B, C, and D in exon 1 of the *MBL2* gene.

Alleles (exon 1)	**Patients ALL**	**Control group**	**p****
	N (F*)	**N (F*)**	
MBL A*	81 (0.38)	58 (0.38)	1.00
MBL B*	67 (0.31)	44 (0.30)	0.73
MBL C*	-	-	-
MBL D*	66 (0.31)	50 (0.33)	0.73
Total	214	152	
Genotypes (exon 1)			
homozygotes			
A/A	16 (0.15)	8 (0.11)	0.65
B/B	8 (0.07)	2 (0.03)	0.13
C/C	-	-	-
D/D	NR†	NR†	-
Heterozygotes	N (F*)	N (F*)	
A/B	17 (0.16)	16 (0.21)	0.44
A/C	-	-	-
A/D	32 (0.30)	25 (0.33)	1.00
B/D	34 (0.32)	25 (0.33)	0.63
B/C	-	-	-
C/D	-	-	-
Total	107	76	

*F = frequency. **p-values are from two-sided Fisher's exact tests. – = not detected: the MBL*C allele was not observed in either group. † NR = not resolvable. The MBL*D allele was typed by semi-nested PCR, which detects the D allele by presence only and cannot distinguish D/D homozygotes (*Steffensen et al., 2000*); D dosage and the D/D genotype were therefore not determined. For this reason, exon 1 was analysed at the biallelic A/O level (*Table 2*), under which Hardy–Weinberg equilibrium was satisfied.*

3.2 Distribution of recorded multilocus profiles

By grouping the variants in exon 1 and the promoter region, 17 diplotypes were identified in this study: HYA/HYA, HYA/LYA, LYA/LYA, HYA/HYO, HYA/LXA, HYA/LYO, LYA/LXA, LYA/LYO, HYO/LXA, HYO/HYO, HYO/LYO, LXA/LYO, LYO/LXO, LYO/LYO, HYO/LXO, LXA/LXO, and LXO/LXO (Fig. 1; Supplementary Table S1). The most frequent haplotypes in patients with ALL and in the control group were those described in the literature as being associated with intermediate and low serum MBL levels (Fig. 1; Supplementary Table S1). Among these, the haplotypes with the highest frequencies in both groups were HYA/LYO, HYO/LYO, and LYO/LYO (Fig. 1; Supplementary Table S1). The HYA/LXA and LXO/LXO haplotypes were identified only in patients with ALL, with frequencies of 0.03 and 0.01, respectively. Meanwhile, the LXA/LXO haplotype was found only in the control group (0.01).

3.3 Association of MBL2 gene polymorphisms with seropositivity for CMV and EBV infections

Anti-CMV IgG seropositivity was 98/107 (91.6%) in patients with ALL and 69/76 (90.8%) in controls, and anti-EBV IgG seropositivity was 86/107 (80.4%) and 67/76 (88.2%), respectively. Dual CMV/EBV IgG seropositivity was present in 80/107 patients (74.8%) and 62/76 controls (81.6%). The high CMV seroprevalence in this population reflects near-universal lifetime exposure in the Amazon region, limiting the usefulness of IgG seropositivity as an outcome because it indicates prior exposure rather than ALL-specific reactivation or disease.

Six patients with ALL were seropositive for both anti-CMV IgM and IgG, compatible with, but not confirmatory of, active or recurrent infection. However, no IgG avidity testing was performed to distinguish between these possibilities. No IgM seropositive cases were detected in the control group, and no individuals were seropositive for anti-EBV IgM in either group. Although CMV IgM positivity is clinically more relevant than IgG seropositivity in ALL, the small number of positive cases precluded formal association testing, highlighting a priority for future studies.

Among patients with ALL, CMV IgG was recorded in 86/91 O-allele carriers (A/O or O/O) and 12/16 participants with A/A. O-allele carriage was associated with higher odds of seropositivity (adjusted OR 5.12, 95% CI 1.24–20.53; nominal p = 0.025). Among controls, CMV IgG was recorded in 14/19 X-allele carriers and 55/57 participants with Y/Y (adjusted OR 0.09, 95% CI 0.014–0.462; nominal p = 0.003). The X/Y-versus-Y/Y comparison gave a similar estimate (13/18 versus 55/57; adjusted OR 0.09, 95% CI 0.014–0.459; nominal p = 0.003). A formal genotype×group interaction term was not statistically significant for either the − 221 X allele (p = 0.22) or the exon 1 O allele (p = 0.08). The opposite directions seen in the separate group analyses are therefore interpreted as exploratory rather than as demonstrated effect-modification.

Individuals carrying haplotypes associated with low or intermediate serum MBL expression (such as HYO/LXA, HYO/HYO, HYO/LYO, LXA/LYO, LYO/LXO, LYO/LYO, LXA/LXO, LXO/LXO, HYO/LXO, HYA/HYO, HYA/LXA, HYA/LYO, LYA/LXA, and LYA/LYO) showed the highest seropositivity for CMV and EBV infections in both patients with ALL and controls. However, when haplotypes were grouped by functional

classification (high, intermediate, and low serum MBL expression), no significant association was observed with seropositivity for CMV or EBV infections in either group (Supplementary Table S3).

3.4 Exploratory predictions of monomer stability

Across all three exon 1 positions, DUET predicted a destabilising effect on the modelled monomer, with negative ΔΔG values at codon 52 (p.Arg52Cys, − 0.264 kcal/mol), codon 54 (p.Gly54Asp, − 0.409 kcal/mol) and codon 57 (p.Gly57Glu, − 0.502 kcal/mol). The two component methods agreed in direction at every position (mCSM: −0.219, − 0.607 and − 0.768 kcal/mol; SDM: −0.41, − 0.88 and − 0.53 kcal/mol, respectively; Table 4). The consistent negative sign across positions and methods indicates a coherent destabilising tendency in the collagen-like region; the magnitudes, all below 1 kcal/mol, fall within the reported error of these predictors and are therefore interpreted qualitatively, as a direction of effect rather than a quantitative measure. Local structural changes at positions 52, 54 and 57 are shown in Supplementary Figure S5.

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Table 4
Predicted effect (ΔΔG, kcal/mol) of exon 1 variants on MBL monomer stability. Negative values indicate a destabilizing effect.

Method	Codon 52 (p.Arg52Cys)	Codon 54 (p.Gly54Asp)	Codon 57 (p.Gly57Glu)
DUET	-0.264	-0.409	-0.502
mCSM	-0.219	-0.607	-0.768
SDM	-0.41	-0.88	-0.53

The consistent negative sign across positions and methods indicates a coherent destabilising tendency in the collagen-like region; the magnitudes, all below 1 kcal/mol, fall within the reported error of these predictors and are therefore interpreted qualitatively, as a direction of effect rather than a quantitative measure. The modeled side-chain substitutions at positions 52, 54 and 57 are shown in Fig. 4.

4. DISCUSSION

Variants in the MBL protein coding gene are frequently associated with an increased risk of infections in various clinical settings, many of which remain poorly understood (Mullighan et al., 2002; Eisen and Minchinton, 2003; Pana et al., 2014). In this context, we assessed the frequency of *MBL2* gene variants and their association with CMV and EBV seropositivity in patients with ALL.

In this study, no statistically significant differences were observed in the frequency of *MBL2* gene variants between patients with ALL and controls, suggesting no association between these variants and the development of ALL. While one previous study reported a higher frequency of genotypes associated with low serum MBL concentrations in children with ALL compared to controls (Schmiegelow et al., 2002), other studies, such as Oguz et al. (2024), have also found no association between *MBL2* gene variants and the development of pediatric ALL, consistent with our findings.

The *MBL*B* and *MBL*D* variants in the coding region (exon 1) were observed at high frequencies, while the *MBL*C* allele was not detected, as also reported in other studies conducted in patients with ALL (Pana et al., 2014; Oliveira et al., 2025). On the other hand, a previous study conducted in the state of Amazonas reported the presence of the C allele in patients with cutaneous leishmaniasis (CL) (18%) and in healthy controls (10%) (Araujo et al., 2015).

This variation across studies may be partly explained by the genetic composition of the population in northern Brazil, which is characterized by historical admixture among Europeans, Africans, and Amerindians, resulting in higher frequencies of alleles B and D (Pena et al., 2011). In contrast, allele C is more common in Sub-Saharan African populations and relatively rare elsewhere (Cruz et al., 2013). The proportion of African ancestry in northern Brazil is approximately 10.9% (Pena et al., 2011), which, combined with the low frequency of allele C and the relatively small sample size, may have contributed to its non-detection in this study.

Structural variants in patients with different types of cancer have been linked to an increased incidence of infections, negatively affecting patient prognosis (Moreto et al., 2014; Fekete et al., 2016). In this study, the O allele was associated with CMV seropositivity in patients with ALL, consistent with findings in non-cancer populations where the O allele has been linked to increased risk of viral infections (Giang et al., 2020; Queiroz et al., 2023). In the ALL group, carriage of the exon 1 O allele was associated with higher odds of CMV IgG seropositivity. As this allele is predicted to destabilise MBL, it is possible that reduced MBL function may facilitate viral exposure or impair early immune control, particularly in the context of immunosuppression due to the neoplasm and its treatment. This aligns with previous reports associating MBL variants with infection risk in patients with neoplasms (Horiuchi et al., 2005; Kennedy-Nasser et al., 2008; Vekemans et al., 2007; Moreto et al., 2014). However, since serum MBL was not measured and IgG indicates prior exposure rather than active infection, this mechanistic interpretation remains hypothetical.

The in silico analysis provides exploratory support for a possible structural rationale for the association between the O allele and CMV seropositivity. The substitutions at codons 52, 54, and 57 are located within the collagen-like domain, which is essential for the triple-helical assembly and oligomerisation required for MBL function; these substitutions are known to disrupt this region and impair oligomerisation rather than directly destabilising the monomer (Sumiya et al., 1991; Garred et al., 2003). Our monomer-level predictions, which consistently indicate a destabilising effect at all three positions

(negative ΔΔG by DUET, mCSM, and SDM), serve as an indirect indicator of this structural defect, in agreement with previous computational analyses (Kalia et al., 2016; Behairy et al., 2022).

These predictions have important limitations: they pertain to the monomer rather than the oligomerisation step, the substitutions are located in the N-terminal region where model confidence is lower, and the ΔΔG values are small and within the error margin of the predictors. Therefore, the structural data provide only exploratory support for the genetic association and do not constitute evidence of functional deficiency; confirmation by serum MBL quantification and assays of oligomerisation or complement activation is needed.

The X allele and the XY genotype in the − 221 region of the promoter were found to be associated with lower odds of CMV seropositivity in immunocompetent individuals. A similar association was observed in Moroccan children, where carriers of the X allele (X/X or X/Y) had a lower chance of developing visceral leishmaniasis (Hamdi et al., 2013). However, haplotypes carrying the X allele are generally associated with intermediate to low serum MBL concentrations (Madsen et al., 1995; Medeiros et al., 2021), which has been linked to increased susceptibility to infections in various clinical contexts (Hu et al., 2010; Cruz et al., 2013). Therefore, these findings should be interpreted with caution, as limited statistical power, potential confounding by age and sex, lack of correction for multiple testing, and the absence of serum MBL measurements restrict conclusions about the functional consequences of *MBL2* variants.

Although haplotypes associated with low and intermediate serum MBL levels were highly prevalent in the study population, and higher seropositivity for CMV and EBV infections was observed among carriers of these haplotypes, no association was detected between these haplotypes and seropositivity for these infections. Other studies have also demonstrated an absence of association between genotypes/haplotypes associated with MBL deficiency and the risk of infections in patients with hematologic diseases (Holanda et al., 2014; Swierzko et al., 2018; Sokołowska et al., 2020).

Nevertheless, the high prevalence of viral infection among patients carrying haplotypes associated with reduced MBL expression in this study highlights the clinical importance of infection management in immunocompromised patients. A study of ALL patients in Amazonas found low serum MBL concentrations regardless of genotype (Oliveira et al., 2025), although only exon 1 genotypes were assessed; it is recognized that different *MBL2* haplotypes can regulate serum MBL levels (Garred et al., 2003; Vasconcelos et al., 2011; Figueiredo et al., 2016). Further studies with larger sample sizes, more detailed clinical and laboratory characterization, and measurement of serum MBL levels are needed to clarify the relationship between *MBL2* variants, functional MBL deficiency, and susceptibility to viral infections in these patients.

Furthermore, the active CMV infection observed in six patients with ALL reinforces their increased vulnerability to viral reactivation or reinfection, possibly favored by the immunosuppression associated with the disease and chemotherapy, corroborating the study by Silva et al. (2021), who reported high rates of recurrent CMV infections in patients with ALL in the western Brazilian Amazon. In this context,

quantitative or functional MBL deficiency could represent an additional susceptibility factor for recurrent infections in these patients, a hypothesis that requires confirmation in studies with larger casuistic and serum protein dosage.

Some limitations constrain inference. First, the small number of CMV-seronegative participants (nine patients and seven controls) limits statistical power, and while Firth penalization reduces small-sample bias, it cannot fully address this limitation. The retrospective design precludes temporal inference. Data on genetic ancestry, treatment phase, chemotherapy regimen, transplantation, antiviral prophylaxis, and transfusion or immunoglobulin exposure were not available and could not be included as covariates. Finally, serum MBL concentration and activity were not measured, and the monomer-level structural predictions do not substitute for functional testing.

In this context, prospective and longitudinal studies involving clinical follow-up of patients and serum MBL measurement are needed to provide a more robust assessment of the relationship between *MBL2* gene variants and seropositivity for CMV and EBV. Despite its limitations, this study makes an important scientific contribution by characterizing the frequency profile of *MBL2* variants in patients with ALL and by suggesting possible implications of these variants for susceptibility to viral infections, particularly CMV. These findings may aid in understanding the role of MBL in the innate immune response in patients with ALL and provide insights for future risk stratification and clinical management strategies.

5. CONCLUSIONS

In this study of patients with ALL and controls from Amazonas, MBL2 exon 1 and promoter variants showed context-dependent associations with CMV IgG seropositivity after adjustment for age and sex. In the ALL group, O-allele carriage was associated with higher odds of CMV seropositivity, whereas in controls, carriage of the − 221 X allele and the XY genotype was associated with lower odds. No variant was associated with EBV seropositivity, and expression-based haplotypes showed no association with either virus. These associations were nominal and should be regarded as exploratory, given the attenuation under correction for multiple comparisons and the few seronegative participants.

In silico modelling predicted that the exon 1 substitutions could perturb the collagen-like region that governs MBL oligomerisation, offering a plausible but unproven structural rationale; because serum MBL was not measured and the predicted effects were small, this interpretation remains hypothetical and awaits functional confirmation. Overall, these findings characterise MBL2 variation in an Amazonian ALL population, including the first description of promoter variants in this setting, and support a possible context-dependent role of MBL in viral serostatus that larger prospective studies incorporating serum MBL measurement and CMV reactivation endpoints will be needed to confirm.

Declarations

Competing interests

The authors declare no competing interests.

Ethics approval

This study was approved by the Research Ethics Committee of the Amazonas Hematology and Hemotherapy Foundation (FHEMOAM) (CAAE 25710819.0.0000.0009; approval no. 5.081.493). All procedures were conducted in accordance with the Declaration of Helsinki and Brazilian National Health Council Resolution 466/2012.

Consent to participate

Written informed consent was obtained from all participants; for participants under 18 years of age, written informed consent was provided by a parent or legal guardian, together with the participant's assent where applicable.

Consent to publish

Not applicable. This article does not contain any individual person's identifiable data in any form.

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Author Contribution

Victória Karoline da Silva Araújo contributed to study conception and design, methodology, formal analysis, manuscript drafting, review, and editing. Leonardo Calheiros de Oliveira, Kerollayne Costa da Silva, Paulo Henrique Rodrigues de Souza, and Lucas Bezerra Palheta contributed to methodology, formal analysis and manuscript draft. Catarina Marques Pereira contributed to formal analysis, in silico structural modelling, manuscript drafting, review, and editing. Gemilson Soares Pontes contributed to study conception and design, supervision, project administration, resources, funding acquisition, and manuscript review and editing. All authors commented on previous versions of the manuscript and read and approved the final version.

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Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

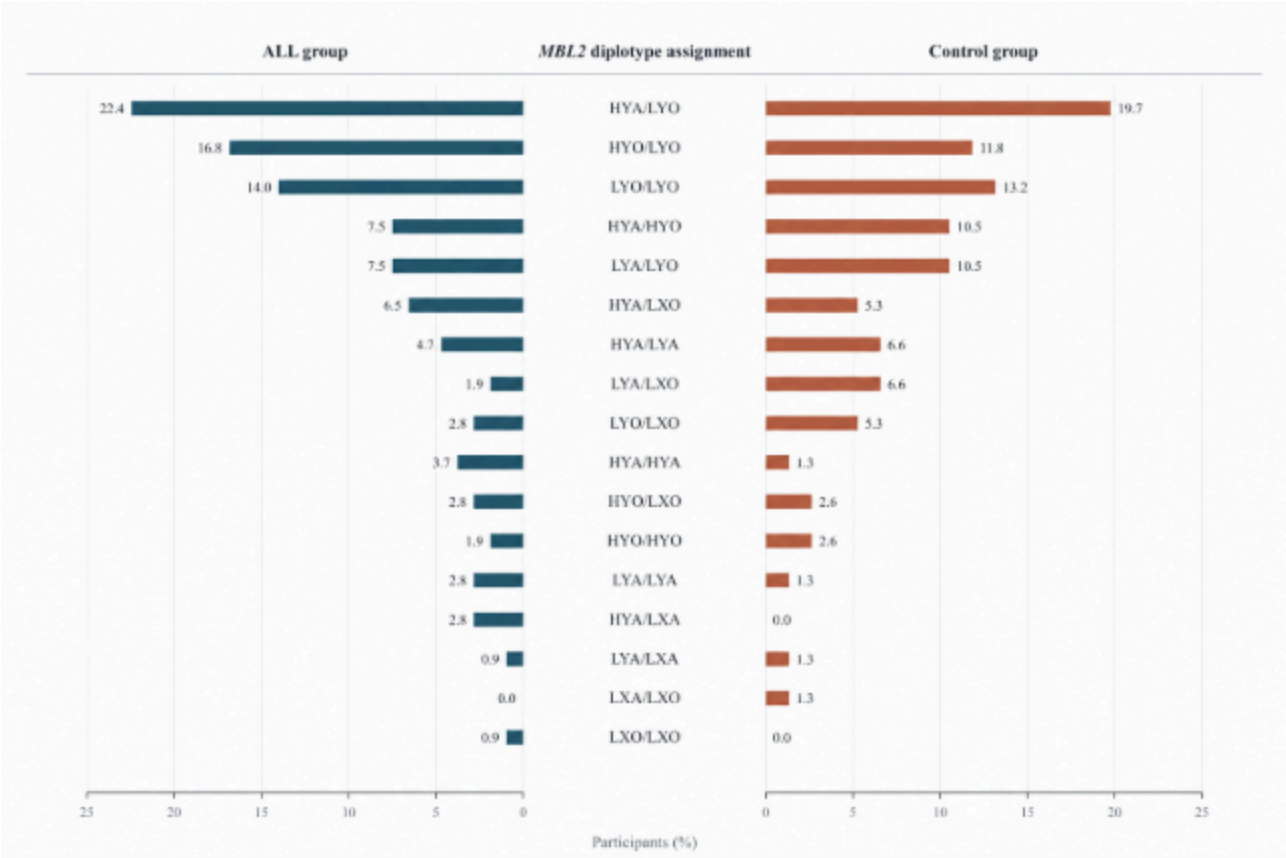


Figure 1

Distribution of recorded putative MBL2 diplotype assignments in patients with acute lymphoblastic leukemia (ALL) and controls. Bars show the percentage of participants assigned to each label within each group. These are pairs of assigned haplotypes, not chromosome-level haplotype frequencies.

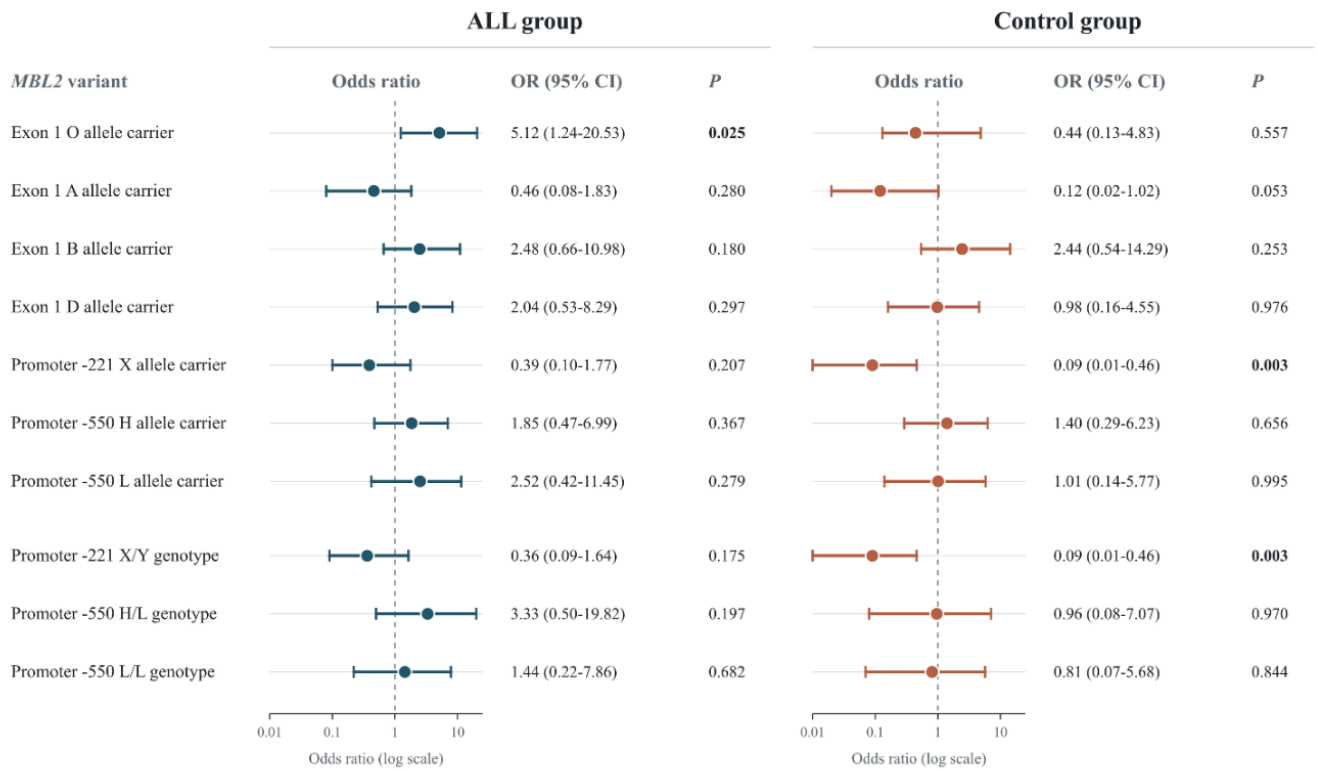


Figure 2

Exploratory associations of MBL2 variants with CMV IgG seropositivity in patients with acute lymphoblastic leukemia (ALL) and controls. Odds ratios and 95% profile penalized-likelihood confidence intervals were estimated by Firth logistic regression adjusted for age and sex. Carrier models compare carriers with noncarriers. Genotype models are separate pairwise comparisons with Y/Y or H/H; other genotypes are excluded from each comparison. The dashed line indicates OR = 1. Bold p-values denote nominal p <0.05. The full association data, including Epstein-Barr virus and haplotype analyses, are provided in Supplementary Tables S2-S4.

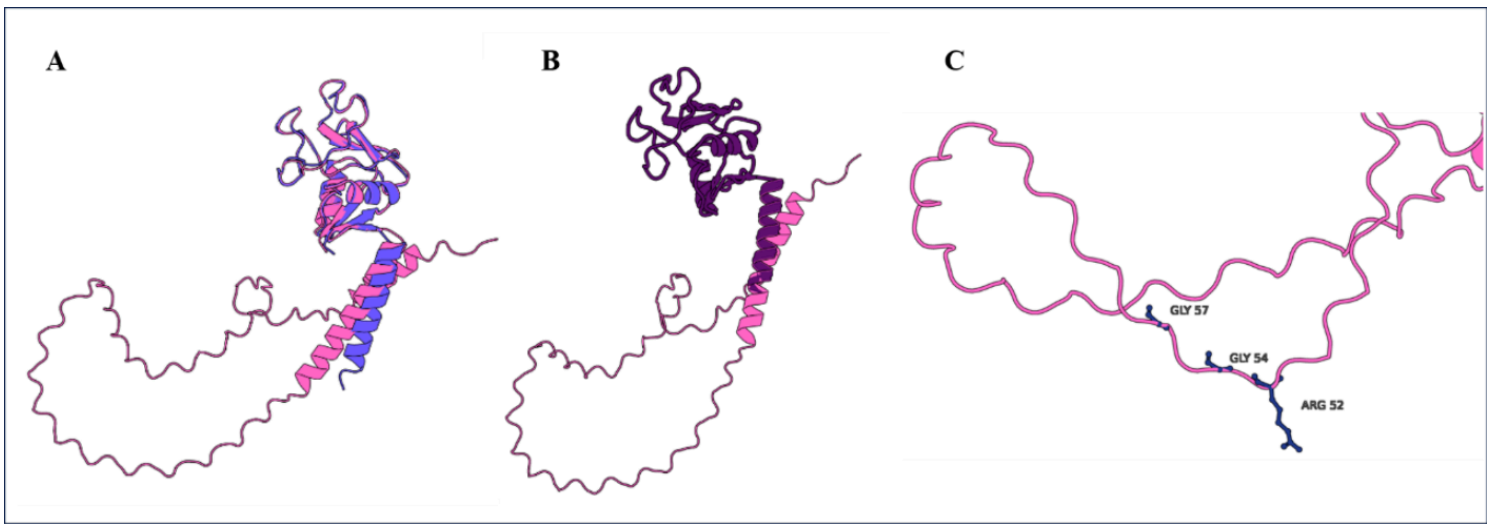


Figure 3

C**omparison between the experimental structure (PDB 1HUP) and the AlphaFold prediction of the MBL monomer**. **A**: Structural alignment over the common neck+CRD region, showing high overlap between the AlphaFold model (pink) and 1HUP (purple); **B**: full predicted model, with the N-terminal region (pink) and the neck+CRD domain (dark purple) indicated. **C**: Residues R52, G54 and G57 in the collagen region.

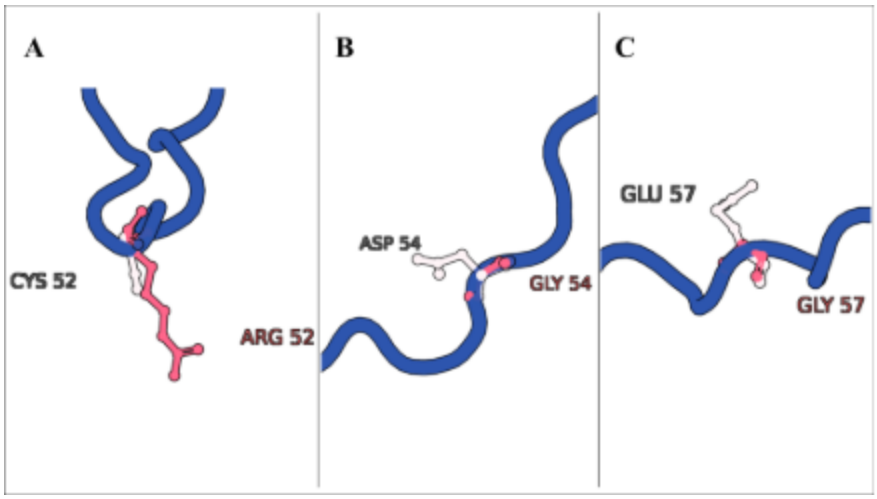


Figure 4

Modeled side-chain substitutions at MBL residues 52, 54 and 57. WT residues are shown in pink, variant residues in white, and the backbone in blue. A: p.Arg52Cys. B: p.Gly54Asp. C: p.Gly57Glu, included as a literature-defined reference variant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryMaterial.docx