

**Multilayer Immunogenomic Decoding within an In Silico Framework:
MBL2 as a Central Susceptibility Factor in Cryptococcal Meningitis**

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Abstract

Introduction:

Cryptococcal meningitis (CM) is a life-threatening opportunistic fungal infection with variable host susceptibility, yet the role of immunogenomic variation remains poorly defined. This study aimed to decode single-nucleotide polymorphism (SNP)-driven susceptibility in key immune-related candidate genes (*MBL2*, *FCGR2A*, *FCGR2B*, *FCGR3A*, *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR9*, *CLEC6A*) using an integrated, multi-layer *in silico* framework.

Materials and Methods:

The multi-layer analytical framework incorporated pathogenicity predictions, molecular modeling, protein stability assessment, post-translational modification mapping, evolutionary conservation, regulatory evaluations (eQTL/pQTL), and system-level network analyses (*GeneMANIA*).

Result:

Three *MBL2* missense variants (R52C, G54D, G57E) consistently demonstrated the strongest deleterious signatures, including structural destabilization, disruption of critical collagen-like and lectin-binding residues, and promoter dysfunction. Expression analyses revealed a discordant pattern with reduced circulating protein levels despite upregulated hepatic *MBL2* transcripts, indicating post-translational destabilization and defective secretion. In contrast, *FcγR* variants exhibited largely benign effects due to functional redundancy, while *TLR* variants preserved signaling capacity. Network integration positioned *MBL2* as the central vulnerability node connecting lectin-complement activation, humoral immunity, phagocytic pathways, and apoptotic cell clearance.

Conclusion:

This multi-layer *in silico* framework highlights *MBL2* as a key determinant of host susceptibility to CM by linking sequence variation to structural and network-level functional disruptions. These findings provide mechanistic insights into individual immune variability, establishing a foundation for biomarker development and risk stratification.

Keywords (3-6 keywords): Cryptococcal meningitis; Fcγ receptors; immunogenomics; *MBL2*; SNP; Toll-like receptors

1. Introduction

Meningitis occurs as a severe inflammatory response taking place within the protective membranes around the central nervous system. A wide array of pathogens—including bacteria, viruses, and parasites can trigger this condition, though fungal agents represent a particularly difficult challenge in clinical settings [1]. Among fungal species, *Cryptococcus* represents a primary public health concern. What often begins as a localized pulmonary infection can rapidly spread to central nervous tissues. How far the infection escalates depends heavily on the host's underlying immune status, a dynamic clearly reflected in immunosuppressed animal models and broad epidemiological surveys [2, 3]. Its most severe impact occurs when it invades the central nervous system, manifesting as cryptococcal meningitis. Globally, cryptococcosis remains a significant public health challenge, causing substantial morbidity and mortality among immunocompromised populations worldwide [4].

Although rare in healthy individuals, *Cryptococcus* infects immunocompromised hosts by deploying evasion mechanisms that resist immune clearance and fungal killing [5]. Upon surviving these host defences, *Cryptococcus* can cross the blood-brain barrier via Trojan horse, paracellular, or transcellular pathways, compromising its integrity to invade the central nervous system and induce meningitis [6].

Mounting an effective defence against *Cryptococcus* requires seamless coordination across multiple cellular receptor networks. At the cell surface, various pattern recognition molecules such as TLR1, TLR2, TLR4, TLR6, CLEC6A, MBL2, alongside FCGR2A, FCGR2B, and FCGR3A bind directly to structural components of the fungal cell wall. This engagement kicks off vital immune events, including complement activation, signalling cascades, and cell clearance [7]. Meanwhile, inside the cell, endosomal receptors such as TLR9 scan for foreign nucleic acids to direct secondary immune signalling [8].

Genetic diversity within these primary defence receptors often dictates how effectively a host combats fungal invasion. Host response to fungal invasion varies widely from person to person, largely driven by underlying single-nucleotide polymorphisms (SNPs) [9]. Depending on its exact genomic location, a SNP alters cellular function through distinct biological routes. For instance, non-synonymous mutations swap amino acid residues to alter a protein's 3D architecture or functional binding, whereas synonymous mutations typically exert regulatory control by modifying transcript stability, codon usage, or baseline expression levels [10].

Genetic association studies have linked host susceptibility to both non-synonymous and synonymous regulatory variants across these key receptors. Notably, significant signals cluster within genes such as *FCGR2A*, *FCGR3A*, *FCGR2B*, *MBL2*, *TLR1*, *TLR2*, *TLR4*, *TLR6*, *TLR9*, and *CLEC6A* [11-16].

However, the majority of current research inspects these variants individually, overlooking how they operate together within an integrated biological system [17]. To address this gap, we implemented an integrated, multi-layered *in silico* framework, combining pathogenicity predictions, 3D structural modelling, PTM mapping, evolutionary conservation, and eQTL/pQTL profiling across ten core candidate immune genes (*MBL2*, *FCGR2A/2B/3A*, *TLR1/2/4/6/9*, and *CLEC6A*). Ultimately, this study aims to decode SNP-driven host susceptibility to cryptococcal meningitis at a systems level.

2. Materials and Methods

We set up an observational, *in silico* workflow to screen candidate variants, build 3D structural models, annotate regulatory features, and map functional gene networks. Between June and October 2025, we screened potential candidate SNPs identified from published literature through a series of dry-lab computational analyses. All target genomic positions were first

aligned to the GRCh37/hg19 human assembly, ensuring coordinate compatibility across HaploReg, RegulomeDB, and public QTL resources. In contrast, functional damage prediction algorithms, such as SIFT, PolyPhen-2, and PROVEAN, together with 3D structural modeling packages, calculated their impact scores independently of specific reference builds.

2.1 Non-Synonymous Analysis

To evaluate how non-synonymous variants affect protein function, we ran a panel of eight complementary prediction algorithms: SIFT (<https://sift.bii.a-star.edu.sg/>), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), PhD-SNP (<https://SNP.biofold.org/phd-snp/phd-snp.html>), FATHMM (<https://fathmm.biocompute.org.uk/>), PROVEAN (<http://provean.jcvi.org>), PANTHER (<http://www.pantherdb.org/>), SNAP (<https://roslab.org/services/snap/>), and Meta-SNP (<https://snps.biofold.org/meta-snp>). Alongside these functional predictions, we estimated shifts in protein thermodynamic stability using MuPro (<http://mupro.proteomics.ics.uci.edu/>) and I-Mutant Suite (<https://folding.biofold.org/i-mutant>). Tool predictions were compiled into a binary matrix, scoring neutral, benign, or tolerated outputs as 0 and damaging or deleterious outputs as 1. Visualizations relied on GraphPad Prism (v9) for bar plots and Morpheus for heatmaps (<https://software.broadinstitute.org/morpheus/>). Amino acid substitution loci were cross-referenced with PTM motifs via PhosphoSitePlus (<https://www.phosphosite.org>) and dbPTM (<https://biomics.lab.nycu.edu.tw/dbPTM/>). Wild-type and mutant 3D atomic structures modeled through SWISS-MODEL (<https://swissmodel.expasy.org>) were inspected in PyMOL (v3.1.6.1, Schrödinger, LLC) to pinpoint conformational shifts. We also ran Project HOPE (<https://www3.cmbi.umcn.nl/hope/>) to quantify structural disruptions caused by alterations in molecular weight and side-chain van der Waals volume ($\text{\AA}^3$).

2.2 Synonymous Analysis

Although synonymous SNPs leave amino acid sequences intact, their capacity to alter mRNA splicing or expression levels was evaluated through the Ensembl Variant Effect Predictor (<https://www.ensembl.org/info/docs/tools/vep/index.html>; VEP). We also gathered composite functional impact metrics, specifically CADD, PHRED, and DANN scores through Varsome (<https://varsome.com>). Finally, we checked non-coding regulatory capacity using RegulomeDB (v2.2) (<https://www.regulomedb.org>) and HaploReg (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>) to spot functional signals in immune-related loci.

2.3 Evolutionary Constraint Analysis of Genomic Variants

Evolutionary conservation often highlights functionally critical genomic regions. To measure selective pressures at candidate SNP sites, we retrieved GERP, PhastCons, and PhyloP scores through Varsome. These metrics allowed us to assess site-specific substitution rates and alignment-based conservation profiles across both coding and regulatory variants.

2.4 Expression and Protein QTL Analysis

To determine how selected variants influence transcript and protein levels, eQTL and pQTL summary statistics were gathered from QTLbase. Our analysis focused on primary immune loci, specifically the Fc gamma receptor family (*FCGR2A*, *FCGR2B*), C-type lectin *CLEC6A*, and *MBL2*, alongside Toll-like receptors (*TLR1*, *TLR2*, *TLR4*, *TLR6*, and *TLR9*). Cohorts with low statistical power were removed during dataset curation, leaving only associations where the observed significance level satisfied $p < 0.05$.

2.5 Gene-gene Interaction

To map functional connectivity among these immune targets, we used interaction networks using GeneMANIA. The platform determined overall biological connectivity by synthesizing evidence across multiple layers, such as co-expression patterns, physical protein interactions, shared domain structures, cellular co-localization, and pathway annotations.

3. Results

Candidate variants across ten immune loci associated with cryptococcal meningitis risk, specifically *MBL2*, *FCGR2A*, *FCGR2B*, *FCGR3A*, *CLEC6A*, *TLR1*, *TLR2*, *TLR4*, *TLR6*, and *TLR9*, were identified by searching the literature and querying the NCBI dbSNP database. Variant rsIDs, exact genomic positions, and alternate alleles are tabulated in Table S1 as our primary baseline dataset.

3.1 Non-Synonymous Analysis

In silico functional evaluation of non-synonymous variants showed clear score divergence across algorithms. All three predictive platforms, SIFT, PolyPhen-2, and PROVEAN, flagged the *MBL2* variants R52C, G54D, and G57E as deleterious, returning scores of 0.000 (under the 0.05 cutoff), over 0.98 (probably damaging), and below -2.5. On the flip side, *FCGR2A* H167R and *TLR6* V327M were uniformly classified as benign across these software packages.

These conservative amino acid changes occur at evolutionary positions tolerant to variation, leaving side-chain charge, core hydrophobic packing, and overall 3D backbone topology intact. Thermodynamic modeling via I-Mutant and MuPro indicated reduced protein stability ($\Delta\Delta G < 0$) across most variants in *FCGR2A*, *FCGR3A*, *FCGR2B*, and *MBL2* (Table 1c), though *MBL2* G57E showed minor directional variance between software.

Table 1. In silico prediction of the functional consequences of SNP-induced amino acid substitutions using multiple pathogenicity and stability assessment tools.

(A) SIFT, PolyPhen-2, PhD-SNP, SNP&GO, and SNAP predictions for the deleteriousness of each variant.

Genes	Residue	SIFT	Polyphen 2.0		PhD SNP	SNP&GO	SNAP
			HumDiv	HumVar			
FCGR2A	H167R	Tolerated	Benign	Benign	Neutral	Neutral	Diseases
FCGR3A	F176V	NA	Benign	Benign	Neutral	Neutral	NA
FCGR2B	I232T	Tolerated	NA	NA	Neutral	Neutral	Diseases
MBL2	R52C	Deleterious	ProDam	ProDam	Diseases	Neutral	Diseases
MBL2	G54D	Deleterious	ProDam	ProDam	Diseases	Diseases	Diseases
MBL2	G57E	Deleterious	ProDam	ProDam	Diseases	Diseases	Diseases
TLR6	V327M	Tolerated	Benign	Benign	Neutral	Neutral	Neutral

* NA, data unavailable; ProDam, probably damaging (PolyPhen-2 classification).

(B) FATHMM, PROVEAN, Meta-SNP, and PANTHER classifications integrating evolutionary and functional impact scores.

Genes	Residue	FATHMM	PROVEAN	Meta-SNP	PANTHER
FCGR2A	H167R	Tolerated	Deleterious	Neutral	Neutral
FCGR3A	F176V	Tolerated	Neutral	Neutral	Neutral
FCGR2B	I232T	Tolerated	Neutral	Neutral	Neutral
MBL2	R52C	Damaging	Deleterious	Diseases	Diseases
MBL2	G54D	Damaging	Deleterious	Diseases	Diseases
MBL2	G57E	Damaging	Deleterious	Diseases	Diseases
TLR6	V327M	Tolerated	Neutral	Neutral	NA

* NA, data unavailable; ProDam, probably damaging (PolyPhen-2 classification).

(C) Protein stability predictions based on I-Mutant Suite and MUPro, expressed as $\Delta\Delta G$ values, indicate stability increase or decrease upon mutation.

Genes	Residue	I-Mutant Suite		MuPro	
		Stability	$\Delta\Delta G$ (Kcal/mol)	Prediction	ΔG
FCGR2A	H167R	Decrease	-0.32	Decrease	-0.67
FCGR3A	F176V	Decrease	-3.45	Decrease	-0.83
FCGR2B	I232T	Decrease	-1.02	Decrease	-1.55
MBL2	R52C	Decrease	-0.99	Decrease	-1.09
MBL2	G54D	Decrease	-1.37	Decrease	-0.70
MBL2	G57E	Increase	+0.32	Decrease	-0.09
TLR6	V327M	Decrease	-1.53	Decrease	-1.44

* $\Delta\Delta G$, Delta Delta G (Change in Gibbs Free Energy); ΔG , Delta G (Gibbs Free Energy)

Integrated cumulative scoring across multiple *in silico* tools confirmed that *MBL2* variants (R52C, G54D, G57E) possess the highest cumulative impact, whereas substitutions in *FCGR2A* (H167R), *FCGR3A* (F176V), *FCGR2B* (I232T), and *TLR6* (V327M) exhibited low scores, reflecting minimal functional risk (**Figure S2a–2c**).

Post-translational modification (PTM) analysis using NetPhos, NetOGlyc-4.0, NetNGlyc-1.0, iPTMNet, PhosphoSitePlus, and dbPTM revealed that all evaluated non-synonymous variants lie strictly outside predicted or validated phosphorylation and glycosylation motifs (**Table S2**). Thus, functional perturbations in *MBL2* stem directly from local domain destabilization rather than impaired PTM attachment. Structural modeling yielded high-reliability 3D protein structures, sharing >95% sequence identity with their respective templates (**Figure 1a–g, Table S3**).

Model quality metrics demonstrated robust backbone geometry and minimal steric clashes across all candidates, with QMEAN scores of 0.65–0.82, GMQE > 0.55, Errat > 85%, MolProbity scores of 0.82–1.79, and >81% of residues in Ramachandran-favored regions, validating their suitability for downstream functional analyses.

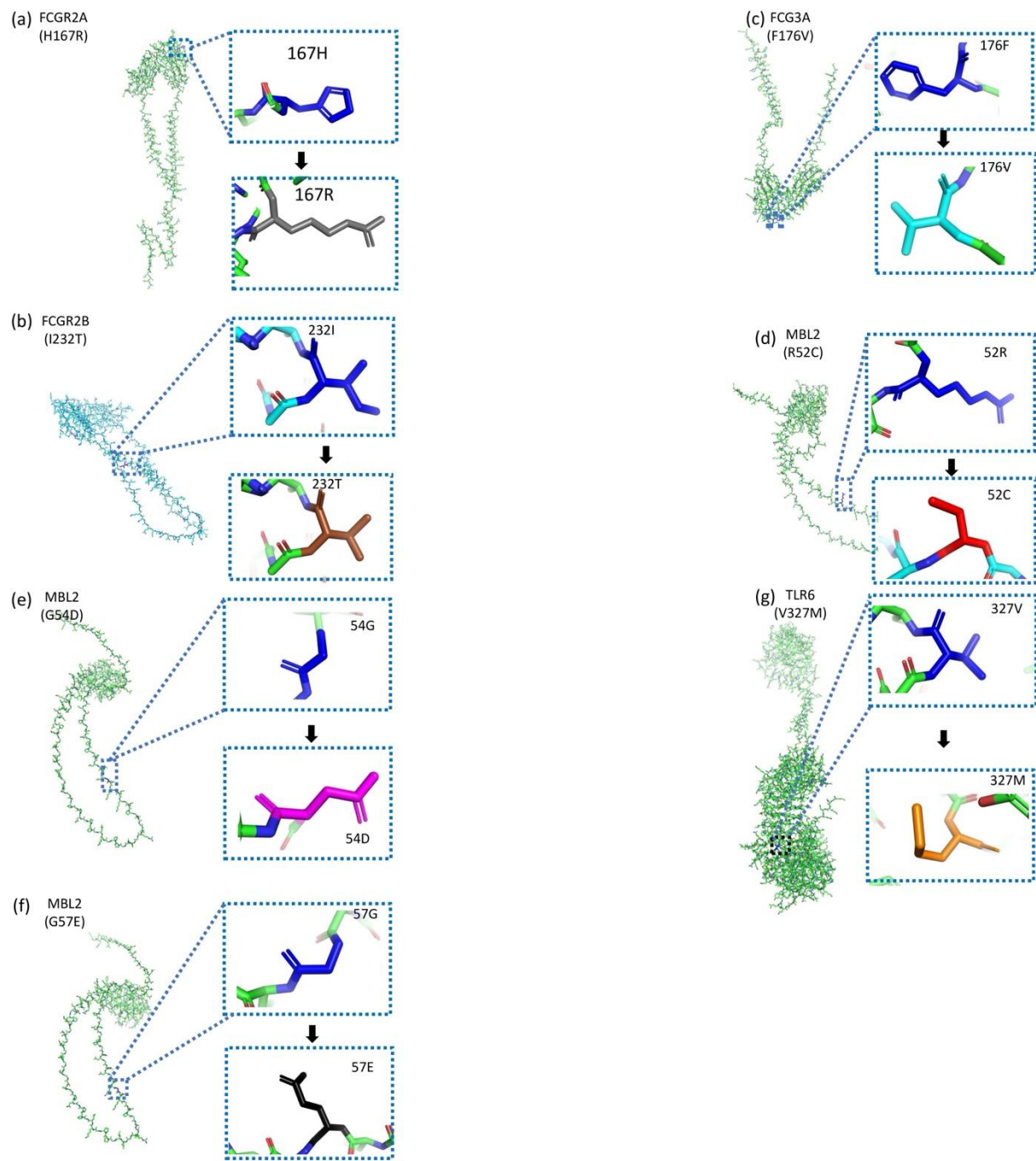


Figure 1. Three-dimensional structural visualization of wild-type and mutant residues for SNPs across immunogenetic genes. Structural models were generated to illustrate local conformational changes introduced by each amino acid substitution. Wild-type residues are presented in the top zoomed inset of each panel, with mutated side chains displayed underneath:

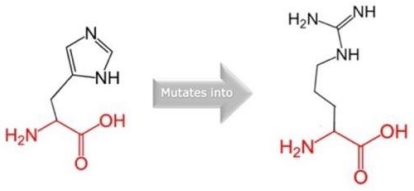
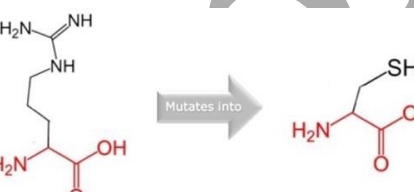
(a) *FCGR2A* H167R, (b) *FCGR2B* I232T, (c) *FCGR3A* F176V, (d) *MBL2* R52C, (e) *MBL2* G54D, (f) *MBL2* G57E, and (g) *TLR6* V327M.

Local physical environment changes varied by mutation. Changes in local microenvironments varied across substitution sites. When we looked at the 3D models, each mutation changed the local protein structure in its own way. In *FCGR2A*, swapping histidine for arginine (H167R) puts a larger, positively charged side chain on the surface, altering the local charge. In *FCGR2B*, replacing isoleucine with a smaller threonine (I232T) leaves a space inside the hydrophobic core, which loosens domain packing.

For *FCGR3A*, the F176V change replaces phenylalanine with a smaller valine, giving the local peptide backbone more flexibility. The three *MBL2* substitutions we examined trigger even sharper physical shifts: R52C eliminates a positive charge while increasing local hydrophobicity, whereas G54D and G57E introduce negatively charged, hydrophobic residues right at critical binding boundaries, directly disturbing contact stability. TLR6 V327M replaced a valine with a slightly larger methionine, which may subtly affect local structural arrangement.

Collectively, these observations suggest that the variants have the potential to induce structural perturbations with functional consequences, providing a mechanistic link between sequence variation and protein behavior, consistent with predictions from Project HOPE and the validated high-quality protein models.

229 **Table 2.** Predicted structural and physicochemical effects of amino acid substitutions based
230 on HOPE analysis

Protein	Residue	Size mutant	Structure	WT charge	Mutant Charge	Hydrophobicity wild type
FCGR2A	H167R	Bigger		Neutral	Positive	-
FCGR2B	I232T	Smaller		-	-	More Hydrophobic
FCGR3A	F176V	Smaller		-	-	-
MBL2	R52C	Smaller		Positive	Neutral	More Hydrophobic
MBL2	G54D	Bigger		Neutral	Negative	More Hydrophobic
MBL2	G57E	Bigger		Neutral	Negative	More Hydrophobic
TLR6	V327M	Bigger		-	-	-

3.2 Synonymous Analysis

When we evaluated the non-coding intronic and synonymous SNPs across our dataset, the vast majority showed negligible structural consequences (Table S4). For instance, intronic variants in *MBL2* (rs11003125, rs7095891), *TLR1*, *TLR4*, *TLR6*, and *CLEC6A*, along with synonymous changes in *TLR2* and *TLR9*, consistently returned low CADD and DANN scores, pointing to functionally neutral effects. However, our regulatory profiling with RegulomeDB and HaploReg picked up a different pattern for two specific variants: *MBL2* rs7096206 and *TLR6* rs5743794. Both displayed strong regulatory potential (rank 1f, Table S4b), backed by evidence of shifted chromatin accessibility and altered transcription factor binding motifs. So while most non-coding variants we identified appear structurally benign, a few key SNPs might still influence host susceptibility by tweaking gene expression at the transcriptional level.

3.3 Evolutionary Constraint Analysis

Our evolutionary preservation analysis (Table S5) showed consistently high PhyloP, PhastCons, and GERP RS values at non-synonymous positions in *MBL2* (rs1800450, rs1800451) and *FCGR2B* (rs1050501), marking them as functionally conserved across species. On the other hand, the sequence data we gathered for *FCGR2A* and *FCGR3A* point toward faster, accelerated evolution at these loci.

3.4 Gene expression pattern analysis

When we looked into the eQTL and pQTL data, we found that candidate gene regulation varied quite a bit depending on the tissue (Table S6). Take the Fc receptor family, for instance—we saw opposing regulatory trends, where *FCGR2A* showed lower expression in blood, yet *FCGR3A* was upregulated at the protein level. We also noticed discordant expression

for *MBL2*, which showed higher mRNA levels in the liver but lower protein levels in blood. The Toll-like receptors followed their own distinct tissue patterns as well: *TLR6* went up in NK cells but dropped in blood, *TLR1* expression was reduced in the prefrontal cortex, and *TLR4* was elevated in esophageal tissue. Together, these differences point to a fairly complex, tissue-dependent genetic control over immune expression.

3.5 Gene–Gene Interaction Network Analysis

In our network, *MBL2* sits at the center, bridging complement activation and humoral responses with how apoptotic cells get cleared. This aligns with what we know about its double duty in early *Cryptococcus* sensing and keeping immune balance. Receptors in the *FCGR* family split the workload: activating hubs (*FCGR2A*, *FCGR3A*) push opsono-phagocytic signaling forward, while *FCGR2B* provides the necessary negative feedback to keep immune responses from causing excess damage. Across the *TLR* set (*TLR1/2/4/6/9*), *TLR4* and *TLR6* take the lead in fungal detection, connecting directly to IL-8-mediated neutrophil recruitment. Looking at these cross-talks together—from *MBL2* opsonization to *FCGR3A* phagocytosis and *TLR* sensing—suggests that cryptococcal meningitis risk is driven by cumulative network disruptions rather than single-gene defects alone.

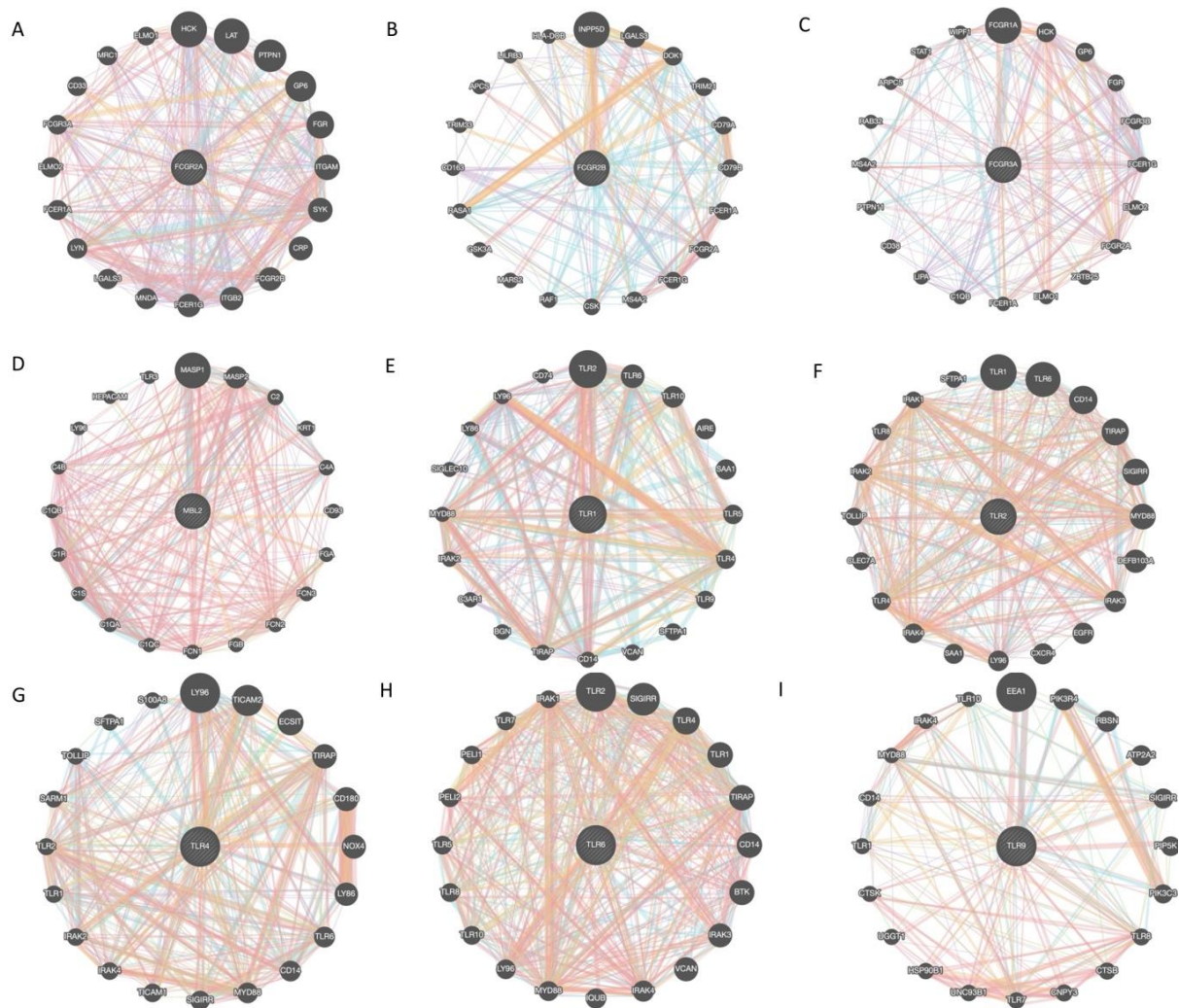


Figure 2. GeneMANIA network map showing functional interactions across our candidate loci.

(A) Network centered on *FCGR2A*, showing clear associations with key signaling adaptors including *SYK*, *LYN*, *LAT*, *HCK*, and *ITGAM*.

(B) Network centered on *FCGR2B*, highlighting interactions with *ITGAX*, *CD79A*, *SYK*, *PIK3R1*, and downstream inhibitory immune regulators.

(C) *FCGR3A* interacts directly with *FCGR1A*, *FCGR3B*, *LYN*, and *HCK*.

(D) We mapped direct linkages between *MBL2* and core lectin pathway factors (*MASP1*, *MASP2*, *C2*, *C3*).

(E) *TLR1* connects with *TLR2*, *TLR6*, *TIRAP*, *MYD88*, *IRAK* family members, and innate sensors.

(F) Our network revealed close functional alignment between *TLR6* and adaptors including *TLR2*, *TLR1*, *CD14*, *TOLLIP*, *SIGIRR*, and *MYD88*.

(G) *TLR4* coordinates with core LPS-sensing molecules such as *LY96* (MD-2), *CD14*, *TIRAP*, *IRAK* proteins, and *TICAM1/TICAM2*.

(H) *TLR5* groups together with shared signaling adaptors (*TLR2*, *TLR4*, *CD14*, *SIGIRR*, *MYD88*, *BTK*).

(I) *TLR9* clusters with endosomal DNA-sensing machinery (*MYD88*, *IRAK1/IRAK4*, *UNC93B1*, *EEA1*) and downstream inflammatory mediators.

287 **Table 3.** Functional enrichment analysis of candidate genes interaction network

Gene	Function	FDR	GIN	GIG
<i>FCGR2A</i>	Phagocytosis	1.83e-29	13	200
	Fc receptor signalling pathway	3.34e-16	12	171
	Immune response-regulating cell surface receptor signalling pathway involved in phagocytosis	1.61e-13	9	76
	Fc-gamma receptor signalling pathway	1.61e-13	9	76
	Fc receptor mediated stimulatory signalling pathway	2.09e-13	9	80
	Immunoglobulin binding	0.00123	3	10
<i>FCGR2B</i>	Antigen receptor-mediated signalling pathway	0.00123	6	222
	Fc receptor signalling pathway	0.0055	5	171
	Cell surface	0.027	5	251
	Negative regulation of immune system process	0.0388	5	281
<i>FCGR3A</i>	Fc receptor signalling pathway	6.68e-16	12	171
	Immune response-regulating cell surface receptor signalling pathway involved in phagocytosis	2.15e-13	9	76
	Fc-gamma receptor signalling pathway	2.15e-13	9	76
	Fc receptor mediated stimulatory signalling pathway	2.62e-13	9	80
	Complement activation	1.83e-29	15	67
<i>MBL2</i>	Humoral immune response	2.81e-27	17	197
	Regulation of humoral immune response	6.78e-12	8	61
	Apoptotic cell clearance	2.38e-9	6	31
	Humoral immune response mediated by circulating Immunoglobulin	9.67e-9	5	15
	Toll-like receptor signalling pathway	7.37e-18	12	119
<i>TLR1</i>	Pattern recognition receptor signalling pathway	7.71e-18	13	190
	Regulation of interleukin-8 production	3.67e-16	10	70
	Interleukin-8 production	4.98e-16	10	74
	Regulation of toll-like receptor signalling pathway	1.15e-14	9	59
	Toll-like receptor signalling pathway	2.79e-20	13	119
<i>TLR2</i>	Pattern recognition receptor signalling pathway	3.99e-20	14	190
	Cellular response to biotic stimulus	1.66e-14	11	161
	Regulation of interleukin-8 production	7.40e-14	9	70
	Interleukin-8 production	8.36e-14	9	74
	Pattern recognition receptor signalling pathway	3.36e-22	15	190
<i>TLR4</i>	Toll-like receptor signalling pathway	1.39e-20	13	119
	Response to molecule of bacterial origin	3.87e-20	14	195
	Cellular response to molecule of bacterial origin	5.77e-20	13	139
	Cellular response to biotic stimulus	3.35e-19	13	161
	Toll-like receptor signalling pathway	4.29e-28	16	119
<i>TLR6</i>	Pattern recognition receptor signalling pathway	5.65e-25	16	190
	Regulation of interleukin-8 production	3.68e-16	10	70
	Interleukin-8 production	4.98e-16	10	74
	Response to molecule of bacterial origin	6.72e-16	12	195
	Pattern recognition receptor signalling pathway	3.36e-22	15	190
<i>TLR9</i>	Toll-like receptor signalling pathway	8.02e-16	11	119
	Endolysosome	8.29e-8	5	20
	Regulation of interleukin-8 production	4.13e-7	6	70
	Interleukin-8 production	4.67e-7	6	74

*GIN, Gene in Network; GIG, Gene in genome

4. Discussion

Multi-layered immunogenomic profiling identified *MBL2* as the primary hub gene driving host susceptibility to cryptococcal meningitis. Structural modeling, evolutionary conservation, and integrated eQTL/pQTL profiling confirmed that three coding variants R52C, G54D, and G57E, exert profound functional impacts by disrupting residues 52–57, a critical region required for collagenous domain oligomerization and mannan recognition [18].

Negative $\Delta\Delta G$ predictions, residue disruptions, conformational changes, and high evolutionary conservation reinforce the essential role of *MBL2* in innate immunity. Combined with the promoter-enhancing variant rs7096206 (RegulomeDB 1f) and pQTL evidence of reduced plasma MBL levels, *MBL2* exhibits structural and regulatory impairments. The observed expression discrepancy, elevated hepatic transcription alongside reduced plasma protein abundance, suggests compensatory transcriptional feedback, post-translational instability, and impaired ER-associated degradation (ERAD)-mediated secretion [19].

The functional dominance of *MBL2* is supported by epidemiological links between deficiency genotypes and cryptococcal meningitis in non-HIV individuals [14], resolving prior population-level discrepancies (Eisen 2008). Our findings establish that pathogenic impact is restricted to variants disrupting critical structural integrity or promoter control rather than non-functional genetic noise [20]. Network topology further validates *MBL2* as a central immunogenetic hub, orchestrating complement activation, humoral response, inflammatory regulation, and apoptotic cell clearance [21].

The FcγR family (*FCGR2A/2B/3A*) and TLR cluster (*TLR1/2/4/6/9*) constitute core functional modules regulating opsono-phagocytosis, inflammatory control, and IL-8-driven fungal sensing [22]. However, most analyzed variants in both pathways exhibit minimal structural or pathogenic effects. This indicates that despite their fundamental necessity in host defense, these

networks possess evolutionary redundancy that buffers individual single-nucleotide polymorphisms against catastrophic functional loss [23].

CLEC6A (Dectin-2) contributes moderately to fungal recognition, with no high-impact variants identified in this study. This finding fits well with the functional redundancy known in the C-type lectin receptor family, where Dectin-2 works alongside Dectin-1 and helps compensate for it during antifungal immune responses [24].

By bringing together SNP pathogenicity scores, 3D structural models, evolutionary conservation metrics, and quantitative trait locus (eQTL/pQTL) data, our study builds a multi-layered immunogenomic framework that directly links genetic variation to host susceptibility in cryptococcal meningitis. Gene interaction networks consistently highlight *MBL2* as a central vulnerability node. While limited by its purely *in silico* nature and lack of wet-lab or epigenetic validation, this novel framework offers valuable mechanistic insights for risk-stratification and biomarker development.

5. Conclusions

This study establishes a multi-layered *in silico* framework mapping SNP-driven susceptibility to cryptococcal meningitis, pinpointing *MBL2* as the primary structural and regulatory vulnerability node in host defense. While FcγR and TLR networks exhibit evolutionary redundancy that buffers individual variants, disease susceptibility stems from cumulative perturbations across interacting immunogenetic pathways. These mechanistic insights provide actionable targets for host risk stratification and personalized therapeutic interventions.

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Author Contribution:

Study concept and design: JJ, RA

Acquisition of data: JJ, RA, SW

Analysis and interpretation of data: JJ, RA

Drafting of the manuscript: JJ, RA, SW

Critical revision of the manuscript for important intellectual content: JJ, RA, SW

Study supervision: SW, RA

Ethics:

This study did not involve human participants, clinical samples, or identifiable personal data.

All analyses were performed using publicly available, Anonymized genomic databases; therefore, ethical approval was not required.

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None Declared

Data availability:

This study used only publicly available genomic and regulatory datasets. No human subjects or clinical samples were used. All database versions and access dates were documented in the Methods. Processed data and analysis were available from the corresponding author upon request.

AI Declaration Statement

Artificial Intelligence (AI) tools were not used in preparing this manuscript. The authors performed all scientific content, data generation, analyses, and interpretations. Computational software and publicly accessible bioinformatics web servers were used exclusively to execute

predefined analytical procedures, while all scientific judgments, interpretations, and conclusions remained the sole responsibility of the authors.

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