Received: 25 June 2021 | Accepted: 16 October 2021

DOI: 10.1111/imm.13425

immunology
insights into immunity in health and disease

REVIEW

Human immune polymorphisms associated with the risk of cryptococcal disease

Chinaemerem U. Onyishi | **Robin C. May**

Institute of Microbiology & Infection, School of Biosciences, University of Birmingham, Birmingham, UK

Correspondence
Robin C. May, Institute of Microbiology & Infection, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.
Email: r.c.may@bham.ac.uk

Senior author: Robin C. May

Funding information
CUO is supported by the Darwin Trust of Edinburgh.

Abstract

Cryptococcus neoformans is an opportunistic fungal pathogen that can cause lethal cryptococcal meningitis in immunocompromised individuals such as those with HIV/AIDS. In addition, cryptococcal infections occasionally arise in immunocompetent individuals or those with previously undiagnosed immunodeficiencies. The course of cryptococcosis is highly variable in both patient groups, and there is rapidly growing evidence that genetic polymorphisms may have a significant impact on the trajectory of disease. Here, we review what is currently known about the nature of these polymorphisms and their impact on host response to *C. neoformans* infection. Thus far, polymorphisms in Fc gamma receptors, mannose-binding lectin, Dectin-2, Toll-like receptors and macrophage colony-stimulating factor have been associated with susceptibility to cryptococcal disease. Notably, however, in some cases the impact of these polymorphisms depends on the genetic background of the population; for example, the *FCGR3A* 158 F/V polymorphism was associated with an increased risk of cryptococcal disease in both HIV-positive and HIV-negative white populations, but not in Han Chinese patients. In most cases, the precise mechanism by which the identified polymorphisms influence disease progression remains unclear, although impaired fungal recognition and phagocytosis by innate immune cells appears to play a major role. Finally, we highlight outstanding questions in the field and emphasize the need for future research to include more diverse populations in their genetic association studies.

KEYWORDS

cryptococcal meningitis, *Cryptococcus neoformans*, genetic susceptibility, genome wide association study, single nucleotide polymorphism

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; BCL10, B-cell lymphoma 10; CARD9, caspase recruitment containing protein 9; CDC, complement-dependent cytotoxicity; CLR, C-type lectin receptor; CM, cryptococcal meningitis; CNS, central nervous system; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin; FcγR, Fc gamma receptor; GWAS, genome wide association study; GXM, glucuronoxylomannan; IRAK, IL-1R-associated kinase; IRF3, interferon regulatory factor; LRR, leucine-rich repeat; MALT1, mucosa-associated lymphoid tissue lymphoma translocation gene 1; MBL, mannose-binding lectin; M-CSF, macrophage colony-stimulating factor; Mincle, macrophage inducible C-type lectin; MR, mannose receptor; MyD88, myeloid differentiation primary response 88; PBMCs, peripheral blood mononuclear cells; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; SYK, spleen tyrosine kinase; TIR, Toll/Interleukin 1 receptor; TLR, Toll-like receptor; TRAF, tumour necrosis factor receptor-associated factors; TRIF, TIR-domain-containing adapter-inducing interferon-β.

INTRODUCTION

Cryptococcus neoformans is an encapsulated fungus that causes a potentially fatal disease called cryptococcosis, primarily in immunocompromised individuals such as HIV patients [1,2]. Infection with *C. neoformans* is thought to begin with the inhalation of fungal cells (either spores or desiccated yeast) from the environment [1]. Within the lungs, *C. neoformans* can be cleared by the immune system or it establishes an asymptomatic latent infection [1]. Following reactivation of latent infection or after successful primary pulmonary infection in immunocompromised individuals, *C. neoformans* can spread from the lungs to the central nervous system (CNS), ultimately leading to fatal cryptococcal meningitis (CM) (Figure 1) [1,3].

Cryptococcosis is an AIDS-defining illness and the leading cause of fungal meningitis in sub-Saharan Africa [1]. The estimated global burden of CM in HIV patients is 223 100 cases per year with 73% of global cases (162 500) occurring in sub-Saharan Africa [4]. HIV-associated CM is estimated to cause 181 100 deaths annually of which 135 900 occur in sub-Saharan Africa. Additionally, CM is estimated to be responsible for 15% of AIDS-related deaths, making it the second-highest cause of death in AIDS patients after tuberculosis [4]. Although CM is typically an opportunistic disease in immunocompromised individuals, there are growing reports of CM in immunocompetent individuals [5–7]. It is also estimated that, globally, only 6% of HIV-infected people with a low (under 100 cells/μl) $CD4^+$ T-cell count are positive for cryptococcal antigens [4]; thus, the risk of cryptococcal disease may be driven by other environmental factors such as alcoholism and diabetes leading to mild states of immunosuppression and/or host genetics [8]. In addition, since the progression of infection can vary significantly even between individuals with apparently similar levels of immunocompromisation, it is likely that host genetic variation has a strong impact on the trajectory of infection. Hence, this review seeks to detail our current understanding of the genetic polymorphisms that are associated with susceptibility to cryptococcal disease and their influence on host response to *C. neoformans* infection.

INNATE IMMUNE RESPONSE TO *C. neoformans* INFECTION

Following the inhalation of *C. neoformans* cells from the environment, fungi are recognized and phagocytosed by professional phagocytes such as macrophages [9]. Phagocytosis is initiated by the recognition of microbial pathogen-associated molecular patterns (PAMPs) by host pattern recognition receptors (PRRs) (Figure 2) [10]. PRRs on the surface of professional phagocytes, such as members of the Toll-like receptor (TLR) family and members of the C-type lectin receptor (CLR) family, have been implicated in the recognition of *C. neoformans* with β-1,3-glucans, mannans and glucuronoxylomannan (GXM) serving as PAMPs [11–14]. The binding of a ligand to a PRR can lead to phagocytosis, the expression of cytokines and type I interferons and the production of reactive oxygen species (ROS), leading to an anti-microbial environment within the host cell.

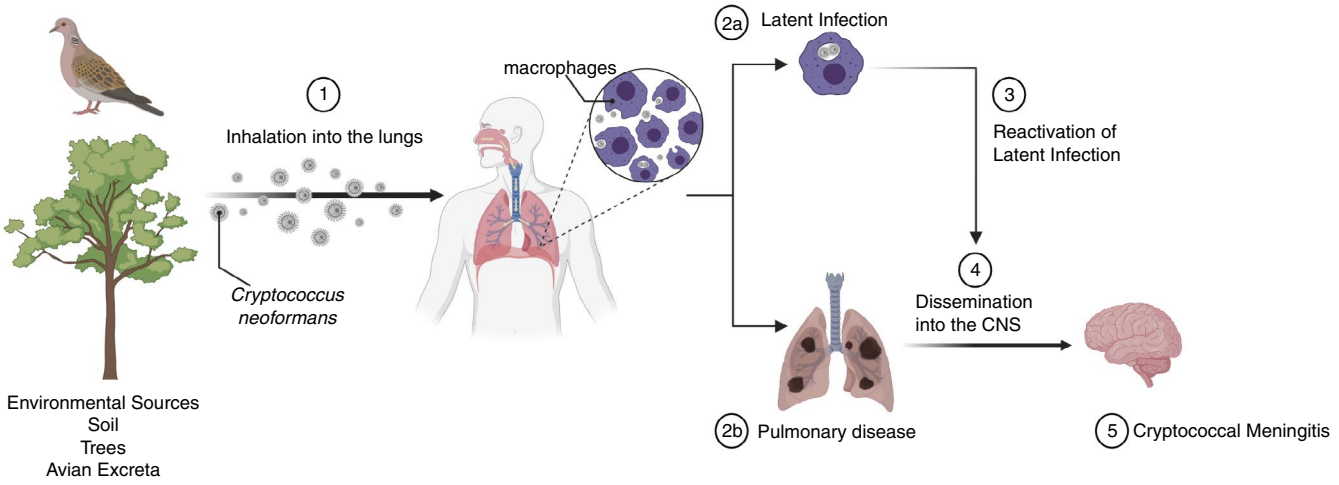

FIGURE 1 *Cryptococcus neoformans* mode of infection. *C. neoformans* is commonly found in soil and avian excreta all over the world. Infection with the fungus begins with the inhalation of fungal cells into the lungs. Within the lungs, *C. neoformans* can establish asymptomatic latent infection or cause pulmonary disease. The fungi can then disseminate to the central nervous system (CNS), cross the blood-brain barrier and infect the meninges, leading to fatal cryptococcal meningitis. Figure created with BioRender.com

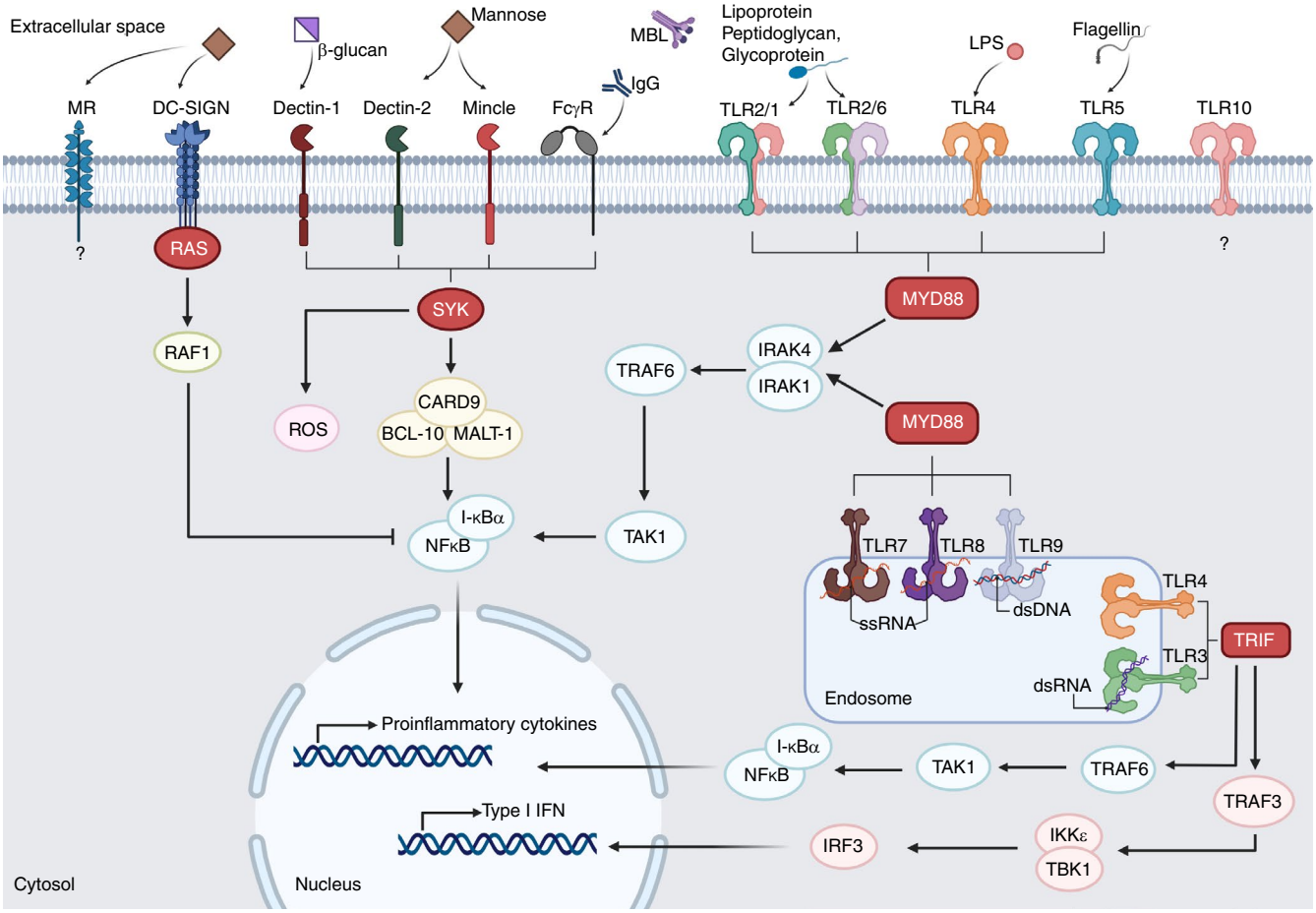

FIGURE 2 Pattern recognition receptors (PRRs) and their signalling pathways. The recognition of foreign particles is initiated by PRR binding to pathogen-associated molecular patterns (PAMPs) unique to microbes. Toll-like receptors (TLRs) recognize a wide range of bacterial, fungal and viral ligands such as lipopolysaccharide (LPS), glycoproteins and nucleic acids. Ligand binding to a TLR activates a signalling cascade that is mediated by the adaptor proteins myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF). The signalling cascade ultimately leads to the activation of transcription factors that induce the expression of proinflammatory cytokines (MyD88- and TRIF-dependent signalling) or type I interferons (TRIF-dependent signalling). The CLR family is composed of receptors such as Dectin-1, Dectin-2, mannose receptor (MR), dendritic cell-specific ICAM-3-grabbing non-integrin (DC-SIGN), macrophage inducible C-type lectin (Mincle) and MBL, which recognize carbohydrate molecules on fungal cells. Ligand binding leads to the phosphorylation of CLRs by spleen tyrosine kinase (SYK) which then drives a signalling cascade involving the caspase recruitment containing protein 9 (CARD9)–B-cell lymphoma 10 (BCL10)–mucosa-associated lymphoid tissue lymphoma translocation gene 1 (MALT1) complex. Foreign agents are also recognized by molecules such as IgG and Mannose Binding Lectin (MBL) that bind and opsonize the pathogen promoting efficient host cell recognition and clearance through Fc γ receptors and the complement pathway, respectively. Figure created with BioRender.com

Toll-like receptors

Toll-like receptors are a family of transmembrane PRRs expressed by a range of immune cells including macrophages and neutrophils [15]. There are 10 functional TLRs in humans (TLR 1–10), and they are characterized by having an intracellular domain composed of a Toll/interleukin 1 receptor (TIR) domain, which interacts with intracellular adaptor molecules, and an extracellular domain containing leucine-rich repeats (LRR) that are responsible for ligand binding [16]. Binding of ligands to TLRs activates a signal transduction pathway mediated by the adaptor protein myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF) (Figure 2) [17]. Both the MyD88- and TRIF-dependent pathways ultimately lead to the activation of NF-κB, which then serves as a transcription factor for the expression of proinflammatory cytokines [18]. On the other hand, TRIF-dependent signalling also activates interferon regulatory factor 3 (IRF3), which serve as a transcription factor to initiate the expression of type I interferons [15,18].

Thus far, only TLR2, TLR4 and TLR9 have been studied in the context of *C. neoformans* infection [19]. TLR2 was shown to detect zymosan (a fungal cell wall extract

composed mainly of β-glucan, but also mannans, chitin, protein and lipids) [20,21] and the *C. neoformans* capsular polysaccharide, GXM [11]. TLR4 was shown to recognize fungal mannans [14] and GXM [11]. Finally, TLR9 was capable of recognizing cryptococcal DNA [12,19]. The current literature on the role of these receptors during cryptococcal infection is limited and contradictory. For example, a study by Biondo et al. [22] showed that TLR2$^{-/-}$ mice had a significantly higher fungal burden, decreased proinflammatory cytokines production and decreased survival rate compared with wild-type mice postintraperitoneal infection with *C. neoformans*. However, Nakamura et al. [23] later showed that there was no significant difference in proinflammatory cytokine production and lung fungal clearance between wild-type and TLR2$^{-/-}$ mice following intratracheal infection. These contradictions may be due to variation in experimental design. Regardless, further research is needed to clarify the role of TLRs in host–fungal interaction.

C-type lectin receptors

Another class of PRRs involved in the recognition of foreign agents is CLRs, which include Dectin-1, Dectin-2, mannose receptor (MR), dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN), macrophage inducible C-type lectin (Mincle) and mannose-binding lectin (MBL) (Figure 2) [24]. Dectin-1 is a phagocytic receptor that is highly expressed on the surface of macrophages and other myeloid cell where it recognizes fungal β-1,3-glucan with or without cross-talk with TLRs [13,25]. Ligand recognition by Dectin-1 activates a signalling pathway mediated by spleen tyrosine kinase (SYK) [26]. This then leads to the activation of NF-κB, the production of a wide range of proinflammatory cytokines, the production of ROS and phagocytosis. The significance of Dectin-1 in regulating macrophage clearance of the fungus *Candida albicans* has been well established [25]. However, it only seems to play a minor or insignificant role in host response to *C. neoformans* infection [23,27,28]. Unlike Dectin-1, Dectin-2 recognizes α-mannans on the fungal cell wall, and its SYK-dependent signalling pathway requires the formation of a heterodimeric complex with the Fc receptor gamma chain (FcRγ) [26,29]. It was recently shown that Dectin-2 was involved in the phagocytosis of *C. neoformans* by dendritic cells [30], although it is important to note that this study used an acapsular *C. neoformans* mutant and therefore may not fully reflect a physiological infection.

Opsonic uptake: Fcγ receptor

Aside from the non-opsonic modes of pathogen recognition and uptake described above, particle uptake can also occur through opsonization, which is the coating of invading microbes with antibodies or complement proteins leading to more efficient phagocytosis [31]. IgG antibody-coated organisms are recognized and phagocytosed by Fcγ receptors (FcγRs), while complement-coated cells are detected and phagocytosed by complement receptors [31]. FcγRs are found on the plasma membrane of immune cells such as macrophages, dendritic cells, neutrophils and B cells [32]. Ligand recognition by FcγRs activates SYK-dependent signalling, leading to responses including phagocytosis, cytotoxicity and cytokine production and release [33,34]. The process of opsonization by IgG antibodies facilitates efficient *C. neoformans* uptake and elimination [27].

ADAPTIVE IMMUNE RESPONSE TO *C. neoformans* INFECTION

The phagocytosis of *C. neoformans* by macrophages and subsequent phagosome maturation can lead to the degradation of the fungus and the presentation of fungal antigens on MHC molecules [26]. These antigens are then recognized by CD4$^+$ T cells, ultimately leading to the activation of the adaptive arm of the immune response [35]. The cytokine profile induced by PRR-ligand interaction polarizes macrophages to adopt different phenotypes: for instance, classically activated macrophages (M1) or alternatively activated macrophages (M2) [19,26]. The M1 phenotype is triggered by the secretion of interferon-γ (IFN-γ) by innate and adaptive immune cells [19]. Following activation, M1 macrophages produce proinflammatory cytokines including IL-12, IFN-γ and tumour necrosis factor-α (TNF-α), which recruits other phagocytes to the site of infection [19,26]. On the other hand, M2 macrophages are activated by the presence of IL-4 and IL-13 and typically permit intracellular fungal survival and proliferation [19,26].

The macrophage polarization state also skews CD4$^+$ T cell towards a Th1 or Th2 response. Similar to the M1 phenotype, the Th1 response is protective against *Cryptococcus*, since mice with defective IL-12 and IL-18 production (markers of Th1 phenotype) showed greater fungal burden in the lungs and decreased survival [36,37]. Meanwhile, the Th2 response results in the production of anti-inflammatory cytokines that are ineffective in clearing the fungi [1,26,38]. This allows *C. neoformans* to escape killing by macrophage, proliferate within phagocytes and establish successful infection.

Aside from the T-cell response, B-cell maturation and antibody production are also involved in anti-cryptococcal response. As previously mentioned, antibodies can opsonize invading pathogens and increase the efficiency

of phagocytosis [27]. The major capsular component in cryptococci is the polysaccharide GXM, and the most abundant anti-GXM antibodies in vivo are typically IgG and IgM [39], with the IgG2 isotype being the major antibody involved in the opsonization of *C. neoformans* [40]. X-linked immunodeficient mice that lack B-1 cells were found to be more susceptible to *C. neoformans* infection than wild-type mice [41,42]. This increase in susceptibility corresponded with a significant decrease in total and GXM-specific IgM and IgG production and impaired phagocytosis [42]. It has also been shown that the expression of IgM by B cells is lower in HIV-positive cryptococcosis patients than in HIV-positive patients with no cryptococcosis, suggesting that IgM expression predicts HIV-associated cryptococcosis status [43].

The remainder of this review will provide an overview of the current literature on the genetic risk of cryptococcal disease in both HIV-infected and HIV-uninfected individuals and will discuss their functional consequence in host response to infection.

GENETIC POLYMORPHISMS IN HUMAN IMMUNE SIGNALLING MOLECULES AND SUSCEPTIBILITY TO CRYPTOCOCCAL DISEASE

Reports of CM in immunocompetent individuals, a lack of CM in many HIV/AIDS patients with low $CD4^+$ T-cell count and the existence of donor-to-donor variation in macrophage response to *C. neoformans* infection suggests that disease risk may be driven by other factors outside of host immune state [17]. It has long been reported that there is a strong genetic component to susceptibility to infectious diseases [44]. Moreover, infectious agents are known to be one of the strongest selection pressures that act on the human genome [45]. Therefore, host genetics may contribute to an individual's susceptibility to cryptococcal disease. Though research in the area is limited, SNPs in various immune signalling proteins have been associated with susceptibility to cryptococcal disease (Table 1).

A 2007 study by Meletiadis et al. [46] investigated the relationship between *FCGR* genes and *Cryptococcus* infection in HIV-negative individuals. They showed that two common allelic variants in Fc receptors, *FCGR2A (CD32a)* 131R/R and *FCGR3A (CD16a)* 158V/V, were associated with an increased risk of cryptococcal disease (OR = 1·67; 95% CI [1·05–2·63]; $p = 0·04$ and OR = 2·04; 95% CI [1·06–4·00]; $p = 0·04$, respectively). Meanwhile, the NA2 copy number variation allele on *FCGR3B (CD16b)* was underrepresented in cryptococcosis patients (28% in cases and 40% in control), suggesting that it may be protective against infection.

The histidine (H) to arginine (R) substitution at position 131 of *FCGR2A* results in reduced affinity of the receptor to IgG2 [47]. Since *FCGR2A* is the major receptor for IgG2, the reduced affinity associated with the arginine substitution likely results in decreased phagocytosis of IgG2-coated fungal cells, ultimately leading to poor fungal clearance. On the other hand, the phenylalanine (F) to valine (V) substitution in amino acid position 158 of *FCGR3A* results in receptors with a higher affinity for IgG1 and IgG3 [48,49], while the NA1 haplotype is more efficient in the binding and phagocytosis of IgG1- and IgG3-coated particles than the protective NA2 haplotype [50,51]. The role of FcγRs during *C. neoformans* infection remains unclear. However, these findings imply that the differential affinity of these FcγRs to monoclonal antibodies impacts the rate at which pathogens are phagocytosed, which could then impact microbe clearance and dissemination. It has been shown that IgG1 and IgG3 are strong inducers of Fc-mediated host responses such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC), while IgG2 induces a more subtle response [52]. This suggests that polymorphisms that increase cryptococcosis risk likely induce an excessive immune response.

Another genetic association study genotyped four polymorphisms in low-affinity *FCGRs* in 117 HIV-uninfected individuals with CM and 190 healthy controls [53]. They found that the *FCGR2B (CD32b)* 232I/I genotype was over-represented in CM patients (OR = 1·65; 95% CI [1·02–2·67]; $p = 0·039$), while the *FCGR2B* 232I/T genotype was less frequently detected when compared to the healthy control group (OR = 0·54; 95% CI [0·33–0·90]; $p = 0·016$) [53]. This indicates that individuals with non-HIV-associated CM are more likely to be homozygous for the dominant allele at this location. The same pattern was replicated when only patients without any predisposing factors such as chronic kidney disease, solid organ transplantation and diabetes mellitus were compared with the control. FcγRIIB is the only known inhibitory FcγR, and its binding to IgG molecules acts to suppress immune cell activation [33]. It has been shown that FcγRIIB with a threonine residue at position 232 has a threefold to fourfold decrease in their affinity to IgG; as a result, they are less able to inhibit immune cell activation, leading to unopposed activatory FcγR signalling and sustained proinflammatory response which can damage healthy tissue [54,55]. The mechanism by which FcγRIIB is involved in *C. neoformans* infection is unclear, making it difficult to explain why the *FCGR2B* 232I/I genotype, though functionally sound, increases risk of CM. Interestingly, it has been repeatedly shown that the *FCGR2B* 232T/T genotype increases susceptibility to the autoimmune disease

TABLE 1 Summary of the genetic polymorphisms found to be associated with risk of cryptococcal disease

#	SNP ID	Closest gene	Major allele	Minor allele	Global MAF	Nucleotide change
1	rs1801274	*FCGR2A (CD32a)*	A	G	0·44	Missense variant; [CAT]>[CGT]
2	rs396991	*FCGR3A (CD16a)*	A	C	0·38[a]	Missense variant; [TTT]>[GTT] (minus strand)
3		*FCGR3B (CD16b)*				Copy number variation
4	rs1050501	*FCGR2B (CD32b)*	T	C	0·19	Missense Variant; [ATT]>[ACT]
5	rs11003125	*MBL2*	G	C	0·31	Intron variant; G>C
6	rs7096206	*MBL2*	C	G	0·20	Intron variant; C>G
7	rs7095891	*MBL2*	G	A	0·29	Intron variant; G>A
8	rs5030737	*MBL2*	G	A	0·03	Missense variant; [CGT]>[TGT] (minus strand)
9	rs1800450	*MBL2*	C	T	0·12	Missense variant; [GGC]>[GAC] (minus strand)
10	rs1800451	*MBL2*	C	T	0·08	Missense variant; [GGA]>[GAA] (minus strand)
11	rs11045418	*CLEC6A (Dectin-2)*	T	C	0·35	Intergenic variant; T>C
12	rs5743563	*TLR1*	A	G	0·18	Intron variant; A>G
13	rs5743604	*TLR1*	A	G	0·47	Intron variant; A>G
14	rs3804099	*TLR2*	T	C	0·41	Synonymous variant; [AAT]>[AAC]
15	rs3796508	*TLR6*	C	T	0·03	Missense variant; [GTG]>[ATG] (minus strand)
16	rs164637	*TWF2*	G	A	0·03	Synonymous variant; [CAC]>[CAT] (minus strand)
17	rs352140	*TLR9*	T	C	0·42	Synonymous variant; [CCG]>[CCT] (minus strand)

Consequence	Odds ratio (95% CI); ethnicity	Significance?
FCGR2A 131 histidine (H) to arginine (R) missense mutation	OR = 1·67 (1·05–2·63); multiple ethnicities [46]	FCGR2A 131 R/R genotype is associated with an increased risk of cryptococcal disease in HIV-negative patients [46]; There is no association between FCGR2A 131 H/R and cryptococcal meningitis in HIV-negative Han Chinese patients [53]; There is no association between FCGR2A 131 H/R polymorphism and cryptococcal disease in HIV-infected patients [58]
FCGR3A 158 phenylalanine (F) to valine (V) missense mutation	OR = 2·04 (1·06–4·00); multiple ethnicities [46] OR = 2·1 (1·2–3·5); multiple ethnicities [58]	FCGR3A 158 V/V genotype is associated with an increased risk of cryptococcal disease in HIV-negative patients [46]; There is no association between FCGR3A 158 F/V and cryptococcal meningitis in HIV-negative Han Chinese patients [53]; FCGR3A 158V allele is associated with an increased risk of cryptococcal disease in HIV-infected patients [58]
FCGR3B shows copy number variation allowing it to exist as the NA1 or NA2 allele	OR = 1·64 (1·02–2·63); multiple ethnicities [46]	FCGR3B NA2/NA2 is protective against cryptococcosis in HIV-negative patients [46]; There is no association between the FCGR3B NA2/NA2 genotype and cryptococcal meningitis in HIV-negative Han Chinese patients [53]
FCGR2B 232 isoleucine (I) to threonine (T) missense mutation	OR = 1·65 (1·02–2·67); Han Chinese [53]	FCGR2B 232 I/I genotype is associated with an increased risk of cryptococcal meningitis in HIV-negative Han Chinese patients [53]
– – – MBL2 52 arginine (R) to cysteine (C) missense mutation MBL2 54 glycine (G) to aspartic acid (D) missense mutation MBL2 57 glycine (G) to glutamic acid (E) missense mutation	OR = 2·09 (0·96–4·51); Han Chinese [61]	Genotypes leading to MBL2 deficiency (homozygous at any of the coding region variants) were associated with an increased risk of cryptococcal meningitis in HIV-negative Han Chinese patients [61]
–	OR = 0·59 (0·37–0·94); Han Chinese [72]	rs11045418 was associated with pulmonary cryptococcosis in HIV-negative patients, but there was no association between the SNP and cryptococcal meningitis [72]
–	OR = 1·66 (1·13–2·46); Han Chinese [78]	rs5743563 T/T was associated with an increased risk of cryptococcal meningitis in HIV-negative patients; rs5743604 C/C was associated with disease severity; rs5743563 was associated with CSF cytokine expression [78]
–	OR = 1·53 (1·02–2·29); Han Chinese [78]	rs5743604 T/T was associated with an increased risk of cryptococcal meningitis in HIV-negative patients [78]
TLR2 199 asparagine to asparagine synonymous mutation	OR = 1·47 (1·02–2·11); Han Chinese [78]	rs3804099 T/T was associated with an increased risk of cryptococcal meningitis in HIV-negative patients; rs3804099 C/T was associated with disease severity; rs3804099 was associated with CSF cytokine expression [78]
TLR6 valine (V) to methionine (M) missense mutation	OR = 1·79 (1·04–3·10); Han Chinese [78]	rs3796508 G/A was associated with an increased risk of cryptococcal meningitis in HIV-negative patients [78]
TWF2 histidine (H) to histidine synonymous mutation	OR = 15·03 (1·74–129·67); Han Chinese [78]	rs164637 C/T allele was associated with an increased risk of cryptococcal meningitis in HIV-negative patients [78]
TLR9 proline (P) to proline synonymous mutation	OR = 1·69 (1·04–2·75); Han Chinese [78]	rs352140 T/T was associated with an increased risk of cryptococcal meningitis in HIV-negative patients; rs352140 was associated with CSF cytokine expression [78]

(Continues)

TABLE 1 (Continued)

#	SNP ID	Closest gene	Major allele	Minor allele	Global MAF	Nucleotide change
18	rs1927907	*TLR4*	C	T	0·18	Intron variant; C>T
19	rs5743794	*TLR6*	C	T	0·18	Intron variant; C>T
20	rs1999713	*CSF1*	T	C	0·47	Intergenic variant; T>C
21	rs12121374	*CSF1*	T	C	0·48	LINC01768 intron variant
22	rs1999715	*CSF1*	C	A	0·48	Intergenic variant; C>A
23	rs1999713	*CSF1*	T	C	0·47	Intergenic variant; T>C
24	rs12124202	*CSF1*	G	A	0·46	Enhancer variant; G>A
25	rs2064163	*UTP25*	G	T	0·37	Regulatory region variant; G>T

Note: SNP information was collected from dbSNP database and Ensembl. Chromosome locations are from build 38 genome assembly (GRCh38); Minor Allele Frequencies (MAF) are from the 1000 Genomes Project combined population.

[a]MAF from the ALFA Allele Frequency project due to SNP absence in the 1000 Genomes project.

systemic lupus erythematosus (SLE), but has a protective function against malaria infection [56,57]. This implies that the threonine substitution increases the risk of autoimmune disease, but is protective against infectious disease, supporting the underrepresentation of the *FCGR2B* 232I/T genotype in CM patients.

Other polymorphisms that were genotyped in this study include *FCGR2A* 131H/R, FCGR3A 158F/V and *FCGR3B* NA1/NA2 [53]. They found no association between these polymorphisms and CM in this cohort of Han Chinese patients. This is in contrast with the findings by Meletiadia et al. [46] that showed that these polymorphisms were associated with cryptococcal disease in non-HIV-infected participants, of whom 68% were of European descent. This suggests that ethnic differences may influence the impact of particular genetic polymorphisms on *C. neoformans* infection.

The studies discussed thus far have focused on cryptococcosis in HIV-negative individuals. Therefore, to explore the genetic factors that influence susceptibility to cryptococcosis in HIV-infected patients, Rohatgi et al. [58] genotyped the *FCGR2A* 131H/R and the *FCGR3A* 158F/V polymorphisms in 164 mostly white male volunteers. They found that the *FCGR3A* 158V allele was associated with an increased risk of cryptococcal disease (OR = 2·1; 95% CI [1·2–3.5]; $p = 0{\cdot}005$). They went on to show that heterozygotes had a 2·1-fold increased risk of developing cryptococcal disease while *FCGR3A* 158V/V homozygotes had a 21-fold increase in infection risk. Similar to the study by Hu et al. [53] with a cohort of Chinese volunteers, but in contrast to the study by Meletiadis et al. [46] that also used a mostly white cohort, they found no association between *FCGR2A* 131H/R polymorphism and cryptococcal disease. An investigation into the functional consequence of the *FCGR3A* 158 polymorphism revealed that CHO-K1 cells engineered to express *FCGR3A* 158V allele bound human serum- or IgG-opsonized *C. neoformans* more effectively than cells expressing the *FCGR3A* 158F allele. Moreover, natural killer (NK) cells expressing *FCGR3A* 158V allele induced greater ADCC towards *C. neoformans*-infected monocytes than those expressing 158F allele. It is known that monocytes secrete chemokines that damage the blood–brain barrier, and thus, the elevated cytotoxicity caused by cells expressing *FCGR3A* 158V allele may promote *C. neoformans* dissemination into the CNS. Since the *FCGR3A* 158V allele increases *C. neoformans* binding to CHO cells, the increased risk of HIV-associated cryptococcal disease may also be caused by an increased phagocytosis of *C. neoformans* by phagocytes (Figure 3). One may assume that elevated phagocytosis would promote fungal clearance; however, it was shown that clinical *C. neoformans* strains that were more easily phagocytosed (termed high uptake *C. neoformans*) led to higher CNS fungal burden and elevated expression

Consequence	Odds ratio (95% CI); ethnicity	Significance?
–	OR = 0·66 (0·44–0·97); Han Chinese [78]	rs1927907 G/A was associated with a decreased risk of cryptococcal meningitis in HIV-negative patients; rs1927907 was associated with CSF cytokine expression [78]
–	OR = 0·57 (0·32–0·99); Han Chinese [78]	rs5743794 A/A was associated with a decreased risk of cryptococcal meningitis in HIV-negative patients; rs5743794 was associated with CSF cytokine expression [78]
–	OR = 0·50; South African Xhosa [79]	The top six SNPs associated with cryptococcal disease in HIV-infected patients were located within 2·5 kb upstream of the colony-stimulating factor 1 (*CSF1*) gene; These SNPs are also near the *LINC01768* long non-coding RNA gene [79]
–	OR = 0·52; South African Xhosa [79]	
–	OR = 0·53; South African Xhosa [79]	
–	OR = 0·53; South African Xhosa [79]	
–	OR = 0·53; South African Xhosa [79]	
–	OR = 0·55; South African Xhosa [79]	

of the Th2 cytokines, which is not protective against fungal infection [59,60]. In essence, increased phagocytosis counterintuitively predisposes to poor disease outcome. This supports the 'Trojan horse' hypothesis that states that *C. neoformans* hijack macrophages to disseminate throughout the host, cross the blood–brain barrier and promote pathogenesis [1].

Moving on from *FCGRs*, a 2011 genetic association study investigated the relationship between SNPs in the *MBL2* gene and non-HIV-associated CM using volunteers of Chinese ethnicity [61]. MBL is a soluble PRR in the CLR family that binds microbial carbohydrates and activates the lectin pathway of the complement system. It has been shown that the expression of functional MBL protein is highly dependent on MBL genotypes [62]. Additionally, genotypes leading to a deficiency of MBL protein have been linked to increased susceptibility to *C. albicans*, *Aspergillus* and HIV infection [63–66]. To explore the relationship between MBL deficiency and CM, Ou et al. [61] genotyped six alleles (three coding region non-synonymous SNPs and three promoter region SNPs) of the *MBL2* gene and found that triple homozygosity for the three coding region SNPs, which results in MBL2 deficiency, was associated with non-HIV CM (OR = 4·29; 95% CI [1·11–19·99]; $p = 0·023$). In a 2019 case report by Wagemakers et al. [67], a 60-year-old HIV-negative man with chronically relapsing CM had a decreased concentration of MBL in his serum, supporting the finding that MBL deficiency increases CM risk. Unfortunately, the patient's MBL2 gene was not genotyped; otherwise, it may have revealed whether the observed deficiency in serum MBL had a genetic basis.

Polymorphisms in CLRs have also been associated with risk of various fungal diseases [68–70]. Hu et al. [71] sought to explore the association of Dectin-2 polymorphisms with cryptococcal disease using a HIV-negative Chinese cohort. A total of 464 healthy controls and 251 HIV-negative patients with cryptococcosis were genotyped for the rs11045418 SNP located at the 5′-flanking region of the Dectin-2 gene. They found a significant association between the rs11045418 SNP and pulmonary cryptococcosis when comparing controls and immunocompetent patient with no predisposing factor such as diabetes, autoimmune disease or solid organ transplantation recipient (OR = 0·59; 95% CI [0·37–0·94]; $p = 0·026$). When all patients were included in the analysis, there was no association between the SNP and risk of pulmonary infection (OR = 0·77; 95% CI [0·53–1·12]; $p = 0·17$). There was also no association between the SNP and CM when comparing the control group with either overall patients or immunocompetent patients. The functional consequence of this SNP was not investigated; however, it has been shown that Dectin-2 and FcγR co-expression by CHO cells enables binding to *C. neoformans* spores [28].

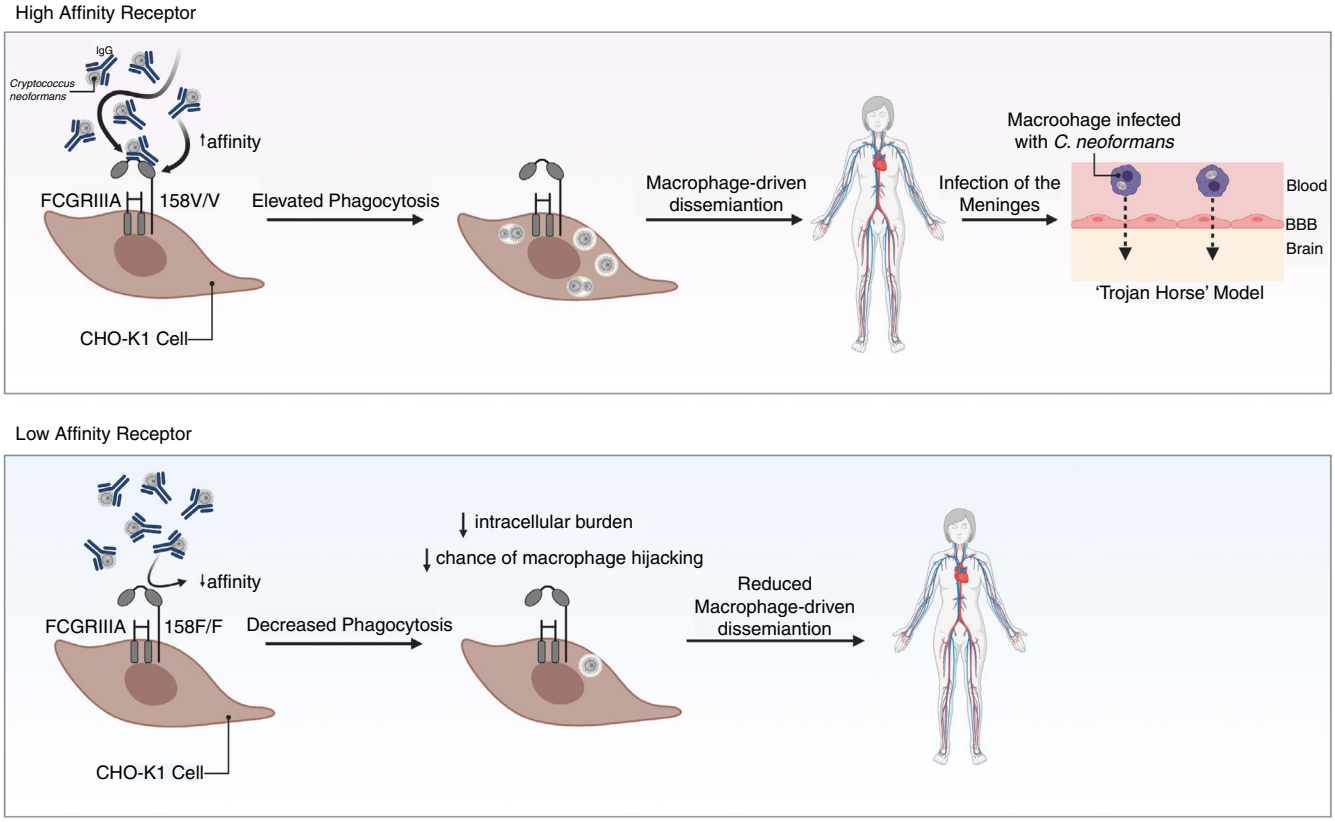

FIGURE 3 Proposed consequence of *FCGR3A (CD16a)* 158F/V polymorphism on host response to infection. Chinese hamster ovary (CHO-K1) cells engineered to express *FCGR3A* 158V allele had a higher affinity to IgG-opsonized *Cryptococcus neoformans* than those expressing the *FCGR3A* 158F allele. The high-affinity FCGR3A 158V receptor may result in elevated phagocytosis by phagocytes. Intracellular *C. neoformans* can then use macrophages as a vehicle to cross the blood–brain barrier (BBB) in what is called the 'Trojan Horse' model and infect the meningitis leading to fatal cryptococcal meningitis (CM). Meanwhile, those expressing the low-affinity *FCGR3A* 158F receptor have decreased phagocytosis, decreased intracellular burden of *C. neoformans* and, therefore, a decreased risk of macrophage-driven dissemination to the brain leading to decreased risk of CM. Figure created with BioRender.com

Moreover, Dectin-$2^{-/-}$ mice had elevated Th2 response compared with WT mice, implying some role for Dectin-2 in anti-cryptococcal immune response [72].

Toll-like receptors are a highly polymorphic protein family, and various studies have implicated TLRs in susceptibility to infections such as malaria [73], tuberculosis [74], herpes simplex virus [75] and even sepsis [76]. In a 2018 study, Jiang et al. [77] carried out the first study to investigate the impact of genetic polymorphisms in TLR genes on *C. neoformans* infection. The study looked at SNPs in the *TLR1*, *TLR2*, *TLR4*, *TLR6* and *TLR9* genes of individuals with non-HIV-associated CM of Chinese ancestry. They identified eight TLR SNPs that were associated with CM susceptibility. Among the eight SNPs, six were associated with an increased risk of CM, five were associated with cerebrospinal fluid (CSF) cytokine concentration and two were associated with disease severity. The only SNP that was associated with CM risk, disease severity and CSF cytokine concentration was the rs3804099 SNP on *TLR2* which causes a synonymous mutation. Analysis of the CSF identified 18 cytokines that were overexpressed in severely ill patients. The C/T genotype of the rs3804099 SNP is associated with lower expression of 12 out of the 18 cytokines shown to be associated with severe disease. This genotype was also rare in individuals with severe disease (OR = 0·39; 95% CI [0·15–1·00]; $p = 0·046$), suggesting that heterozygosity of the rs3804099 SNP decreases risk of severe disease by preventing the over-expression of these cytokines in the CNS. Meanwhile, the T/T genotype of rs3804099 was associated with increased risk of CM (OR = 1·47; 95% CI [1·02–2·11]; $p = 0·036$). Overall, the study concluded that polymorphisms in TLRs have a causal role in *C. neoformans* pathogenesis [77]. The verdict is still out on whether TLRs have an important role in *C. neoformans* infection; however, this study supports the need for further research to clarify the mechanisms by which TLRs are involved in macrophage response to infection.

The genetic studies described so far have mainly included volunteers of Han Chinese or European Ancestry.

Despite sub-Saharan Africa having the highest burden of cryptococcal disease, few genetic studies have focused on people of African descent. To combat this lack of representation, Kannambath et al. [78] performed the first genome wide association study (GWAS) of genetic susceptibility to cryptococcosis in HIV-positive people of African descent. The discovery cohort was composed of 524 cases and controls recruited in Cape Town, South Africa. No SNP was associated with *C. neoformans* at the genome wide significance level of $p < 5 \times 10^{-8}$. This was likely due to the relatively small sample size. However, they identified 49 SNPs associated with cryptococcosis at the significance level of $p < 1 \times 10^{-5}$. Among these, the top six susceptibility SNPs were located within 2·5 kb upstream of the colony-stimulating factor 1 (*CSF1*) gene which codes for macrophage (M)-CSF, a cytokine that promotes monocyte or macrophage differentiation, activation and phagocytosis.

To explore the implications of this finding, the researchers isolated peripheral blood mononuclear cells (PBMCs) from six healthy volunteers, stimulated the cells with heat killed *C. neoformans* for 24 h and performed RNA-seq. They found that 653 genes were up- or downregulated in stimulated cells compared with unstimulated PBMCs and one gene that was highly up regulated after stimulation was *CSF1*. Gene ontology analysis and pathway enrichment analysis revealed the significance of genes, including *CSF1*, involved in cytokine activity, phagocytosis, TLR signalling and macrophage differentiation in anti-cryptococcal immune response in this population. Finally, they isolated PBMCs from five HIV-positive individuals and stimulated the cells with exogenous M-CSF. M-CSF treatment significantly increased the phagocytosis and killing of *C. neoformans* by these PBMCs. Meanwhile, antibody-mediated inhibition of M-CSF receptor resulted in comparable phagocytosis and killing with the control. Other studies have also reported a role for exogenous M-CSF in promoting anti-cryptococcal immune response [79].

Unlike previous studies that targeted specific genes, the study by Kannambath et al. [78] implemented a genome wide, hypothesis-free approach. Although the sample size was small for a GWAS, the researchers were still able to identify SNPs associated with cryptococcosis in people of African descent and took steps towards functional validation. In the future, extending this analysis to PBMCs from genotyped HIV-positive patients with and without cryptococcosis might provide stronger evidence linking disease outcome with some of these differentially expressed genes.

The functional consequence of the *CSF1* SNPs identified by Kannambath et al. remains unclear; however, a search on the GTEx Portal revealed that one of the top 6 identified SNPs, rs2064163, is associated with the expression of TRAF3IP3 and IRF6. There is currently no evidence that these SNPs impact *CSF1* gene expression directly. The authors propose that genotypes that increase macrophage uptake of *C. neoformans* and promote intracellular survival lead to an increased risk of cryptococcosis. This aligns closely with the research mentioned above that showed that infection with high uptake *C. neoformans* resulted in greater CNS fungal burden and elevated expression of Th2 cytokines than low uptake *C. neoformans* [59,60]. The findings that exogenous M-CSF drives anti-cryptococcal immune response suggest that polymorphisms in and around the *CSF1* gene that result in decreased expression of the gene may increase risk of cryptococcal disease.

Continued research into identifying polymorphisms that increase cryptococcosis risk will have clinical applications as well as revealing unexpected genes and pathways involved in the host interaction with *C. neoformans*. Although there are only a limited number of studies investigating the genetic risk factors for *C. neoformans*, building on discoveries made using other pathogens, particularly other fungal pathogens, may reveal genes that could also impact host susceptibility to cryptococcosis. In that context, readers are pointed towards a detailed review of the genetic risk of the fungal pathogens *C. albicans* and *Aspergillus fumigatus* published elsewhere [80].

In addition to the human genotypic factors that impact disease outcome, it is relevant to note that there also exist cryptococcal genetic factors that contribute to pathogenicity and virulence [81]. *Cryptococcus* strains vary in their ability to infect mammalian cells, their ability to disseminate from the lungs to the CNS and the severity of the disease they cause [82–84]. Several studies have investigated the role of fungal factors during infection. One such study compared human survival and other clinical parameters with the whole-genome sequence of 38 *C. neoformans* isolates [85]. They identified 40 *Cryptococcus* genes that were significantly associated with patient survival, cytokine expression and clinical parameters. To examine the biological relevance of these genes, 17 deletion strains were created and used to infect mice. Compared to the control strain, three of the deletion strains led to increased mice survival, while three led to decreased mice survival [85]. A more in-depth review of the association between cryptococcal genotypes and phenotypes and clinical outcome can be found in a recent paper by Montoya et al. [81].

IMPORTANCE OF DIVERSITY IN GENETIC RESEARCH

Despite bearing the largest burden of infectious diseases, individuals of African descent are underrepresented in

research on the genetic basis of disease susceptibility, a problem that is widespread in the field [86]. To date, the majority of the cryptococcosis and CM genetic association studies have involved people of European and Han Chinese ancestry, even though 73% of HIV-associated CM cases and 75% of deaths were in sub-Saharan Africa [4]. More generally, it is estimated that 78% of individuals in GWAS studies are of European ancestry, even though they make up only 16% of the global population [87,88]. Due to the lack of ethnic diversity, drawing broad conclusions from these studies is misleading and may exacerbate health disparities.

It is known that there is diversity in genome architecture between populations, such that a risk allele in one population may have no association with disease or have a protective effect on disease in another population. On the other hand, allele frequencies vary significantly between populations [89]. Such variation in SNP distribution between ethnic groups will likely contribute to differences in susceptibility to infection; thus, results from association studies are not entirely generalizable [90,91].

A GWAS study by Wojcik et al. [91] that sought to demonstrate the importance of having a diverse cohort in genomic studies showed that critical variants may be missed if they exist at a low frequency or are completely absent in Europeans. Consequently, discoveries made using populations that are mostly of European ancestry may lead to a bias in the risk variants that are identified. Additionally, there is significant evidence of effect size heterogeneity across ancestries [91–93], meaning that the disease risk prediction scores derived from effect sizes will only be clinically relevant and personalized if a diverse group of people are used in genomic studies.

Diversity in genomic research is also crucial when genetic risk factors are being applied to therapeutics and drug development. It was recently shown that people of African descent produce a stronger inflammatory response to infection than those of European descent, which also makes Africans more susceptible to autoimmune diseases [94]. This is important during therapeutic administration to avoid the prescription of drugs that excessively induce inflammation. The effect of the drug might be redundant in those of African ancestry and could even worsen any underlying autoimmune and inflammatory conditions. Moreover, acute inflammation may contribute to the onset of new autoimmune or autoinflammatory disease in these patients [95]. Alternatively, a therapy that dampens the immune response could increase the risk of infection due to the generally higher burden of infectious diseases in the region [96]. Evolutionarily, the 'price' of having a robust inflammatory response to clear infection is an increased risk of autoinflammatory disease, demonstrating the way that the cost and benefit of the inflammatory response depends on environmental selection pressures [95]. Understanding this trade-off is critical for genetic-based therapies and requires the involvement of diverse, multi-ethnic populations.

CONCLUSION

The dual observation that not all HIV-positive individuals develop cryptococcosis, while some immunocompetent individuals do, led researchers to speculate about a role for host genetic variation in disease risk. Both hypothesis-driven and hypothesis-free genetic association studies have identified host polymorphisms that increase or decrease risk of cryptococcal disease. Most of the polymorphisms identified were in PRRs, including FcγRs, Dectin-2, MBL and TLRs, although other immune-related genes, such as *CSF1*, have also been associated with cryptococcal disease. The association of these proteins with cryptococcal disease suggests that they play a mechanistic role in host response to infection. Therefore, this review also identifies potential candidate proteins to be investigated further.

Not only do these studies help us identify novel molecules and pathways involved in host-pathogen interaction, but they also lay the foundation for the implementation of genetic data in the clinical setting. Polymorphisms that increase cryptococcosis risk may serve as biomarkers to identify people that would benefit from frequent check-ups and/or earlier treatment. It would make risk stratification possible and contribute to the development of personalized therapeutic approaches for the treatments of cryptococcal disease both in HIV-infected people and in otherwise healthy people. However, to achieve this goal, more genetic association studies involving a diverse group of people are needed to identify novel SNPs. Moreover, significant emphasis needs to be placed on investigating the functional consequences of identified SNPs. The field is young and there is lots of room for fascinating discoveries.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

CUO conceived of the review topic, performed the literature search, drafted the manuscript and revised the manuscript critically. RCM contributed to the refinement of the review topic, reviewed drafts of the manuscript and provided feedback.

ORCID

Chinaemerem U. Onyishi https://orcid.org/0000-0001-8292-9873

Robin C. May https://orcid.org/0000-0001-5364-1838

REFERENCES

1. May RC, Stone NRH, Wiesner DL, Bicanic T, Nielsen K. *Cryptococcus*: from environmental saprophyte to global pathogen. Nat Rev Microbiol. 2016;14(2):106–17.
2. Maziarz EK, Perfect JR. Cryptococcosis. Infect Dis Clin North Am. 2016;30(1):179–206.
3. Srikanta D, Santiago-Tirado FH, Doering TL. *Cryptococcus neoformans*: historical curiosity to modern pathogen. Yeast. 2014;31(2):47–60.
4. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017;17(8):873–81.
5. Pappas PG. Cryptococcal infections in non-HIV-infected patients. Trans Am Clin Climatol Assoc. 2013;124:61–79.
6. Mada P, Nowack B, Cady B, Chandranesan ASJ. Disseminated cryptococcosis in an immunocompetent patient. Case Rep. 2017;2017:bcr2016218461.
7. Poley M, Koubek R, Walsh L, McGillen B. Cryptococcal meningitis in an apparent immunocompetent patient. J Investig Med High Impact Case Rep. 2019;7:1–5.
8. Cunha C, Carvalho A. Host genetics takes a Toll on immunity to *Cryptococcus*. EBioMedicine. 2018;37:9–10.
9. Johnston SA, May RC. *Cryptococcus* interactions with macrophages: evasion and manipulation of the phagosome by a fungal pathogen. Cell Microbiol. 2013;15(3):403–11.
10. Freeman SA, Grinstein S. Phagocytosis: receptors, signal integration, and the cytoskeleton. Immunol Rev. 2014;262(1):193–215.
11. Shoham S, Huang C, Chen J-M, Golenbock DT, Levitz SM. Toll-like receptor 4 mediates intracellular signaling without TNF-α release in response to *Cryptococcus neoformans* polysaccharide capsule. J Immunol. 2001;166(7):4620–6.
12. Nakamura K, Miyazato A, Xiao G, Hatta M, Inden K, Aoyagi T, et al. Deoxynucleic acids from *Cryptococcus neoformans* activate myeloid dendritic cells via a TLR9-dependent pathway. J Immunol. 2008;180(6):4067–74.
13. Brown GD, Gordon S. A new receptor for β-glucans. Nature. 2001;413(6851):36–7.
14. Tada H, Nemoto E, Shimauchi H, Watanabe T, Mikami T, Matsumoto T, et al. *Saccharomyces cerevisiae*- and *Candida albicans*-derived mannan induced production of tumor necrosis factor alpha by human monocytes in a CD14- and toll-like receptor 4-dependent manner. Microbiol Immunol. 2002;46(7):503–12.
15. Vidya MK, Kumar VG, Sejian V, Bagath M, Krishnan G, Bhatta R. Toll-like receptors: significance, ligands, signaling pathways, and functions in mammals. Int Rev Immunol. 2018;37(1):20–36.
16. Gay NJ, Symmons MF, Gangloff M, Bryant CE. Assembly and localization of Toll-like receptor signalling complexes. Nat Rev Immunol. 2014;14(8):546–58.
17. Garelnabi M, May RC. Variability in innate host immune responses to cryptococcosis. Mem Inst Oswaldo Cruz. 2018;113(7):e180060.
18. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5:461.
19. Wager CML, Wormley FL. Classical versus alternative macrophage activation: the Ying and the Yang in host defense against pulmonary fungal infections. Mucosal Immunol. 2014;7(5):1023–35.
20. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol. 2010;11(5):373–84.
21. Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, et al. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. Nature. 1999;401(6755):811–5.
22. Biondo C, Midiri A, Messina L, Tomasello F, Garufi G, Catania M, et al. MyD88 and TLR2, but not TLR4, are required for host defense against *Cryptococcus neoformans*. Eur J Immunol. 2005;35(3):870–8.
23. Nakamura K, Miyagi K, Koguchi Y, Kinjo Y, Uezu K, Kinjo T, et al. Limited contribution of Toll-like receptor 2 and 4 to the host response to a fungal infectious pathogen, *Cryptococcus neoformans*. FEMS Immunol Med Microbiol. 2006;47(1):148–54.
24. Heung LJ. Innate immune responses to *Cryptococcus*. J Fungi. 2017;3(3):35.
25. Gantner BN, Simmons RM, Underhill DM. Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. EMBO J. 2005;24(6):1277–86.
26. Campuzano A, Wormley F. Innate immunity against *Cryptococcus*, from recognition to elimination. J Fungi. 2018;4(1):33.
27. Lim J, Coates CJ, Seoane PI, Garelnabi M, Taylor-Smith LM, Monteith P, et al. Characterizing the mechanisms of non-opsonic uptake of cryptococci by macrophages. J Immunol Author Choice. 2018;200(10):3539–46.
28. Walsh NM, Wuthrich M, Wang H, Klein B, Hull CM. Characterization of C-type lectins reveals an unexpectedly limited interaction between *Cryptococcus neoformans* spores and Dectin-1. PLoS One. 2017;12(3):e0173866.
29. Li K, Underhill DM. C-type lectin receptors in phagocytosis. In: Yamasaki S, editor. Current topics in microbiology and immunology. Berlin, Heidelberg: Springer; 2020. p. 1–18. https://doi.org/10.1007/82_2020_198
30. Kitai Y, Sato K, Tanno D, Yuan X, Umeki A, Kasamatsu J, et al. Role of Dectin-2 in the phagocytosis of *Cryptococcus neoformans* by dendritic cells. Infect Immun. 2021;89(10):e0033021.
31. Saylor CA, Dadachova E, Casadevall A. Murine IgG1 and IgG3 isotype switch variants promote phagocytosis of *Cryptococcus neoformans* through different receptors. J Immunol. 2010;184(1):336–43.
32. Li X, Ptacek TS, Brown EE, Edberg JC. Fcγ receptors: structure, function and role as genetic risk factors in SLE. Genes Immun. 2009;10(5):380–9.
33. Hargreaves CE, Rose-Zerilli MJJ, Machado LR, Iriyama C, Hollox EJ, Cragg MS, et al. Fcγ receptors: genetic variation, function, and disease. Immunol Rev. 2015;268(1):6–24.
34. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. Nat Rev Immunol. 2008;8(1):34–47.
35. Rohatgi S, Pirofski L. Host immunity to *Cryptococcus neoformans*. Future Microbiol. 2015;10(4):565–81.
36. Decken K, Köhler G, Palmer-Lehmann K, Wunderlin A, Mattner F, Magram J, et al. Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. Infect Immun. 1998;66(10):4994–5000.
37. Kawakami K, Koguchi Y, Qureshi MH, Miyazato A, Yara S, Kinjo Y, et al. IL-18 contributes to host resistance against

infection with *Cryptococcus neoformans* in mice with defective IL-12 synthesis through induction of IFN-γ production by NK cells. J Immunol. 2000;165(2):941–7.
38. Müller U, Stenzel W, Köhler G, Werner C, Polte T, Hansen G, et al. IL-13 induces disease-promoting type 2 cytokines, alternatively activated macrophages and allergic inflammation during pulmonary infection of mice with *Cryptococcus neoformans*. J Immunol. 2007;179(8):5367–77.
39. Houpt DC, Pfrommer GS, Young BJ, Larson TA, Kozel TR. Occurrences, immunoglobulin classes, and biological activities of antibodies in normal human serum that are reactive with *Cryptococcus neoformans* glucuronoxylomannan. Infect Immun. 1994;62(7):2857–64.
40. Zhong Z, Pirofski LA. Opsonization of *Cryptococcus neoformans* by human anticryptococcal glucuronoxylomannan antibodies. Infect Immun. 1996;64(9):3446–50.
41. Marquis G, Montplaisir S, Pelletier M, Mousseau S, Auger P. Genetic resistance to murine cryptococcosis: increased susceptibility in the CBA/N XID mutant strain of mice. Infect Immun. 1985;47(1):282–7.
42. Szymczak WA, Davis MJ, Lundy SK, Dufaud C, Olszewski M, Pirofski L. X-linked immunodeficient mice exhibit enhanced susceptibility to *Cryptococcus neoformans* infection. MBio. 2013;4(4):e00265-13.
43. Subramaniam K, Metzger B, Hanau LH, Guh A, Rucker L, Badri S, et al. IgM+ memory B cell expression predicts HIV-associated cryptococcosis status. J Infect Dis. 2009;200(2):244–51.
44. Sørensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med. 1988;318(12):727–32.
45. Fumagalli M, Sironi M, Pozzoli U, Ferrer-Admetlla A, Ferrer-Admettla A, Pattini L, et al. Signatures of environmental genetic adaptation pinpoint pathogens as the main selective pressure through human evolution. PLoS Genet. 2011;7(11):e1002355.
46. Meletiadis J, Walsh TJ, Hwa Choi E, Pappas PG, Ennis D, Douglas J, et al. Study of common functional genetic polymorphisms of FCGR2A, 3A and 3B genes and the risk for cryptococcosis in HIV-uninfected patients. Med Mycol. 2007;45(6):513–8.
47. Warmerdam PA, van de Winkel JG, Vlug A, Westerdaal NA, Capel PJ. A single amino acid in the second Ig-like domain of the human Fc gamma receptor II is critical for human IgG2 binding. J Immunol. 1991;147(4):1338–43.
48. Koene HR, Kleijer M, Algra J, Roos D, von dem Borne AE, de Haas M. Fc gammaRIIIa-158V/F polymorphism influences the binding of IgG by natural killer cell Fc gammaRIIIa, independently of the Fc gammaRIIIa-48L/R/H phenotype. Blood. 1997;90(3):1109–14.
49. Wu J, Edberg JC, Redecha PB, Bansal V, Guyre PM, Coleman K, et al. A novel polymorphism of FcgammaRIIIa (CD16) alters receptor function and predisposes to autoimmune disease. J Clin Invest. 1997;100(5):1059–70.
50. Salmon JE, Millard SS, Brogle NL, Kimberly RP. Fc gamma receptor IIIb enhances Fc gamma receptor IIa function in an oxidant-dependent and allele-sensitive manner. J Clin Invest. 1995;95(6):2877–85.
51. Bredius RG, Fijen CA, De Haas M, Kuijper EJ, Weening RS, Van de Winkel JG, et al. Role of neutrophil Fc gamma RIIa (CD32) and Fc gamma RIIIb (CD16) polymorphic forms in phagocytosis of human IgG1- and IgG3-opsonized bacteria and erythrocytes. Immunology. 1994;83(4):624–30.
52. de Taeye SW, Rispens T, Vidarsson G. The ligands for human IgG and their effector functions. Antibodies. 2019;8(2):30–47.
53. Hu X-P, Wu J-Q, Zhu L-P, Wang X, Xu B, Wang R-Y, et al. Association of Fcγ receptor IIB polymorphism with cryptococcal meningitis in HIV-uninfected Chinese patients. PLoS One. 2012;7(8):e42439.
54. Floto RA, Clatworthy MR, Heilbronn KR, Rosner DR, MacAry PA, Rankin A, et al. Loss of function of a lupus-associated FcγRIIb polymorphism through exclusion from lipid rafts. Nat Med. 2005;11(10):1056–8.
55. Hu W, Zhang Y, Sun X, Zhang T, Xu L, Xie H, et al. FcγRIIB-I232T polymorphic change allosterically suppresses ligand binding. eLife. 2019;8:e46689.
56. Willcocks LC, Smith KGC, Clatworthy MR. Low-affinity Fcγ receptors, autoimmunity and infection. Expert Rev Mol Med. 2009;11:e24.
57. Kyogoku C, Dijstelbloem HM, Tsuchiya N, Hatta Y, Kato H, Yamaguchi A, et al. Fcgamma receptor gene polymorphisms in Japanese patients with systemic lupus erythematosus: contribution of FCGR2B to genetic susceptibility. Arthritis Rheum. 2002;46(5):1242–54.
58. Rohatgi S, Gohil S, Kuniholm MH, Schultz H, Dufaud C, Armour KL, et al. Fc gamma receptor 3A polymorphism and risk for HIV-associated cryptococcal disease. MBio. 2013;4(5):e00573-13.
59. Hansakon A, Mutthakalin P, Ngamskulrungroj P, Chayakulkeeree M, Angkasekwinai P. *Cryptococcus neoformans* and *Cryptococcus gattii* clinical isolates from Thailand display diverse phenotypic interactions with macrophages. Virulence. 2018;10(1):26–36.
60. Sabiiti W, Robertson E, Beale MA, Johnston SA, Brouwer AE, Loyse A, et al. Efficient phagocytosis and laccase activity affect the outcome of HIV-associated cryptococcosis. J Clin Invest. 2014;124(5):2000–8.
61. Ou X-T, Wu J-Q, Zhu L-P, Guan M, Xu B, Hu X-P, et al. Genotypes coding for mannose-binding lectin deficiency correlated with cryptococcal meningitis in HIV-uninfected Chinese patients. J Infect Dis. 2011;203(11):1686–91.
62. Bouwman LH, Roep BO, Roos A. Mannose-binding lectin: clinical implications for infection, transplantation, and autoimmunity. Hum Immunol. 2006;67(4–5):247–56.
63. Tan Y, Liu L, Luo P, Wang A, Jia T, Shen X, et al. Association between mannose-binding lectin and HIV infection and progression in a Chinese population. Mol Immunol. 2009;47(2–3):632–8.
64. Babula O, Lazdane G, Kroica J, Ledger WJ, Witkin SS. Relation between recurrent vulvovaginal candidiasis, vaginal concentrations of mannose-binding lectin, and a mannose-binding lectin gene polymorphism in Latvian women. Clin Infect Dis. 2003;37(5):733–7.
65. Held K, Thiel S, Loos M, Petry F. Increased susceptibility of complement factor B/C2 double knockout mice and mannan-binding lectin knockout mice to systemic infection with *Candida albicans*. Mol Immunol. 2008;45(15):3934–41.
66. Crosdale DJ, Poulton KV, Ollier WE, Thomson W, Denning DW. Mannose-binding lectin gene polymorphisms as a susceptibility factor for chronic necrotizing pulmonary aspergillosis. J Infect Dis. 2001;184(5):653–6.
67. Wagemakers A, Ang CW, Hagen F, Bot JCJ, Bomers MK, Visser MC, et al. Case report: chronic relapsing cryptococcal meningitis in a patient with low mannose-binding lectin and a low naïve CD4 cell count. BMC Infect Dis. 2019;19(1):846.

68. Sainz J, Lupiáñez CB, Segura-Catena J, Vazquez L, Ríos R, Oyonarte S, et al. Dectin-1 and DC-SIGN polymorphisms associated with invasive pulmonary aspergillosis infection. PLoS One. 2012;7(2):e32273.
69. Chai LYA, de Boer MGJ, van der Velden WJFM, Plantinga TS, van Spriel AB, Jacobs C, et al. The Y238X stop codon polymorphism in the human β-glucan receptor dectin-1 and susceptibility to invasive aspergillosis. J Infect Dis. 2011;203(5):736–43.
70. Plantinga TS, Hamza OJM, Willment JA, Ferwerda B, van de Geer NMD, Verweij PE, et al. Genetic variation of innate immune genes in HIV-infected African patients with or without oropharyngeal candidiasis. J Acquir Immune Defic Syndr. 2010;55(1):87–94.
71. Hu X-P, Wang R-Y, Wang X, Cao Y-H, Chen Y-Q, Zhao H-Z, et al. Dectin-2 polymorphism associated with pulmonary cryptococcosis in HIV-uninfected Chinese patients. Med Mycol. 2015;53(8):810–6.
72. Nakamura Y, Sato KO, Yamamoto H, Matsumura K, Matsumoto I, Nomura T, et al. Dectin-2 deficiency promotes Th2 response and mucin production in the lungs after pulmonary infection with *Cryptococcus neoformans*. Infect Immun. 2015;83(2):671–81.
73. Mockenhaupt FP, Cramer JP, Hamann L, Stegemann MS, Eckert J, Oh N-R, et al. Toll-like receptor (TLR) polymorphisms in African children: common TLR-4 variants predispose to severe malaria. Proc Natl Acad Sci USA. 2006;103(1):177–82.
74. Ben-Ali M, Barbouche M-R, Bousnina S, Chabbou A, Dellagi K. Toll-like receptor 2 Arg677Trp polymorphism is associated with susceptibility to tuberculosis in Tunisian patients. Clin Diagn Lab Immunol. 2004;11(3):625–6.
75. Zhang S-Y, Jouanguy E, Ugolini S, Smahi A, Elain G, Romero P, et al. TLR3 deficiency in patients with herpes simplex encephalitis. Science. 2007;317(5844):1522–7.
76. Wurfel MM, Gordon AC, Holden TD, Radella F, Strout J, Kajikawa O, et al. Toll-like receptor 1 polymorphisms affect innate immune responses and outcomes in sepsis. Am J Respir Crit Care Med. 2008;178(7):710–20.
77. Jiang Y-K, Wu J-Q, Zhao H-Z, Wang X, Wang R-Y, Zhou L-H, et al. Genetic influence of Toll-like receptors on non-HIV cryptococcal meningitis: an observational cohort study. EBioMedicine. 2018;37:401–9.
78. Kannambath S, Jarvis JN, Wake RM, Longley N, Loyse A, Matzaraki V, et al. Genome-wide association study identifies novel colony stimulating factor 1 locus conferring susceptibility to cryptococcosis in human immunodeficiency virus-infected South Africans. Open Forum Infect Dis. 2020;7(11):ofaa489.
79. Nassar F, Brummer E, Stevens DA. Effect of in vivo macrophage colony-stimulating factor on fungistasis of bronchoalveolar and peritoneal macrophages against *Cryptococcus neoformans*. Antimicrob Agents Chemother. 1994;38(9):2162–4.
80. Campos CF, van de Veerdonk FL, Gonçalves SM, Cunha C, Netea MG, Carvalho A. Host genetic signatures of susceptibility to fungal disease. In: Rodrigues ML, editor. Fungal physiology and immunopathogenesis (Current topics in microbiology and immunology; vol. 422). Cham: Springer International Publishing; 2018. p. 237–63.
81. Montoya MC, Magwene PM, Perfect JR. Associations between *Cryptococcus* genotypes, phenotypes, and clinical parameters of human disease: a review. J Fungi. 2021;7(4):260.
82. Kwon-Chung KJ, Boekhout T, Wickes BL, Fell JW. Systematics of the genus *Cryptococcus* and its type species *C. neoformans*. In: Heitman J, Kozel TR, Kwon-Chung KJ, Perfect JR, Casadevall A, editors. *Cryptococcus* [Internet]. Hoboken, NJ: John Wiley & Sons, Ltd; 2010. p. 1–17 [cited 2021 Aug 20]. Available from: https://onlinelibrary.wiley.com/doi/abs/ https://doi.org/10.1128/9781555816858.ch1
83. Esher SK, Zaragoza O, Alspaugh JA. Cryptococcal pathogenic mechanisms: a dangerous trip from the environment to the brain. Mem Inst Oswaldo Cruz. 2018;113:e180057.
84. Litvintseva AP, Mitchell TG. Most environmental isolates of *Cryptococcus neoformans* var. *grubii* (serotype A) are not lethal for mice. Infect Immun. 2009;77(8):3188–95.
85. Gerstein AC, Jackson KM, McDonald TR, Wang Y, Lueck BD, Bohjanen S, et al. Identification of pathogen genomic differences that impact human immune response and disease during *Cryptococcus neoformans* infection. MBio. 2019;10(4):e01440-19.
86. Africa's people must be able to write their own genomics agenda. Nature. 2020;586(7831):644.
87. Sirugo G, Williams SM, Tishkoff SA. The missing diversity in human genetic studies. Cell. 2019;177(1):26–31.
88. Genetics for all. Nat Genet. 2019;51(4):579.
89. Choudhury A, Aron S, Botigué LR, Sengupta D, Botha G, Bensellak T, et al. High-depth African genomes inform human migration and health. Nature. 2020;586(7831):741–8.
90. Ioana M, Ferwerda B, Plantinga TS, Stappers M, Oosting M, McCall M, et al. Different patterns of Toll-like receptor 2 polymorphisms in populations of various ethnic and geographic origins. Infect Immun. 2012;80(5):1917–22.
91. Wojcik GL, Graff M, Nishimura KK, Tao R, Haessler J, Gignoux CR, et al. Genetic analyses of diverse populations improves discovery for complex traits. Nature. 2019;570(7762):514–8.
92. Martin AR, Gignoux CR, Walters RK, Wojcik GL, Neale BM, Gravel S, et al. Human demographic history impacts genetic risk prediction across diverse populations. Am J Hum Genet. 2017;100(4):635–49.
93. Carlson CS, Matise TC, North KE, Haiman CA, Fesinmeyer MD, Buyske S, et al. Generalization and dilution of association results from European GWAS in populations of non-European ancestry: the PAGE study. PLoS Biol. 2013;11(9):e1001661.
94. Nédélec Y, Sanz J, Baharian G, Szpiech ZA, Pacis A, Dumaine A, et al. Genetic ancestry and natural selection drive population differences in immune responses to pathogens. Cell. 2016;167(3):657–69.
95. Okin D, Medzhitov R. Evolution of inflammatory diseases. Curr Biol. 2012;22(17):733–40.
96. Bhutta ZA, Sommerfeld J, Lassi ZS, Salam RA, Das JK. Global burden, distribution, and interventions for infectious diseases of poverty. Infect Dis Poverty. 2014;3(1). https://doi.org/10.1186/2049-9957-3-21

How to cite this article: Onyishi CU, May RC. Human immune polymorphisms associated with the risk of cryptococcal disease. Immunology. 2022;165:143–157. https://doi.org/10.1111/imm.13425