

Review Article

Ficolins and the Recognition of Pathogenic Microorganisms: An Overview of the Innate Immune Response and Contribution of Single Nucleotide Polymorphisms

Stefan Bidula ¹, Darren W. Sexton ², and Silke Schelenz³

¹School of Pharmacy, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ, UK

²School of Pharmacy and Biomolecular Science, Liverpool John Moores University, Byrom Street, Liverpool, L3 3AF, UK

³Department of Microbiology, Royal Brompton Hospital, Sydney Street, London SW3 6NP, UK

Correspondence should be addressed to Stefan Bidula; s.bidula@uea.ac.uk

Received 31 July 2018; Revised 29 November 2018; Accepted 24 December 2018; Published 5 February 2019

Academic Editor: Eirini Rigopoulou

Copyright © 2019 Stefan Bidula et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Ficolins are innate pattern recognition receptors (PRR) and play integral roles within the innate immune response to numerous pathogens throughout the circulation, as well as within organs. Pathogens are primarily removed by direct opsonisation following the recognition of cell surface carbohydrates and other immunostimulatory molecules or via the activation of the lectin complement pathway, which results in the deposition of C3b and the recruitment of phagocytes. In recent years, there have been a number of studies implicating ficolins in the recognition and removal of numerous bacterial, viral, fungal, and parasitic pathogens. Moreover, there has been expanding evidence highlighting that mutations within these key immune proteins, or the possession of particular haplotypes, enhance susceptibility to colonization by pathogens and dysfunctional immune responses. This review will therefore encompass previous knowledge on the role of ficolins in the recognition of bacterial and viral pathogens, while acknowledging the recent advances in the immune response to fungal and parasitic infections. Additionally, we will explore the various genetic susceptibility factors that predispose individuals to infection.

1. Introduction

Ficolins are innate pattern recognition receptors (PRRs) similar to the collectin, the mannose-binding lectin (MBL), and the surfactant proteins (SP). Like the collectins, ficolins consist of an N-terminal rich in cysteine residues, a collagen-like domain composed of glycine-X-Y repeats, and a neck region. However, in the ficolins, the carbohydrate-recognition domain (CRD) of the collectins is replaced by a C-terminal fibrinogen-like domain (FBG; Figure 1(a)). In their native form, ficolin monomers assemble to form trimers via their collagen-like domains, before further assembling into oligomeric bouquet-like structures of between 4 and 6 trimers. In humans, there are three ficolins termed M-, L-, and H-ficolin (also referred to as ficolin-1, ficolin-2, and ficolin-3) whereas rodents only possess two, termed ficolin-A and -B, which are the orthologues of human L- and M-ficolin,

respectively. The H-ficolin gene is present in rodents as a pseudogene [1].

Ficolins function within innate immunity via the recognition of pathogen-associated molecular patterns (PAMPs) on microbial pathogens. Binding to acetylated polysaccharides on microbial pathogens, in particular *N*-acetylglucosamine (GlcNAc) and *N*-acetylgalactosamine (GalNAc), is a common characteristic shared amongst the ficolins. However, ficolins have also been observed to recognise specific microbial patterns such as sialic acid, lipopolysaccharides, bacterial peptidoglycan, and fungal 1,3- β -D-glucan [2–6]. Following the recognition of cell surface structures during infection, ficolins can function as opsonins, potentiating the functions of leukocytes and the lung epithelium [7–9].

To date, all of the human and rodent ficolins have been observed to activate the lectin-complement pathway (Figures 1(b) and 1(c)) following the association with

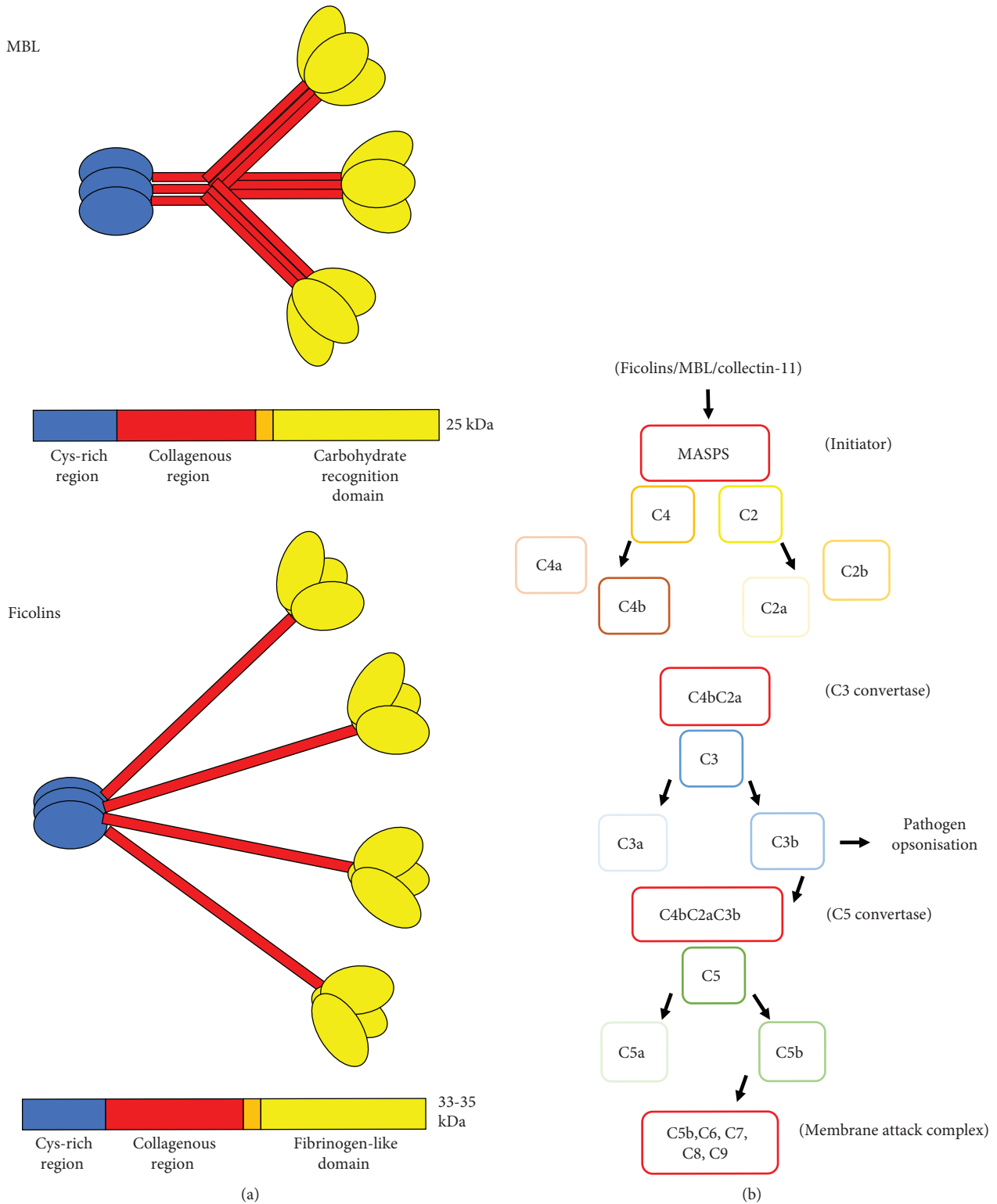


FIGURE 1: Ficolin structure and the lectin complement pathway. (a) MBL is composed of a cysteine-rich region, a MASP-interacting collagenous region, and a pathogen-binding carbohydrate recognition domain. Ficolins have structural similarity to MBL, albeit the carbohydrate recognition domain is replaced by a fibrinogen-like domain. (b) There are three main pathways of complement activation: the classical, lectin, and alternative pathways. Ficolins interact with MASPs to cleave C4 and C2 to form the C3 convertase C4bC2a. This results in the cleavage of C3 into C3a and C3b. C3b then functions as an opsonin or enters the alternative pathway forming an amplification loop. Each pathway can also result in the formation of the membrane attack complex following the cleavage of C5 by the C5 convertases C4bC2aC3b or C3BbC3b and subsequent association with C6, C7, C8, and C9.

MBL-associated serine proteases (MASPs), a characteristic shared with MBL and the recently discovered collectin-11 (CL-11) [10–17]. Ficolins associate with three serine proteases, MASP-1, MASP-2, and MASP-3, in addition to two nonenzymatic fragments MAp19 and MAp44 [18–20]. Ficolin-MASP complexes can then cleave C4 and C2 to form the C3 convertase C4bC2a [11, 21]. Following the deposition of C3b on C4bC2a, C2a gains C5 convertase activity leading to the production of C5a and C5b. C3b itself acts as a marker to facilitate phagocytosis, and C5b initiates the formation of the membrane attack complex (MAC) in collaboration with C6, C7, C8, and C9, which directly lyses pathogens [22].

In this review, we describe updates on the opsonic activity of human and rodent ficolins and explore their role within innate immune responses against pathogens. Moreover, we briefly discuss the effects of single nucleotide polymorphisms on pathogen susceptibility.

2. The Ligand-Binding Properties of Ficolin Fibrinogen-like (FBG) Domains

The FBG of ficolins is composed of a number of different binding sites that can work synergistically or alone in a complex interaction that allow ficolins to distinguish nonself structures from self. This allows ficolins to play an integral role in the opsonisation of various pathogens whereby they can recognise a vast number of ligands on the microbial cell-surface.

In recent years, it has been discovered that the binding range of M-ficolin is a lot broader than was first anticipated, and the tethering of M-ficolin to the leukocyte surface is due to the recognition of sialic acid by the FBG, in particular 9-*O*-acetylated sialic acid [2, 23–25]. Moreover, like other ficolins, M-ficolin can bind to a vast range of acetylated moieties including *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), *N*-acetylglucosamine (LacNAc), *N*-acetylcysteine (CysNAc), and acetylated human serum albumin [10, 23, 26]. A glycan array further advanced our knowledge on the recognition properties of M-ficolin and highlighted two novel ligands in the form of gangliosides and sialylated biantennary N-linked type glucans [25]. M-ficolin has also recently been implicated in neutrophil responses, including cell polarization, adhesion, aggregation, and complement activation, following interaction with the membrane sialoprotein CD43 [27]. The M-ficolin recognition domain has been studied in great detail. The structure is similar to the other ficolins; however, within a predicted ligand-binding site, the peptide bond between Asp282 and Cys283 is in a normal *trans* conformation, compared to the *cis* conformation exhibited by the other ficolins [28]. The difference between active and nonactive function was suggested to be due to a *cis-trans* isomerization of the Asp282 and Cys283 peptide bond [29], with an acidic environment gearing M-ficolin towards the nonfunctional *trans* conformation. Using various histidine mutants, the protonation of His284 was found to be associated with the *trans* to *cis* change to a functional conformation and the ability to regulate GlcNAc binding [30, 31].

L-ficolin is the best characterised of all of the ficolins and binds to a wide range of antigens, thus allowing L-ficolin to recognise an array of microorganisms. L-ficolin shares a common binding specificity to GlcNAc and GalNAc but also binds to a wider range of structures such as lipoteichoic acid (LTA), 1,3- β -D-glucan, N-glycans, hemagglutinin (HA), and neuraminidase [5, 6, 32–34]. Due to the large recognition spectrum of L-ficolin, the incorporation of sites other than the S1 binding site, termed S2-S4, is of great importance. The S1 site, important in the recognition of GlcNAc for the other ficolins, contains a phenylalanine residue in place of a GlcNAc stabilising tyrosine in L-ficolin and is less involved [35]. Alternatively, GlcNAc, CysNAc, and neutral galactose were found in the S2 site; various *N*-acetylated structures in the S3 site and a cooperation of the S3 and S4 sites were involved in the recognition of 1,3- β -D-glucan, altogether producing a unique recognition surface for the recognition of pathogens [35].

H-ficolin shares common binding specificities with the other ficolins, namely, the recognition of the acetylated polysaccharides GlcNAc and GalNAc, but, additionally D-fucose and galactose [3, 35]. Structurally, H-ficolin shares characteristics with L-ficolin such as the common *cis* conformation of the Asp282 and Cys283 peptide bond [35, 36]. Garlatti et al. [35] characterised the binding of H-ficolin and elucidated the S1 site which was involved in binding to both D-fucose and galactose. As in the other ficolins, this site lies within the vicinity of the Ca²⁺-binding site and is homologous to the GlcNAc-binding site in tachylectin 5A, involving Cys235, Tyr236, Tyr254, and Val264 residues [35]. Zacho et al. [37] further characterised the binding profile of H-ficolin reporting binding to acetylsalicylic acid, *N*-acetyl glycine (GlyNAc), and CysNAc and reporting the Ca²⁺ dependence of H-ficolin binding. However, the sites involved in this recognition need to be investigated further.

3. The Role of M-Ficolin in Immunity to Pathogenic Microorganisms

3.1. Bacteria. As a consequence of its recognition spectrum (Table 1), it has been suggested that M-ficolin could be involved in immunity against bacterial infection. Dose-dependent binding of serum M-ficolin has been observed to capsular *Streptococcus agalactiae* serotype VI, which presents sialic acid as the terminal side-chain residues of the capsular polysaccharides, but does not bind to the noncapsulated strain B848/64 [38]. This concentration-dependent binding was inhibited following the addition of GlcNAc or treatment of bacteria with sialidase. Recombinant M-ficolin also exhibited the same binding preferences and activated complement only on serotype VI streptococci [38]. The same group reported that M-ficolin was unable to bind to either the capsulated or noncapsulated strains of *Staphylococcus aureus*, contradicting a previous observation by Liu et al. [26]. To further characterize M-ficolin binding, more than 100 different strains of *Streptococcus pneumoniae* and *Streptococcus mitis* were screened for M-ficolin binding. M-ficolin was only observed to bind to three strains: the pneumococcal serotypes 19B and 19C and a single *S. mitis*

TABLE 1: Expression, sugar specificity, and target pathogens of human and rodent ficolins.

	Tissues of origin	Gene localization	Sugars	PAMPs	Endogenous/artificial ligands	Pathogen interactions
<i>Human</i>						
M-ficolin	Cell surface, serum	9q34	GlcNAc, GalNAc, LacNAc, SiaLacNAc, CysNAc, sialic acid, gangliosides	Ebola virus glycoprotein, chitin, β -1, 3-glucans	Acetylated human albumin CD43	<i>S. agalactiae</i> , <i>S. aureus</i> , <i>S. pneumoniae</i> , <i>S. mitis</i> , <i>E. coli</i> , IAV, <i>T. cruzi</i> , <i>Zaire Ebola virus</i> , <i>A. fumigatus</i>
L-ficolin	Serum	9q34	GlcNAc, GalNAc, ManNAc, CysNAc, GlyNAc, NeuNAc, acetylcholine, elastin	β -1, 3-glucans, N-glycans, HA, neuraminidase, teichoic acid, LPS	Acetylcholine, elastin, corticosteroids	<i>S. aureus</i> , <i>S. pyogenes</i> , <i>S. agalactiae</i> , <i>B. subtilis</i> , <i>S. typhimurium</i> , <i>E. coli</i> , <i>S. pneumoniae</i> , <i>L. monocytogenes</i> , <i>M. bovis</i> BCG, <i>M. tuberculosis</i> , <i>M. smegmatis</i> , <i>E. faecalis</i> , <i>A. fumigatus</i> , HCV, IAV, <i>T. cruzi</i> , <i>G. intestinalis</i> , <i>Leptospira biflexa</i> , <i>Pasteurella pneumotropica</i>
H-ficolin	Serum, bronchus, alveolus, bile	1p36.11	GlcNAc, GalNAc, fucose, glucose, acetylsalicylic acid, sialic acid, D-mannose, GlyNAc, CysNAc	LPS, PSA, Ag85	—	<i>S. typhimurium</i> , <i>S. minnesota</i> , <i>E. coli</i> O111, <i>Hafnia alvei</i> , <i>A. fumigatus</i> , IAV, <i>T. cruzi</i> , <i>G. intestinalis</i> , <i>P. pneumotropica</i> , <i>M. bovis</i> BCG, <i>M. kansasii</i>
<i>Rodent</i>						
Ficolin-A	Serum	2A3	GlcNAc, GalNAc	LPS	Fibrinogen	<i>S. pneumoniae</i> , <i>S. aureus</i> , <i>E. coli</i> O157:H7, <i>P. aeruginosa</i> , <i>C. neoformans</i> , <i>A. fumigatus</i> , <i>A. flavus</i> , <i>A. terreus</i> , <i>A. niger</i>
Ficolin-B	Peritoneal MØ	2A3	GlcNAc, GalNAc, LacNAc, SiaLacNAc, LDL, NeuNAc	—	LDL, fetuin	Nk

BM, bone marrow; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; LacNAc, *N*-acetylactosamine; SiaLacNAc, sialylated *N*-acetylactosamine; CysNAc, *N*-acetylcysteine; ManNAc, *N*-acetylmannosamine; GlyNAc, *N*-acetylglycine; NeuNAc, *N*-acetylneuraminic acid; HA, hemagglutinin; LPS, lipopolysaccharide; IAV, influenza A virus; LDL, low-density lipoprotein; LTA, lipoteichoic acid; HCV, hepatitis C virus; Nk, not known.

strain [39]. This binding exhibited the common characteristic of GlcNAc inhibition and in conjunction with MASP-2, mediated the cleavage of C4. Kjaer et al. [39] postulated that binding to these pneumococcal strains was via an *N*-acetylmannosamine residue linked by glycoside linkage only present in serotypes 19B and 19C. In addition, M-ficolin can function as an opsonin and enhance the action of phagocytes, as phagocytosis of *Escherichia coli* by U937 cells was observed to be inhibited by antibodies against M-ficolin [23].

Bartłomiejczyk and colleagues also demonstrated M-ficolin binding to *Mycobacterium bovis* and *M. tuberculosis*, although this binding had little effect on biological responses [40].

3.2. Viruses. Verma et al. [41] identified that M-ficolin could recognise and inhibit the infectivity of the Phil82 and PR-8 strains of the influenza A virus (IAV) to levels comparable to L-ficolin. H-ficolin in this study was also capable of inhibiting the infectivity of the pandemic Cal09 H1N1 strain. Furthermore, M-ficolin was also observed to interact with acute phase proteins which could potentiate the immune response against pathogens. Using surface plasmon

resonance spectroscopy and electron microscopy, the interaction of M-ficolin with the long pentraxin, pentraxin 3 (PTX3), was investigated. M-ficolin was shown to bind PTX3 in a Ca^{2+} -dependent manner in an interaction inhibited by GlcNAc. The M-ficolin-PTX3 interaction was attributed to sialic acid, and the activation of the lectin-complement pathway was observed [42]. Functionally, the M-ficolin-PTX3 interaction has been observed to decrease the infectivity of the PR-8 and Phil82 strains of IAV [41]. Conversely, interactions of M-ficolin with the mucin-like domain of the Zaire Ebola virus glycoprotein results in enhanced infectivity of host cells [43].

3.3. Fungi. Until recently, there had been no reports of M-ficolin binding to *A. fumigatus*; however, Jensen et al. [44] identified that M-ficolin can interact with chitin and β -1,3-glucans, contributing to complement activation and potentiation of IL-8 from a lung epithelial cell line. The ability of M-ficolin to recognise such key components of the fungal cell wall, as well as its production by peripheral blood leukocytes and type II alveolar cells, is suggestive that its importance in antifungal immunity will be further unveiled in time.

3.4. *Parasites*. There has been a paucity of information regarding the role of ficolins in immunity to parasites, but due to its recognition spectrum, it would seem likely that M-ficolin would interact with parasites and is an area that needs to be explored.

4. The Role of L-Ficolin in Immunity to Pathogenic Microorganisms

4.1. *Bacteria*. L-ficolin is undoubtedly the most widely investigated ficolin, and studies have identified important roles within infection and immunity. L-ficolin was first observed to enhance the opsonophagocytosis of *Salmonella typhimurium* leading to complement activation [7]. It should be noted that in this study, L-ficolin was demonstrated to bind to the *S. typhimurium* Ra strain lacking LPS O-specific polysaccharide but not with the LPS smooth-type strain. L-ficolin recognises LTA expressed by a range of staphylococcal and streptococcal strains, including *S. aureus* serotypes 1, 8, 9, 11, and 12 and *S. pneumoniae* serotypes 11A, 11D, 11F, 20, 35A, and 35C, subsequently leading to activation of the lectin pathway on some serotypes [5, 39]. This binding could be due to the recognition of the pneumococcal surface virulence factors of the choline-binding protein (Cbp) family by the FBG domain [45]. Recent evidence has been provided which demonstrates that L-ficolin recognises O-acetylated epitopes on pneumococcal serotype 11A and contributes to reduced invasiveness [46]. Further delineation of the binding specificity led to the observation that L-ficolin could bind to the PCho residue in teichoic acid [47]. An important study by Ali et al. additionally provided evidence that L-ficolin can activate the lectin pathway of complement via binding pneumolysin, a major toxin of *S. pneumoniae* [48].

Group B streptococci (GBS), in particular capsular polysaccharide (CPS) from serotypes Ib, III, V, VI, and VIII, is also avidly recognised by L-ficolin, leading to a significant increase in opsonophagocytosis and C3b deposition via the lectin pathway working synergistically with the alternative complement pathway [49, 50]. The binding of L-ficolin to GBS is suggested to be irrespective of the amount of LTA or group B-specific polysaccharide (GBPS) content but shows a directly proportional decrease in binding following the removal of N-acetylneuraminic acid (NeuNAc) [51]. L-ficolin and serotype-specific IgG from cord serum have been observed to increase opsonophagocytic killing of serotype III and V [50]. However, recognition of GBS by L-ficolin is a result currently under debate as another group reported no binding to any serotypes investigated [38]. L-ficolin also binds to *Enterococcus faecalis*, *Leptospira biflexa*, *Listeria monocytogenes*, *Pasteurella pneumotropica*, and enteropathogenic or enteroaggregative *E. coli*; however, the functional consequences of these interactions are not fully elucidated [52, 53]. Recent evidence is indicative that L-ficolin may play an important role in immunity against enteroaggregative *E. coli* via activation of the lectin-complement pathway [54]. Moreover, the interaction of L-ficolin with C-reactive protein (CRP) significantly enhanced the complement deposition on *P. aeruginosa* [55].

L-ficolin has arisen as an important defence molecule within the liver in particular, whereby lower L-ficolin levels are correlated with an increased incidence of bacterial infections and disease severity during sepsis [56–58].

Additionally, L-ficolin has been observed to bind to mycobacteria. L-ficolin recognises *Mycobacterium bovis* BCG, leading to complement activation and significant C3b deposition [59]. In this case, C3b deposition could be involved in the virulence of *M. bovis* BCG to allow entry into macrophages where they reside. Moreover, Luo and colleagues identified that L-ficolin could bind more effectively to the virulent *M. tuberculosis* strain H37Rv, comparatively to nonvirulent *M. bovis* BCG and *M. smegmatis* [60]. Notably, an insufficiency of L-ficolin in humans was attributed to enhanced susceptibility to infection with TB [60].

4.2. *Viruses*. A role for L-ficolin in viral defence is now also starting to emerge. Liu et al. [33] found that L-ficolin in patients with hepatitis C virus (HCV) was elevated and was able to bind to N-glycans of the envelope glycoproteins E1 and E2. This interaction further led to the activation of the lectin pathway. Recent evidence has been provided which displays the ability of L-ficolin to directly inhibit HCV entry into cells and demonstrated that apolipoprotein E3 (ApoE3) blocks this effect, mediating immune escape [61, 62]. Recent evidence has indicated that L-ficolin concentrations are elevated in the serum of chronic hepatitis B patients also [63]. Additionally, L-ficolin has been observed to bind to HA and neuraminidase via their FBG and has been shown to have an inhibitory effect on the invasion of kidney cells by IAV *in vitro* [34]. Using mice deficient of the mouse orthologue of L-ficolin, ficolin-A, it was also observed that these mice demonstrated a greatly decreased survival rate in comparison to WT. However, reconstitution of L-ficolin into a ficolin-A knockout mouse could significantly reduce mortality. Chimeric lectins whereby part of the L-ficolin collagen-like domain was added in place of MBLs have proven beneficial in defence against both the IAV and the Ebola virus [64, 65].

4.3. *Fungi*. L-ficolin has been observed to bind to the pathogenic fungus, *Aspergillus fumigatus*, leading to lectin-complement pathway activation [66]. Complement activation can be further potentiated by an L-ficolin-PTX3 complex. This group also highlighted that the classical and lectin-complement pathways can complement each other, with the classical pathway the preferred method of initiation but the lectin pathway capable of initiating complement in the absence of anti-*Aspergillus* antibodies [67]. Additionally, we showed that the recognition of *A. fumigatus* by the L-ficolin FBG also enhances the association of *A. fumigatus* to the A549 type II epithelial cell line, human primary neutrophils, and monocyte-derived macrophages (MDM) [9, 68]. This interaction was observed to enhance fungal killing and modulate the inflammatory cytokine response, leading to increased production of IL-8 by epithelial cells, while conversely decreasing the production of IL-1 β , IL-6, IL-8, and TNF- α from neutrophils and MDM *in vitro*. We have since observed the presence of L-ficolin in the bronchoalveolar lavage fluid

of lung transplant patients with fungal lung infections, hinting at an important role in antifungal defence [68]. Recently, Genster et al. have highlighted that the absence of both the rodent orthologues ficolin-A and ficolin-B sensitised mice to *A. fumigatus* infections [69]. However, the absence of either one alone was not sufficient to enhance susceptibility. Furthermore, very little is understood about the recognition of other pathogenic fungi by L-ficolin, and this could be an important area to investigate, although it has been reported that there is no association observed between levels of any ficolin and intra-abdominal *Candida albicans* infection [70].

4.4. Parasites. Parasitic binding is also a characteristic of L-ficolin. L-ficolin has been observed to bind to glycosylated proteins on the cell surface of *T. cruzi* and recognise *Giardia intestinalis*, leading to complement activation [71, 72]. Recent data has shown that *T. cruzi* is also able to manipulate L-ficolin as a virulence factor. *T. cruzi* calreticulin can bind L-ficolin directly and in doing so is observed to inhibit lectin pathway activation in a dose-dependent manner [73].

5. The Role of H-Ficolin in Immunity to Pathogenic Microorganisms

5.1. Bacteria. Early work from Sugimoto et al. [3] characterised the ability of H-ficolin to induce agglutination of human erythrocytes by recognition of lipopolysaccharides (LPS) from *S. typhimurium*, *Salmonella minnesota*, and *E. coli* O111 coated on their surface. Binding to bacteria has proven to be restricted to only a few species, the most characterised of these is binding to PSA, a polysaccharide of *Aerococcus viridans*, which is now often used as a control for H-ficolin binding and complement activation [12, 74, 75]. However, even H-ficolin recognition of *A. viridans* and *E. coli* is strain specific as binding to the strains *A. viridans* Ring 44 and *E. coli* 74285 was not observed [75]. Recent work by Swierzko et al. [4] increased our current knowledge of H-ficolin-bacteria interactions. They showed that LPS from only four strains of *Hafnia alvei* was recognised by H-ficolin, in particular via their O-specific polysaccharides, leading to C4b deposition in a calcium and magnesium-dependent manner. The interaction between H-ficolin and *H. alvei* has since been investigated further, whereby H-ficolin can augment phagocytosis and promote bacterial killing [76]. In stark contrast, H-ficolin has not been observed to recognise any *S. pneumoniae*, *S. agalacticae*, *S. mitis*, or *S. aureus* strains [38, 39, 75]. In addition, H-ficolin does not bind to other bacteria such as *L. monocytogenes*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [52]. Recently, H-ficolin binding has been observed to *Pasteurella pneumotropica* and enteropathogenic and enteroaggregative *E. coli* [53]. Bartłomiejczyk and colleagues demonstrated that H-ficolin could bind to *M. tuberculosis*, *M. bovis*, and *M. kansasii*, with binding to the former resulting in bacterial agglutination and enhanced phagocytosis [40]. The mycobacterial antigen Ag85 has arisen as a novel antigen for the ficolins, in particular H-ficolin, where it might influence the interaction of *Mycobacterium* with the extracellular matrix [77].

5.2. Viruses. Observations regarding the role of H-ficolin in the defence against viruses are encouraging. Recent studies have exhibited the ability of recombinant H-ficolin, H-ficolin from human serum, and from bronchoalveolar lavage to bind to IAV, the mouse-adapted PR-8H1N1 and a pandemic H1N1 strain [41]. Following recognition, a decrease in the ability of IAV to cause infection *in vitro* was observed. The role of sialic acid in these mechanisms was suggested to be important, as following sialidase treatments and removal of the sialic acid residues decorating H-ficolin, inhibition of IAV was abolished [41]. In addition, H-ficolin was capable of activating the lectin pathway on a surface coated with IAV. As observed for M- and L-ficolin, H-ficolin has the ability to interact with PTX3 in a dose-dependent manner, although it exhibits the weakest binding of the three [66]. This H-ficolin-PTX3 interaction, as for M-ficolin, exhibited the ability to inhibit HA activity and infectivity of IAV [41]. A more recent study further consolidated these earlier observations by demonstrating that H-ficolin can inhibit the replication of pandemic IAV by enhancing uptake and dampening TNF- α response in monocytes [78].

5.3. Fungi. Similar to L-ficolin, H-ficolin has been implicated in the recognition of *A. fumigatus*. This interaction led to the activation of the lectin pathway of complement, enhanced association of conidia with A549 epithelial cells, and increased IL-8 production [79]. Moreover, H-ficolin has been shown to be recruited to the lungs during inflammation, and we have observed increased concentrations of H-ficolin in the lungs of transplant patients with proven or probable *A. fumigatus* infection [79, 80].

5.4. Parasites. As for L-ficolin, H-ficolin has also recently been observed to bind to the parasites *T. cruzi* and *G. intestinalis* and play a role in complement activation [71, 72]. Depletion of ficolins and MBL led to a 70% decrease in C3b and C4b deposition on *T. cruzi* [81].

6. The Role of Rodent Ficolin-A and Ficolin-B in Immunity to Pathogenic Microorganisms

6.1. Bacteria. Until recently, the recognition spectrum of ficolin-A with microorganisms was relatively unknown. However, there has been much progress in characterising the interaction of ficolin-A with pathogens. Hummelshøj et al. [52] have greatly expanded current knowledge on the pathogen specificity. They showed ficolin-A to recognise a plethora of microorganisms including pathogenic gram-positive and -negative bacteria such as *S. aureus* and the pathogenic *E. coli* strain O157:H7. They also exhibited the ability of ficolin-A to bind LPS from *E. coli* and *P. aeruginosa*. However, recognition of LPS did not equate to protection or inflammatory modulation in an *in vivo* model of LPS-induced systemic inflammation [82].

Ficolin-A has been observed to partake in the activation of the lectin-complement pathway via the association with MASPs. Further interactions with fibrinogen and thrombin have been observed to potentiate the activation

of complement on *S. aureus* [83]. It has also proven to be an essential activator of the lectin-complement pathway in the defence against *S. pneumoniae*. Ficolin-A and -B opsonisation of *S. pneumoniae* leads to complement deposition in the presence of ficolin-A and only weakly in its absence [15]. The role of the lectin-complement pathway in pneumococcal defence has been shown to be important [84].

6.2. Viruses. To date, there have been very few studies implicating ficolin-A or -B in viral recognition. Pan et al. [34] however did demonstrate that reconstitution of a ficolin-A KO mouse with either L-ficolin or ficolin-A could ameliorate the effects of IAV and protect against mortality and inflammation.

6.3. Fungi. We have further characterised the interactions of ficolin-A with *Aspergillus* spp. *in vitro*. Ficolin-A was observed to recognise *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* with its FBG in a calcium-independent manner. In addition to this, recognition of the most pathogenic species, *A. fumigatus*, was greatly increased in acidic conditions, an interaction which led to enhanced association with the lung epithelium and immobilisation of the fungus [9]. Ficolin-A has also been observed to enhance the phagocytosis of *A. fumigatus* and *A. flavus* by RAW macrophages (unpublished observation), neutrophils, and human macrophages [85]. As previously mentioned, Genster et al. have elucidated an important role for ficolin-A *in vivo* against *A. fumigatus* infection [69]. In addition to this, we have also characterised the binding to the pathogenic yeast *Cryptococcus neoformans*. This interaction shared many of the characteristics of binding to *Aspergillus* such as pH dependence and Ca²⁺ independence, in addition to increased epithelial cell adherence [86].

We also recently elucidated a role of the lectin pathway of complement in the defence against *A. fumigatus* *in vitro*. In the absence of ficolin-A, complement was activated, but in the absence of MBL-A and -C, no complement activation could be observed. MBL-C but not MBL-A was able to bind to *A. fumigatus*; therefore, we postulated that MBL-C could be the activator of the lectin pathway in the defence against *Aspergillus* [9]. Indeed, fungal clearance in ficolin-A knockout mice appeared to be independent of complement activation [69].

Ficolin-A has also exhibited both pro- and anti-inflammatory potential. Recent evidence suggests that ficolin-A may be capable of binding to LPS- and inhibiting TLR-4-mediated inflammation on mast cells [87]. We have previously observed that ficolin-A is capable of enhancing IL-8 secretion from A549 cells challenged with *A. fumigatus* and others have attributed decreased cytokine production to be the cause of higher fungal burdens in knockout mice [9, 69].

6.4. Parasites. Akin to its human orthologue, ficolin-A is also important in the defence against parasites. In an *in vivo* mouse model, ficolin-A was shown to enhance the immunoprotective activity of the 19kDa fragment of merozoite surface protein-1 of *Plasmodium berghei* which led to a

reduction in invasion and an increase in mouse survival [88, 89]. Conversely, upregulated transcripts of ficolin-A were observed in macrophages obtained from mice with tropical pulmonary eosinophilia [90].

The current knowledge on the role of ficolin-B in the recognition and defence against pathogens is largely unknown, but Endo et al. [15] have suggested a synergistic role of ficolin-A and -B in the defence against *S. pneumoniae*. [91].

7. Ficolin Single Nucleotide Polymorphisms and Haplotypes Contribute to Pathophysiology

Single nucleotide polymorphisms (SNPs) can have significant effects on the susceptibility to various infections by altering the function or concentration of ficolins found within serum or organs. Early research in the Garred laboratory indicated that there were large ethnic differences in the distribution of SNPs [92]. Moreover, the number of SNPs found in *FCN3* was very low in comparison to *FCN1* and *FCN2*. Many SNPs were predicted to have a major effect on the function of their respective proteins, with one in *FCN3* for example, completely disrupting the FBG. Since then, there has been much evidence implicating the effects of SNPs within ficolins (Table 2).

7.1. SNPs in *FCN1*. To date, there have been very few polymorphisms identified in the *FCN1* gene, but polymorphisms and haplotypes have been identified which are linked to antibacterial immunity.

Polymorphisms in *FCN1* have been directly associated with M-ficolin levels, with the +7895T>C mutation resulting in an inability to produce M-ficolin and a mutation at -144C>A resulting in significantly increased levels [93]. The same group identified two other nonsynonymous mutations, one at position +6658G>A and the other at +7959A>G that were associated with low M-ficolin levels, poor ligand-binding capacity, and low binding to group B streptococcus [93]. These observations were made using HEK293 cells transfected with plasmids for the various mutated M-ficolin receptors, albeit computational predictions were made that suggested that these SNPs would be potentially damaging in patients.

Cystic fibrosis patients heterozygous or homozygous for mutant alleles for two SNPs in *FCN1* at position 1981G>A and +7918G>A were more susceptible to earlier colonization by *P. aeruginosa* [94]. Moreover, patients heterozygous for two further SNPs in *FCN2* were also more susceptible to earlier onset of colonization [94].

Boldt and colleagues [95] were the first to implicate *FCN1* SNPs as a risk factor for mycobacterial infections. They identified a combination of SNPs (*FCN1**-542A-144C) to have an additive protective effect against *M. leprae* infection, albeit there was a negative association of the *FCN1** 3A haplotype with lepromatous leprosy. Genotyping of Danish individuals with known M-ficolin levels highlighted that the *FCN1**3A haplotype resulted in higher than average M-ficolin levels, which could be an explanation for enhanced susceptibility.

TABLE 2: Single nucleotide polymorphisms in ficolin genes contributing to pathophysiology and colonization.

Ficolin gene	rs number	Position	Gene region	Amino acid change	Reference
M-ficolin (<i>FCN1</i>)	rs2989727	-1981G>A	Promoter	—	[93, 94]
	rs10120023	-542G>A	Promoter	—	[95]
	rs10117466	-144C>A	Promoter	—	[95]
	rs148669884	+6658G>A	Exon 8	Ala218Thr	[93]
	rs150625869	+7895T>C	Exon 9	Ser268Pro	[93]
	rs1071583	+7918G>A	Exon 9	Gln275Gln	[94]
	rs138055828	+7959A>G	Exon 9	Asn289Ser	[93]
L-ficolin (<i>FCN2</i>)	rs3124952	-986G>A	Promoter	—	[111, 112]
	rs3124953	-602G>A	Promoter	—	[103]
	rs3811140	-557A>G	Promoter	—	[104, 106]
	rs28969369	-64A>C	Promoter	—	[96, 104]
	rs175141136	-4A>G	Promoter	—	[103, 111, 112]
	rs17549193	+6359C>T	Exon 8	Thr236Met	[96, 98–100, 108]
	rs7851696	+6424G>T	Exon 8	Ala258Met	[96, 97, 102, 104, 106, 110]
H-ficolin (<i>FCN3</i>)	rs28357092	+1637CdC	Exon 5	Leu117fs	[113, 114]
	rs4494157	+4473C>A	Intron 7	—	[107]

7.2. SNPs in *FCN2*. L-ficolin polymorphisms have been the most widely reported, with polymorphisms in the *FCN2* gene contributing to susceptibility to numerous bacterial pathogens and some viruses.

Cedzynski and colleagues initially reported four polymorphisms that were linked to extremes in L-ficolin concentrations in a cohort of Polish children suffering from recurrent respiratory infections [96]. Low L-ficolin levels were associated with variant alleles for -64A>C and +6424G>T but normal alleles for -4A>G and +6359C>T. Conversely, high L-ficolin levels were associated with variant alleles of -4A>G and +6359C>T. *FCN2* polymorphisms were not identified as major risk factors for community acquired pneumonia, albeit there was an association between the +6424G>T polymorphism and patient colonization with *Coxiella burnetii* [97].

The *FCN2* exon 8 +6359 C>T polymorphism has arisen as an important SNP involved in susceptibility to bacterial infections, especially following organ transplants [98, 99]. Wan and colleagues [98] correlated the SNP with increased incidence of bacteraemia in kidney transplants, whereas de Rooij et al. [99] demonstrated an increased incidence of significant bacterial infections and mortality in liver transplant patients. Moreover, the negative effects were cumulative if patients also had SNPs in the lectin pathway components, MBL-2 and MASP-2 [99].

The +6359C>T SNP was also reported as a significant risk factor in patients on continuous ambulatory peritoneal dialysis with a history of staphylococcal peritonitis [100], potentially due to decreased ability to bind to staphylococci.

B acute lymphoblastic leukemia patients were observed to be at a greater risk of bacterial infections and present prolonged episodes of febrile neutropenia if they possessed a medium-/high-risk haplotype for *FCN2* of GGATG, GGACG, or AGACG (all haplotypes composed of -986/-602/-4/+6359/+6424) [101]. Notably, the risk of

bacterial infections was further enhanced if patients possessed both a medium-/high-risk haplotype of *FCN2* and a medium-/high-risk genotype of *MBL2* [101].

Following genotyping of 219 severely injured patients admitted to a level 1 trauma centre over a period of 3 years, a *FCN2* +6424G>T SNP was identified that predisposed patients to positive wound cultures and septic shock [102]. Additionally, this *FCN2* SNP, but not the -4A>G or -602G>A polymorphisms, was significantly associated with chronic adenotonsillitis in young children [103].

Although the vast majority of SNPs are associated with a negative output, three SNPs within *FCN2* have been associated with protection against pulmonary tuberculosis. In this study, the frequency of the -557A>G, -64A>C, and +6424G>T SNPs was found to be lower in the pulmonary TB group in comparison to the control group [104]. In opposition, research from Chalmers et al. suggests that there is no relationship between lectin pathway proteins and susceptibility to tuberculosis, leaving the subject open for debate [105]. Conversely, in a Chinese cohort of leprosy patients, genetic variants of *FCN2* (-557A>G and +6424G>T) in the promoter region and exon 8, respectively, were linked to low L-ficolin levels and had a positive association with leprosy susceptibility [106], which is in opposition to both H- and M-ficolin, where higher concentrations of either of these results in enhanced susceptibility [95, 107].

SNPs in *FCN2* have also been reported to be correlated with a predisposition to parasitic infections, including, leishmaniasis, malaria, Chagas disease, and schistosomiasis [108–111].

Elevated levels of L-ficolin observed in leishmaniasis were reported to be due to the +6359C>T structural variant [108]. Conversely, low L-ficolin levels were significantly associated with Chagas disease, which could be attributed to increased incidence of the Ala258Ser amino acid change (+6424G>T in exon 8) [110]. Moreover, heterozygote -4A>G genotypes

with the Ala258Ser variant were more frequent amongst the patients with cardiogestive symptoms [110].

Malaria patients were tested for polymorphisms known to be associated with varying L-ficolin plasma levels, and, although concentrations varied between mild and severe cases of malaria, there was no significant association for any of the haplotypes with disease severity [109].

Ouf et al., [111] identified that the -986G>AA and -4A>G alleles were significantly associated with schistosomiasis. Patients heterozygous for -986G>A or -4A>G, or those with the haplotype AGGG (composed of -986/-602/-4/+6424), were most at risk for schistosomiasis, whereas those homozygous for these mutations, or patients with the haplotype GGAG, were protected against schistosomiasis. Notably, L-ficolin concentrations were higher in both the controls and the GGAG patients.

SNPs in *FCN2* have also been implicated in viral infections, participating in the pathophysiology of hepatitis B infection [112]. There appeared to be significant haplotypic differences between hepatitis B patients and controls, with the AGGG haplotype being found more frequently in controls and the AAAG haplotype being associated with higher L-ficolin concentrations (both composed of -986/-602/-4/+6424) and increased viral loads [112]. Furthermore, strong linkage was associated between the variant -986G>A and -4A>G.

7.3. SNPs in *FCN3*. As aforementioned, *FCN3* was observed to have fewer SNPs comparatively to *FCN1* and *FCN2*, and few have been implicated in pathogen susceptibility.

The first incidence of H-ficolin deficiency was highlighted by Munthe-Fog et al. in 2009 [113], whereby they identified a patient with recurrent infections that was homozygous for a frameshift mutation (+1637C>A) and had undetectable levels of H-ficolin in their serum. Furthermore, Michalski et al. [114] reported a neonate with *S. agalactiae* infection who was homozygous for this frameshift mutation and completely H-ficolin deficient, suggesting an important role of H-ficolin in antibacterial defence.

Recently, there has also been a link to leprosy. Although there was no direct link observed between polymorphisms in *FCN3* and leprosy, H-ficolin levels were higher in patients with the *FCN3* +4473C>A *2B1 haplotype, in addition to being higher in the leprosy and lepromatous patient's comparative to controls [107], indicating that elevated H-ficolin levels may help propagate the disease.

8. Conclusions

There has been ever increasing evidence that ficolins play an integral role in a plethora of infectious diseases, additionally mutations within these proteins generally results in enhanced susceptibility to infection. In recent years, there has been a lot of interest in the role of ficolins within fungal disease, with each of the human ficolins and rodent ficolin-A proving beneficial to antifungal immunity. Furthermore, the recognition spectra of both M- and H-ficolin are increasing, but compared to L-ficolin, are still relatively limited. Future research could therefore focus on further

exploration of the role of M-ficolin within antifungal immunity and enhancing our knowledge on the roles of M- and H-ficolin within innate immunity.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgments

This work was supported by the Faculty of Health, University of East Anglia, UK PhD studentship (FMH 04.4.66 C4).

References

- [1] Y. Endo, Y. Liu, K. Kanno, M. Takahashi, M. Matsushita, and T. Fujita, "Identification of the mouse H-ficolin gene as a pseudogene and orthology between mouse ficolins A/B and human L/M ficolins," *Genomics*, vol. 84, no. 4, pp. 737–744, 2004.
- [2] C. Honoré, S. Rørvig, T. Hummelshøj, M. O. Skjoedt, N. Borregaard, and P. Garred, "Tethering of ficolin-1 to cell surfaces through recognition of sialic acid by the fibrinogen-like domain," *Journal of Leukocyte Biology*, vol. 88, no. 1, pp. 145–158, 2010.
- [3] R. Sugimoto, Y. Yae, M. Akaiwa et al., "Cloning and characterization of the Hakata antigen, a member of the ficolin/opsonin p35 lectin family," *The Journal of Biological Chemistry*, vol. 273, no. 33, pp. 20721–20727, 1998.
- [4] A. Swierzko, J. Lukaszewicz, M. Cedzynski et al., "New functional ligands for ficolin-3 among lipopolysaccharides of *Hafnia alvei*," *Glycobiology*, vol. 22, no. 2, pp. 267–280, 2012.
- [5] N. J. Lynch, S. Roscher, T. Hartung et al., "L-ficolin specifically binds to lipoteichoic acid, a cell wall constituent of Gram-positive bacteria, and activates the lectin pathway of complement," *The Journal of Immunology*, vol. 172, no. 2, pp. 1198–1202, 2004.
- [6] Y. G. Ma, M. Y. Cho, M. Zhao et al., "Human mannose-binding lectin and L-ficolin function as specific pattern recognition proteins in the lectin activation pathway of complement," *Journal of Biological Chemistry*, vol. 279, no. 24, pp. 25307–25312, 2004.
- [7] M. Matsushita, Y. Endo, S. Taira et al., "A novel human serum lectin with collagen- and fibrinogen-like domains that functions as an opsonin," *The Journal of Biological Chemistry*, vol. 271, no. 5, pp. 2448–2454, 1996.
- [8] C. Honoré, T. Hummelshøj, B. E. Hansen, H. O. Madsen, P. Eggleton, and P. Garred, "The innate immune component ficolin 3 (Hakata antigen) mediates the clearance of late apoptotic cells," *Arthritis & Rheumatism*, vol. 56, no. 5, pp. 1598–1607, 2007.
- [9] S. Bidula, H. Kenawy, Y. M. Ali, D. Sexton, W. J. Schwaeble, and S. Schelenz, "Role of ficolin-A and lectin complement pathway in the innate defense against pathogenic *Aspergillus* species," *Infection and Immunity*, vol. 81, no. 5, pp. 1730–1740, 2013.
- [10] P. D. Frederiksen, S. Thiel, C. B. Larsen, and J. C. Jensenius, "M-ficolin, an innate immune defence molecule, binds patterns of acetyl groups and activates complement," *Scandinavian Journal of Immunology*, vol. 62, no. 5, pp. 462–473, 2005.

- [11] M. Matsushita, Y. Endo, and T. Fujita, "Cutting edge: complement-activating complex of ficolin and mannose-binding lectin-associated serine protease," *Journal of Immunology*, vol. 164, no. 5, pp. 2281–2284, 2000.
- [12] M. Matsushita, M. Kuraya, N. Hamasaki, M. Tsujimura, H. Shiraki, and T. Fujita, "Activation of the lectin complement pathway by H-ficolin (Hakata antigen)," *The Journal of Immunology*, vol. 168, no. 7, pp. 3502–3506, 2002.
- [13] Y. Endo, N. Nakazawa, Y. Liu et al., "Carbohydrate-binding specificities of mouse ficolin A, a splicing variant of ficolin A and ficolin B and their complex formation with MASP-2 and sMAP," *Immunogenetics*, vol. 57, no. 11, pp. 837–844, 2005.
- [14] U. V. Girija, D. A. Mitchell, S. Roscher, and R. Wallis, "Carbohydrate recognition and complement activation by rat ficolin-B," *European Journal of Immunology*, vol. 41, no. 1, pp. 214–223, 2011.
- [15] Y. Endo, D. Iwaki, Y. Ishida, M. Takahashi, M. Matsushita, and T. Fujita, "Mouse ficolin B has an ability to form complexes with mannose-binding lectin-associated serine proteases and activate complement through the lectin pathway," *Journal of Biomedicine and Biotechnology*, vol. 2012, Article ID 105891, 7 pages, 2012.
- [16] M. Matsushita and T. Fujita, "Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease," *The Journal of Experimental Medicine*, vol. 176, no. 6, pp. 1497–1502, 1992.
- [17] Y. J. Ma, M. O. Skjoedt, and P. Garred, "Collectin-11 (CL-K1):MASPs complex formation triggers activation of the complement lectin pathway—the fifth lectin pathway initiation complex," *Immunobiology*, vol. 217, no. 11, article 1152, 2012.
- [18] S. Thiel, "Complement activating soluble pattern recognition molecules with collagen-like regions, mannan-binding lectin, ficolins, and associated proteins," *Molecular Immunology*, vol. 44, no. 16, pp. 3875–3888, 2007.
- [19] M.-O. Skjoedt, T. Hummelshoj, Y. Palarasah et al., "A novel mannose-binding lectin/ficolin-associated protein is highly expressed in heart and skeletal muscle tissues and inhibits complement activation," *Journal of Biological Chemistry*, vol. 285, no. 11, pp. 8234–8243, 2010.
- [20] S. E. Degn, A. G. Hansen, R. Steffensen, C. Jacobsen, J. C. Jensenius, and S. Thiel, "MAP44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation," *The Journal of Immunology*, vol. 183, no. 11, pp. 7371–7378, 2009.
- [21] T. Vorup-Jensen, J. C. Jensenius, and S. Thiel, "MASP-2, the C3 convertase generating protease of the MBLectin complement activating pathway," *Immunobiology*, vol. 199, no. 2, pp. 348–357, 1998.
- [22] M. C. Peitsch and J. Tschopp, "Assembly of macromolecular pores by immune defense systems," *Current Opinion in Cell Biology*, vol. 3, no. 4, pp. 710–716, 1991.
- [23] C. Teh, Y. le, S. H. Lee, and J. Lu, "M-ficolin is expressed on monocytes and is a lectin binding to N-acetyl-D-glucosamine and mediates monocyte adhesion and phagocytosis of *Escherichia coli*," *Immunology*, vol. 101, no. 2, pp. 225–232, 2000.
- [24] V. Garlatti, L. Martin, E. Gout et al., "Structural basis for innate immune sensing by M-ficolin and its control by a pH-dependent conformational switch," *The Journal of Biological Chemistry*, vol. 282, no. 49, pp. 35814–35820, 2007.
- [25] E. Gout, V. Garlatti, D. F. Smith et al., "Carbohydrate recognition properties of human ficolins: glycan array screening reveals the sialic acid binding specificity of M-ficolin," *Journal of Biological Chemistry*, vol. 285, no. 9, pp. 6612–6622, 2010.
- [26] Y. Liu, Y. Endo, D. Iwaki et al., "Human M-ficolin is a secretory protein that activates the lectin complement pathway," *Journal of Immunology*, vol. 175, no. 5, pp. 3150–3156, 2005.
- [27] A. N. Moreno-Amaral, E. Gout, C. Danella-Polli et al., "M-ficolin and leukosialin (CD43): new partners in neutrophil adhesion," *Journal of Leukocyte Biology*, vol. 91, no. 3, pp. 469–474, 2012.
- [28] M. Tanio, S. Kondo, S. Sugio, and T. Kohno, "Trivalent recognition unit of innate immunity system. Crystal structure of trimeric human M-ficolin fibrinogen-like domain," *The Journal of Biological Chemistry*, vol. 282, no. 6, pp. 3889–3895, 2007.
- [29] M. Tanio, S. Kondo, S. Sugio, and T. Kohno, "Trimeric structure and conformational equilibrium of M-ficolin fibrinogen-like domain," *Journal of Synchrotron Radiation*, vol. 15, no. 3, pp. 243–245, 2008.
- [30] M. Tanio and T. Kohno, "Histidine-regulated activity of M-ficolin," *The Biochemical Journal*, vol. 417, no. 2, pp. 485–491, 2009.
- [31] L. Yang, J. Zhang, B. Ho, and J. L. Ding, "Histidine-mediated pH-sensitive regulation of M-ficolin:GlcNAc binding activity in innate immunity examined by molecular dynamics simulations," *PLoS One*, vol. 6, no. 5, article e19647, 2011.
- [32] Y. Le, S. H. Lee, O. L. Kon, and J. Lu, "Human L-ficolin: plasma levels, sugar specificity, and assignment of its lectin activity to the fibrinogen-like (FBG) domain," *FEBS Letters*, vol. 425, no. 2, pp. 367–370, 1998.
- [33] J. Liu, M. A. M. Ali, Y. Shi et al., "Specifically binding of L-ficolin to N-glycans of HCV envelope glycoproteins E1 and E2 leads to complement activation," *Cellular & Molecular Immunology*, vol. 6, no. 4, pp. 235–244, 2009.
- [34] Q. Pan, H. Chen, F. Wang et al., "L-ficolin binds to the glycoproteins hemagglutinin and neuraminidase and inhibits influenza A virus infection both in vitro and in vivo," *Journal of Innate Immunity*, vol. 4, no. 3, pp. 312–324, 2012.
- [35] V. Garlatti, N. Belloy, L. Martin et al., "Structural insights into the innate immune recognition specificities of L- and H-ficolins," *The EMBO Journal*, vol. 26, no. 2, pp. 623–633, 2007.
- [36] N. Kairies, H. G. Beisel, P. Fuentes-Prior et al., "The 2.0-Å crystal structure of tachylectin 5A provides evidence for the common origin of the innate immunity and the blood coagulation systems," *Proceedings of the National Academy of Sciences*, vol. 98, no. 24, pp. 13519–13524, 2001.
- [37] R. M. Zacho, L. Jensen, R. Terp, J. C. Jensenius, and S. Thiel, "Studies of the pattern recognition molecule H-ficolin: specificity and purification," *Journal of Biological Chemistry*, vol. 287, no. 11, pp. 8071–8081, 2012.
- [38] T. R. Kjaer, A. G. Hansen, U. B. S. Sørensen, O. Nielsen, S. Thiel, and J. C. Jensenius, "Investigations on the pattern recognition molecule M-ficolin: quantitative aspects of bacterial binding and leukocyte association," *Journal of Leukocyte Biology*, vol. 90, no. 3, pp. 425–437, 2011.

- [39] T. R. Kjaer, A. G. Hansen, U. B. S. Sørensen et al., "M-ficolin binds selectively to the capsular polysaccharides of *Streptococcus pneumoniae* serotypes 19B and 19C and of a *Streptococcus mitis* strain," *Infection and Immunity*, vol. 81, no. 2, pp. 452–459, 2013.
- [40] M. A. Bartłomiejczyk, A. S. Swierzko, A. Brzostek, J. Dziadek, and M. Cedzynski, "Interaction of lectin pathway of complement-activating pattern recognition molecules with mycobacteria," *Clinical and Experimental Immunology*, vol. 178, no. 2, pp. 310–319, 2014.
- [41] A. Verma, M. White, V. Vathipadikeal et al., "Human H-ficolin inhibits replication of seasonal and pandemic influenza A viruses," *Journal of Immunology*, vol. 189, no. 5, pp. 2478–2487, 2012.
- [42] E. Gout, C. Moriscot, A. Doni et al., "M-ficolin interacts with the long pentraxin PTX3: a novel case of cross-talk between soluble pattern-recognition molecules," *The Journal of Immunology*, vol. 186, no. 10, pp. 5815–5822, 2011.
- [43] A. L. Favier, E. Gout, O. Reynard et al., "Enhancement of Ebola virus infection via ficolin-1 interaction with the mucin domain of GP glycoprotein," *Journal of Virology*, vol. 90, no. 11, pp. 5256–5269, 2016.
- [44] K. Jensen, K. P. Lund, K. B. Christensen et al., "M-ficolin is present in *Aspergillus fumigatus* infected lung and modulates epithelial cell immune responses elicited by fungal cell wall polysaccharides," *Virulence*, vol. 8, no. 8, pp. 1870–1879, 2017.
- [45] E. Stermann, M. Lacroix, E. Gout et al., "Molecular and functional characterization of the interaction of L-ficolin with *Streptococcus pneumoniae*," *Immunobiology*, vol. 217, no. 11, pp. 1188–1189, 2012.
- [46] A. M. Brady, J. J. Calix, J. Yu, K. A. Geno, G. R. Cutter, and M. H. Nahm, "Low invasiveness of pneumococcal serotype 11A is linked to ficolin-2 recognition of O-acetylated capsule epitopes and lectin complement pathway activation," *The Journal of Infectious Diseases*, vol. 210, no. 7, pp. 1155–1165, 2014.
- [47] E. Vassal-Stermann, M. Lacroix, E. Gout et al., "Human L-ficolin recognizes phosphocholine moieties of pneumococcal teichoic acid," *Journal of Immunology*, vol. 193, no. 11, pp. 5699–5708, 2014.
- [48] Y. M. Ali, H. I. Kenawy, A. Muhammad, R. B. Sim, P. W. Andrew, and W. J. Schwaible, "Human L-ficolin, a recognition molecule of the lectin activation pathway of complement, activates complement by binding to pneumolysin, the major toxin of *Streptococcus pneumoniae*," *PLoS One*, vol. 8, no. 12, article e82583, 2013.
- [49] Y. Aoyagi, E. E. Adderson, J. G. Min et al., "Role of L-ficolin/mannose-binding lectin-associated serine protease complexes in the opsonophagocytosis of type III group B streptococci," *The Journal of Immunology*, vol. 174, no. 1, pp. 418–425, 2005.
- [50] M. Fujieda, Y. Aoyagi, K. Matsubara et al., "L-ficolin and capsular polysaccharide-specific IgG in cord serum contribute synergistically to opsonophagocytic killing of serotype III and V group B streptococci," *Infection and Immunity*, vol. 80, no. 6, pp. 2053–2060, 2012.
- [51] Y. Aoyagi, E. E. Adderson, C. E. Rubens et al., "L-ficolin/mannose-binding lectin-associated serine protease complexes bind to group B streptococci primarily through N-acetylneuraminic acid of capsular polysaccharide and activate the complement pathway," *Infection and Immunity*, vol. 76, no. 1, pp. 179–188, 2008.
- [52] T. Hummelshøj, Y. J. Ma, L. Munthe-Fog et al., "The interaction pattern of murine serum ficolin-A with microorganisms," *PLoS ONE*, vol. 7, no. 5, article e38196, 2012.
- [53] A. Sahagún-Ruiz, L. C. D. Breda, M. M. Castiblanco Valencia et al., "Studies of the binding of ficolin-2 and ficolin-3 from the complement lectin pathway to *Leptospira biflexa*, *Pasteurella pneumotropica* and Diarrheagenic *Escherichia coli*," *Immunobiology*, vol. 220, no. 10, pp. 1177–1185, 2015.
- [54] C. Adler Sørensen, A. Rosbjerg, B. Hebbelstrup Jensen, K. A. Krogfelt, and P. Garred, "The lectin complement pathway is involved in protection against Enterococcal *Escherichia coli* infection," *Frontiers in Immunology*, vol. 9, article 1153, 2018.
- [55] J. Zhang, J. Koh, J. Lu et al., "Local inflammation induces complement crosstalk which amplifies the antimicrobial response," *PLoS Pathogens*, vol. 5, no. 1, article e1000282, 2009.
- [56] I. Foldi, T. Tornai, D. Tornai et al., "Lectin-complement pathway molecules are decreased in patients with cirrhosis and constitute the risk of bacterial infections," *Liver International*, vol. 37, no. 7, pp. 1023–1031, 2017.
- [57] U. Kessler, L. J. Schlapbach, P. Klimek, and S. M. Jakob, "Low L-ficolin associated with disease severity during sepsis in adult ICU patients," *Liver International*, vol. 37, no. 9, p. 1409, 2017.
- [58] M. Papp, "Response to low L-ficolin associated with disease severity during sepsis in adult ICU patients," *Liver International*, vol. 37, no. 9, p. 1410, 2017.
- [59] M. V. Carroll, N. Lack, E. Sim, A. Krarup, and R. B. Sim, "Multiple routes of complement activation by *Mycobacterium bovis* BCG," *Molecular Immunology*, vol. 46, no. 16, pp. 3367–3378, 2009.
- [60] F. Luo, X. Sun, Y. Wang et al., "Ficolin-2 defends against virulent mycobacteria tuberculosis infection in vivo, and its insufficiency is associated with infection in humans," *PLoS One*, vol. 8, no. 9, article e73859, 2013.
- [61] M. R. Hamed, R. J. P. Brown, C. Zothner et al., "Recombinant human L-ficolin directly neutralizes hepatitis C virus entry," *Journal of Innate Immunity*, vol. 6, no. 5, pp. 676–684, 2014.
- [62] Y. Zhao, Y. Ren, X. Zhang et al., "Ficolin-2 inhibits hepatitis C virus infection, whereas apolipoprotein E3 mediates viral immune escape," *Journal of Immunology*, vol. 193, no. 2, pp. 783–796, 2014.
- [63] T. Chen, Y. Hu, Q. Ding et al., "Serum ficolin-2 concentrations are significantly changed in patients with hepatitis B virus infection and liver diseases," *Virologica Sinica*, vol. 30, no. 4, pp. 249–260, 2015.
- [64] W. C. Chang, K. L. Hartshorn, M. R. White et al., "Recombinant chimeric lectins consisting of mannose-binding lectin and L-ficolin are potent inhibitors of influenza A virus compared with mannose-binding lectin," *Biochemical Pharmacology*, vol. 81, no. 3, pp. 388–395, 2011.
- [65] I. C. Michelow, M. Dong, B. A. Mungall et al., "A novel L-ficolin/mannose-binding lectin chimeric molecule with enhanced activity against Ebola virus," *Journal of Biological Chemistry*, vol. 285, no. 32, pp. 24729–24739, 2010.
- [66] Y. J. Ma, A. Doni, T. Hummelshøj et al., "Synergy between ficolin-2 and pentraxin 3 boosts innate immune recognition and complement deposition," *The Journal of Biological Chemistry*, vol. 284, no. 41, pp. 28263–28275, 2009.

- [67] A. Rosbjerg, N. Genster, K. Pilely, M. O. Skjoedt, G. L. Stahl, and P. Garred, "Complementary roles of the classical and lectin complement pathways in the defense against *Aspergillus fumigatus*," *Frontiers in Immunology*, vol. 7, p. 473, 2016.
- [68] S. Bidula, D. W. Sexton, A. Abdolrasouli et al., "The serum opsonin L-ficolin is detected in lungs of human transplant recipients following fungal infections and modulates inflammation and killing of *Aspergillus fumigatus*," *Journal of Infectious Diseases*, vol. 212, no. 2, pp. 234–246, 2015.
- [69] N. Genster, E. Præstekjær Cramer, A. Rosbjerg, K. Pilely, J. B. Cowland, and P. Garred, "Ficolins promote fungal clearance in vivo and modulate the inflammatory cytokine response in host defense against *Aspergillus fumigatus*," *Journal of Innate Immunity*, vol. 8, no. 6, pp. 579–588, 2016.
- [70] M. Osthoff, A. Wojtowicz, F. Tissot et al., "Association of lectin pathway proteins with intra-abdominal *Candida* infection in high-risk surgical intensive-care unit patients. A prospective cohort study within the fungal infection network of Switzerland," *The Journal of Infection*, vol. 72, no. 3, pp. 377–385, 2016.
- [71] I. D. S. Cestari, A. Krarup, R. B. Sim, J. M. Inal, and M. I. Ramirez, "Role of early lectin pathway activation in the complement-mediated killing of *Trypanosoma cruzi*," *Molecular Immunology*, vol. 47, no. 2-3, pp. 426–437, 2009.
- [72] I. Evans-Osses, E. A. Ansa-Addo, J. M. Inal, and M. I. Ramirez, "Involvement of lectin pathway activation in the complement killing of *Giardia intestinalis*," *Biochemical and Biophysical Research Communications*, vol. 395, no. 3, pp. 382–386, 2010.
- [73] E. Sosoniuk, G. Vallejos, H. Kenawy et al., "Trypanosoma cruzi calreticulin inhibits the complement lectin pathway activation by direct interaction with L-Ficolin," *Molecular Immunology*, vol. 60, no. 1, pp. 80–85, 2014.
- [74] M. Tsujimura, T. Miyazaki, E. Kojima et al., "Serum concentration of Hakata antigen, a member of the ficolins, is linked with inhibition of *Aerococcus viridans* growth," *Clinica Chimica Acta*, vol. 325, no. 1-2, pp. 139–146, 2002.
- [75] A. Krarup, U. B. S. Sorensen, M. Matsushita, J. C. Jensenius, and S. Thiel, "Effect of capsulation of opportunistic pathogenic bacteria on binding of the pattern recognition molecules mannan-binding lectin, L-ficolin and H-ficolin," *Infection and Immunity*, vol. 73, no. 2, pp. 1052–1060, 2005.
- [76] M. Michalski, A. St Swierzko, J. Lukasiewicz et al., "Ficolin-3 activity towards the opportunistic pathogen, *Hafnia alvei*," *Immunobiology*, vol. 220, no. 1, pp. 117–123, 2015.
- [77] A. S. Świerzko, M. A. Bartłomiejczyk, A. Brzostek et al., "Mycobacterial antigen 85 complex (Ag85) as a target for ficolins and mannose-binding lectin," *International Journal of Medical Microbiology*, vol. 306, no. 4, pp. 212–221, 2016.
- [78] M. R. White, S. Tripathi, A. Verma et al., "Collectins, H-ficolin and LL-37 reduce influenza viral replication in human monocytes and modulate virus-induced cytokine production," *Innate Immunity*, vol. 23, no. 1, pp. 77–88, 2017.
- [79] S. Bidula, D. W. Sexton, M. Yates et al., "H-ficolin binds *Aspergillus fumigatus* leading to activation of the lectin complement pathway and modulation of lung epithelial immune responses," *Immunology*, vol. 146, no. 2, pp. 281–291, 2015.
- [80] R. R. Plovsing, R. M. G. Berg, L. Munthe-Fog et al., "Alveolar recruitment of ficolin-3 in response to acute pulmonary inflammation in humans," *Immunobiology*, vol. 221, no. 5, pp. 690–697, 2016.
- [81] I. Cestari and M. I. Ramirez, "Inefficient complement system clearance of *Trypanosoma cruzi* metacyclic trypomastigotes enables resistant strains to invade eukaryotic cells," *PLoS One*, vol. 5, no. 3, article e9721, 2010.
- [82] N. Genster, O. Østrup, C. Schjalm, T. Eirik Mollnes, J. B. Cowland, and P. Garred, "Ficolins do not alter host immune responses to lipopolysaccharide-induced inflammation in vivo," *Scientific Reports*, vol. 7, no. 1, p. 3852, 2017.
- [83] Y. Endo, N. Nakazawa, K. Kanno et al., "Establishment of three lineages of ficolin-deficient mice and their phenotypes," *International Immunology*, vol. 22, supplement, p. 57, 2010.
- [84] Y. M. Ali, N. J. Lynch, K. S. Haleem et al., "The lectin pathway of complement activation is a critical component of the innate immune response to pneumococcal infection," *PLoS Pathogens*, vol. 8, no. 7, article e1002793, 2012.
- [85] S. Bidula, D. W. Sexton, and S. Schelenz, "Serum opsonin ficolin-A enhances host-fungal interactions and modulates cytokine expression from human monocyte-derived macrophages and neutrophils following *Aspergillus fumigatus* challenge," *Medical Microbiology and Immunology*, vol. 205, no. 2, pp. 133–142, 2016.
- [86] S. Schelenz, N. Kirchhof, S. Bidula, R. Wallis, and D. W. Sexton, "Opsonizing properties of rat ficolin-A in the defence against *Cryptococcus neoformans*," *Immunobiology*, vol. 218, no. 4, pp. 477–483, 2013.
- [87] Y. J. Ma, H. J. Kang, J. Y. Kim, P. Garred, M. S. Lee, and B. L. Lee, "Mouse mannose-binding lectin-A and ficolin-A inhibit lipopolysaccharide-mediated pro-inflammatory responses on mast cells," *BMB Reports*, vol. 46, no. 7, pp. 376–381, 2013.
- [88] F. Chen, Q. Liu, Y. Xue et al., "Ficolin-A enhances inhibition of the C-terminal 19 kDa region of merozoite surface protein-1 of *Plasmodium berghei* using test in vivo," *Iranian Journal of Parasitology*, vol. 8, no. 1, pp. 33–39, 2013.
- [89] T. Xiang, T. Xiang, G. Liu, W. A. Dai, Z. Q. Li, and F. Chen, "Study on Ficolin-A against infection of *Plasmodium berghei* in mouse model," *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*, vol. 32, no. 1, pp. 42–45, 2014.
- [90] P. Sharma, A. Sharma, A. L. Vishwakarma, P. K. Agnihotri, S. Sharma, and M. Srivastava, "Host lung immunity is severely compromised during tropical pulmonary eosinophilia: role of lung eosinophils and macrophages," *Journal of Leukocyte Biology*, vol. 99, no. 4, pp. 619–628, 2016.
- [91] Y. Endo, M. Takahashi, D. Iwaki et al., "Mice deficient in ficolin, a lectin complement pathway recognition molecule, are susceptible to *Streptococcus pneumoniae* infection," *Journal of Immunology*, vol. 189, no. 12, pp. 5860–5866, 2012.
- [92] T. Hummelshøj, L. Munthe-Fog, H. O. Madsen, and P. Garred, "Functional SNPs in the human ficolin (FCN) genes reveal distinct geographical patterns," *Molecular Immunology*, vol. 45, no. 9, pp. 2508–2520, 2008.
- [93] C. G. Ammitzbøll, T. R. Kjær, R. Steffensen et al., "Non-synonymous polymorphisms in the FCN1 gene determine ligand-binding ability and serum levels of M-ficolin," *PLoS One*, vol. 7, no. 11, article e50585, 2012.
- [94] F. Haerynck, K. van Steen, T. Cattaert et al., "Polymorphisms in the lectin pathway genes as a possible cause of early chronic *Pseudomonas aeruginosa* colonization in cystic fibrosis patients," *Human Immunology*, vol. 73, no. 11, pp. 1175–1183, 2012.
- [95] A. B. W. Boldt, M. I. N. Sanchez, E. R. S. Stahlke et al., "Susceptibility to leprosy is associated with M-ficolin

- polymorphisms,” *Journal of Clinical Immunology*, vol. 33, no. 1, pp. 210–219, 2013.
- [96] M. Cedzynski, L. Nuytinck, A. P. M. Atkinson et al., “Extremes of L-ficolin concentration in children with recurrent infections are associated with single nucleotide polymorphisms in the FCN2 gene,” *Clinical and Experimental Immunology*, vol. 150, no. 1, pp. 99–104, 2007.
- [97] G. van Kempen, S. Meijvis, H. Endeman et al., “Mannose-binding lectin and l-ficolin polymorphisms in patients with community-acquired pneumonia caused by intracellular pathogens,” *Immunology*, vol. 151, no. 1, pp. 81–88, 2017.
- [98] Q. Q. Wan, Q. F. Ye, and J. D. Zhou, “Mannose-binding lectin 2 and ficolin-2 gene polymorphisms influence the susceptibility to bloodstream infections in kidney transplant recipients,” *Transplantation Proceedings*, vol. 45, no. 9, pp. 3289–3292, 2013.
- [99] B. J. F. de Rooij, B. van Hoek, W. R. ten Hove et al., “Lectin complement pathway gene profile of donor and recipient determine the risk of bacterial infections after orthotopic liver transplantation,” *Hepatology*, vol. 52, no. 3, pp. 1100–1110, 2010.
- [100] S. C. A. Meijvis, B. L. Herpers, H. Endeman et al., “Mannose-binding lectin (*MBL2*) and ficolin-2 (*FCN2*) polymorphisms in patients on peritoneal dialysis with staphylococcal peritonitis,” *Nephrology, Dialysis, Transplantation*, vol. 26, no. 3, pp. 1042–1045, 2011.
- [101] Z. D. Pana, F. Samarah, R. Papi et al., “Mannose binding lectin and ficolin-2 polymorphisms are associated with increased risk for bacterial infections in children with B acute lymphoblastic leukemia,” *Pediatric Blood & Cancer*, vol. 61, no. 6, pp. 1017–1022, 2014.
- [102] M. W. G. A. Bronkhorst, M. A. Z. Lomax, R. H. A. M. Vossen, J. Bakker, P. Patka, and E. M. M. van Lieshout, “Risk of infection and sepsis in severely injured patients related to single nucleotide polymorphisms in the lectin pathway,” *British Journal of Surgery*, vol. 100, no. 13, pp. 1818–1826, 2013.
- [103] A. N. Erkan, I. Oz, Y. K. Terzi et al., “FCN2 c.772G>T polymorphism is associated with chronic adenoiditis and/or tonsillitis, but not -4 A>G and -602 G>A,” *International Journal of Pediatric Otorhinolaryngology*, vol. 87, pp. 1–4, 2016.
- [104] D. D. Xu, C. Wang, F. Jiang et al., “Association of the *FCN2* gene single nucleotide polymorphisms with susceptibility to pulmonary tuberculosis,” *PLoS One*, vol. 10, no. 9, article e0138356, 2015.
- [105] J. D. Chalmers, M. Matsushita, D. C. Kilpatrick, and A. T. Hill, “No strong relationship between components of the lectin pathway of complement and susceptibility to pulmonary tuberculosis,” *Inflammation*, vol. 38, no. 4, pp. 1731–1737, 2015.
- [106] D. F. Zhang, X. Q. Huang, D. Wang, Y. Y. Li, and Y. G. Yao, “Genetic variants of complement genes ficolin-2, mannose-binding lectin and complement factor H are associated with leprosy in Han Chinese from Southwest China,” *Human Genetics*, vol. 132, no. 6, pp. 629–640, 2013.
- [107] F. A. Andrade, M. H. Beltrame, V. B. Bini, L. B. Gonçalves, A. B. W. Boldt, and I. J. . Messias-Reason, “Association of a new FCN3 haplotype with high ficolin-3 levels in leprosy,” *PLoS Neglected Tropical Diseases*, vol. 11, no. 2, article e0005409, 2017.
- [108] A. Mishra, J. S. Antony, P. Sundaravadivel et al., “Association of ficolin-2 serum levels and FCN2 genetic variants with Indian visceral leishmaniasis,” *PLoS One*, vol. 10, no. 5, article e0125940, 2015.
- [109] I. Faik, S. I. Oyedeji, Z. Idris et al., “Ficolin-2 levels and genetic polymorphisms of FCN2 in malaria,” *Human Immunology*, vol. 72, no. 1, pp. 74–79, 2011.
- [110] P. R. Luz, A. B. W. Boldt, C. Grisbach, J. F. J. Kun, T. P. Velavan, and I. J. T. Messias-Reason, “Association of L-ficolin levels and *FCN2* genotypes with chronic Chagas disease,” *PLoS One*, vol. 8, no. 4, article e60237, 2013.
- [111] E. A. Ouf, O. Ojurongbe, A. A. Akindele et al., “Ficolin-2 levels and FCN2 genetic polymorphisms as a susceptibility factor in schistosomiasis,” *The Journal of Infectious Diseases*, vol. 206, no. 4, pp. 562–570, 2012.
- [112] T. V. Hoang, N. L. Toan, H. Song le et al., “Ficolin-2 levels and *FCN2* haplotypes influence hepatitis B infection outcome in Vietnamese patients,” *PLoS One*, vol. 6, no. 11, article e28113, 2011.
- [113] L. Munthe-Fog, T. Hummelshøj, C. Honoré, H. O. Madsen, H. Permin, and P. Garred, “Immunodeficiency associated with *FCN3* mutation and ficolin-3 deficiency,” *New England Journal of Medicine*, vol. 360, no. 25, pp. 2637–2644, 2009.
- [114] M. Michalski, A. Szala, A. St Swierzko et al., “H-ficolin (ficolin-3) concentrations and FCN3 gene polymorphism in neonates,” *Immunobiology*, vol. 217, no. 7, pp. 730–737, 2012.