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Review Article

Human L-Ficolin (Ficolin-2) and Its Clinical Significance

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Human L-ficolin (P35, ficolin-2) is synthesised in the liver and secreted into the bloodstream where it is one of the major pattern recognition molecules of plasma/serum. Like other ficolins, it consists of a collagen-like tail region linked to a fibrinogen-related globular head; a basic triplet subunit arises via a collagen-like triple helix, and this then forms higher multimers (typically a 12-mer, Mr 400K). Unlike other ficolins, it has a complex set of binding sites arranged within an internal cleft enabling it to recognise a variety of molecular patterns including acetylated sugars and certain 1,3-β-glucans. It is one of the few molecules known to activate the lectin pathway of complement. Recently, some disease association studies (at either the DNA or protein level) have implicated L-ficolin in innate immunity, where it might cooperate with pentraxins and collectins. Emerging lines of evidence point to a role for L-ficolin in respiratory immunity, where its affinity for *Pseudomonas aeruginosa* could be significant.

1. Introduction

1.1. Discovery. Ficolins were first discovered as transforming growth factor β-binding proteins present in porcine uterus, characterised by the possession of both fibrinogen-related and collagen-like domains [1]. However, it was the description of P35 as an opsonic, GlcNAc-specific lectin that first indicated that this family could be involved in innate immunity as pattern recognition molecules [2]. A very similar molecule named L- (for liver) ficolin was independently purified from human plasma on GlcNAc-Sepharose [3]. Both resembled two other previously discovered plasma proteins: EBP-37, which bound elastin [4], and hucolin, which bound a corticosteroid derivative [5].

Despite some minor discrepancies in properties, it was clear that the same protein was being isolated on different affinity matrices, and the term L-ficolin (or ficolin-2) is now used for this protein with a rather catholic taste in ligands. In this, as in several other features, it resembles the intensively studied mannan- (or mannose-) binding lectin [6], but by definition a ficolin has a fibrinogen-like domain combined with a collagen-like domain and is therefore not a collectin. (Collectins combine a collagen-like domain with a C-type lectin domain and resemble ficolins and C1q in tertiary structure.)

1.2. The Ficolin Family. Porcine ficolins consist of two homologous molecules, designated ficolin-α and -β. Although first discovered in uterine tissue, porcine ficolin-α is more abundant in liver and blood where two isoforms, "little ficolin" (Mr$\sim$400 000) and "big ficolin" (Mr$\sim$800 000) were described [7]. Ficolin-β, with around 80% identity to ficolin-α, was found to be expressed mainly in neutrophils [8].

A similar situation exists in mice. Ficolin A is present in liver and blood plasma, while ficolin B (60% identical) is expressed in bone marrow and spleen and is associated with macrophages [9, 10].

These findings have prompted the generalisation that ficolins can be classified into soluble serum ficolins and cell-bound ficolins whatever the species. This view is supported by a similar dichotomy in the toad, *Xenopus laevis* [11]. The relationships between ficolins in the above species and others have been reviewed in more detail by Matsushita [12] and by Garred et al. 2010 [13].

1.3. Human Ficolins. Unlike pigs or mice, humans have three ficolins, all of which are present in the bloodstream: M-ficolin (monocyte ficolin or ficolin-1); L-ficolin (liver ficolin or ficolin-2); and H-ficolin (Hakata antigen or ficolin-3). M- and L-ficolin have approximately 80% identity in amino acid sequence; H-ficolin has only about 50% identity with

Table 1: The human ficolins.

	M-ficolin	L-ficolin	H-ficolin
Molecular size (subunit)	35 K	35 K	34 K
Molecular size (native)	900 K	420 K	610 K
Location	Neutrophils, monocytes > serum	liver; serum	liver; bile; lung; serum
Chemical specificity	Acetylated sugars	acetylated compounds; LPS; 1,3-β-glucans; lipoteichoic acids; elastin; steroids	D-fucose > GlcNAc; polysaccharide from *A.viridans*
Microbial specificity	*E. coli; S. aureus; S. agalactiae*	*S. aureus; S. pneumoniae; S. typhimurium*; *E. coli*; *P. aeruginosa; M. bovis; G. lamblia; T. cruzi; A. fumigatus,*	*A. viridans; T. cruzi*
Complement activation	Yes	Yes	Yes
Opsonic activity	?	Yes	?
Collagenase sensitivity	Yes	Yes	No

the other two. M-ficolin, predominantly found in monocytes and granulocytes, is the homologue of murine ficolin-B and porcine ficolin-β; L-ficolin is the homologue of murine ficolin-A and porcine ficolin-α [12].

The third human ficolin, the Hakata antigen originally identified and defined by autoantibodies present in a small minority of lupus patients, is synthesised in both liver (secreted into bile as well as blood) and lung (and secreted into the bronchi). It is the most abundant plasma ficolin and the most potent at activating complement in vitro [14, 15].

A comparison of the properties of the ficolins is summarised in Table 1. All three have the ability to activate the lectin pathway of complement, an activity known to be shared with just two collectins, mannan-binding lectin (MBL) and CL-L1 [16, 17]. All three ficolins seem to recognise acetylated sugars like GlcNAc to some degree. L-ficolin (like MBL) appears to be a major pattern recognition molecule in human plasma [18]. It has a uniquely complex set of binding sites, potentially conferring the ability to recognise and interact with a wide range of microorganisms [19]. Recently, L-ficolin has been the subject of several disease association studies, providing evidence that L-ficolin complements MBL as an important component of innate immunity in the circulation. The structure, properties and function(s) of L-ficolin form the remainder of this paper.

2. Genetics

The human L-ficolin gene (*FCN2*) has been localised to chromosome 9 (9q34) [20], like the M-ficolin gene but in contrast to the H-ficolin gene located on chromosome 1. The L-ficolin gene has eight exons (Figure 1). The first exon encodes a signal sequence and the first nine N-terminal residues. Exons 2 and 3 encode a collagen-like region similar to that found in collectins. The fourth exon encodes a link or connecting region. Exons five to eight encode a domain similar in structure to that of the C-terminal portion of the human fibrinogen β and γ chains that is characterized by the conservation of 24 mainly hydrophobic amino acid residues.

The *FCN2* gene is undoubtedly polymorphic. Hummelshoj et al. [21] first described 5 polymorphisms in the promotor region and 9 in the structural gene from a Danish population. Compatible results were reported by Herpers et al. [22], describing 10 single nucleotide polymorphisms in 1888 Dutch blood donors. A later study compared five different ethnic groups; some ethnic-specific polymorphisms were noted, but most were found in all populations [23].

Considerable linkage disequilibrium exists between pairs of promoter and structural gene dimorphisms, complicating the investigation of the relationship between allele expression and protein concentration, but such relationships certainly exist. High concentrations appear to be associated with the variant (minority) nucleotide at promoter position −4, while low concentrations are associated with the variant allele at position 6424 on exon 8 [24, 25]. It is perhaps surprising, and certainly confusing, that the latter mutation is also associated with an increased lectin activity (GlcNAc binding). L-ficolin has several independent activities, however, associated with a variety of binding sites (see below), and it is unknown if other activities are affected.

The single nucleotide polymorphisms implicated in influencing protein concentration in one or more studies are listed in Table 2. It is clear however that those mutations have a very modest influence on average L-ficolin values, each being associated with large and overlapping ranges [25]. Although circulating L-ficolin levels appear to be reasonably stable in healthy individuals, it remains to be established if nongenetic influences may have as great or greater an effect than the *FCN2* genotype. For example, we found that patients treated for haematological malignancies had significantly lower median serum L-ficolin compared with healthy controls [26]. Moreover, when studying babies with sepsis, we have found large differences in some individuals with time (unpublished).

Most individuals possess one or two of the most common five haplotypes, and Munthe-Fog et al. [25] have helpfully stratified serum L-ficolin concentration according to those haplotypes. From this analysis, it is apparent that *FCN2*

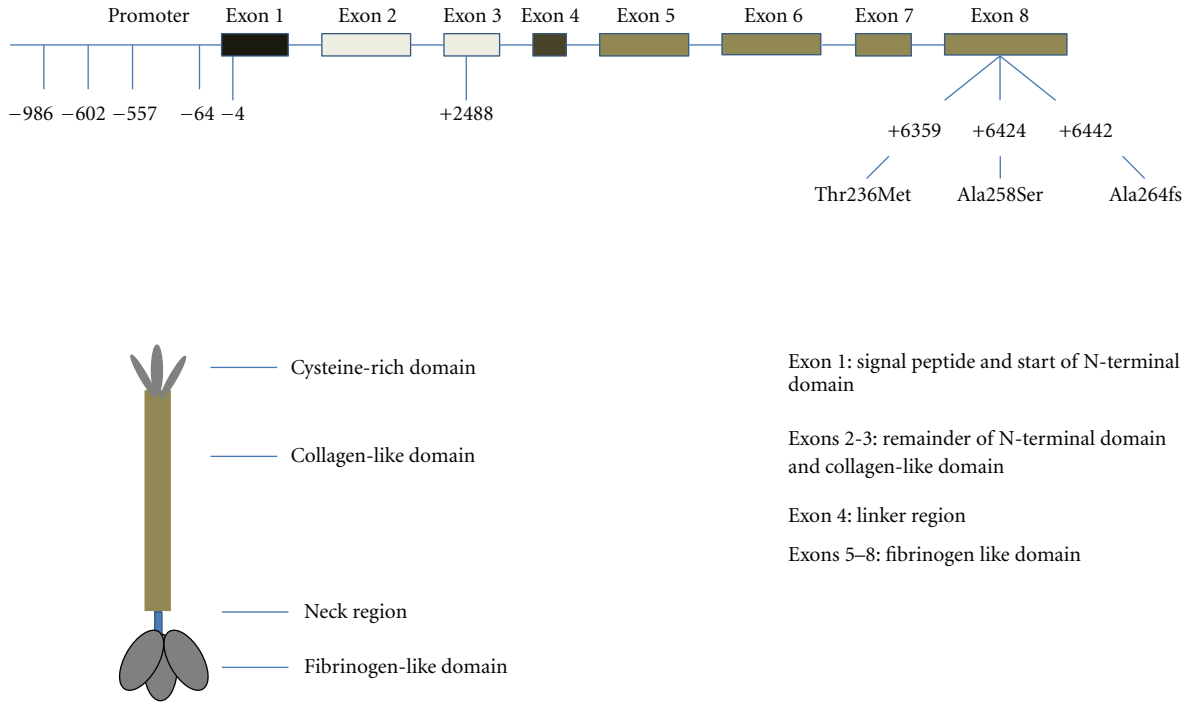

Figure 1: The human *FCN2* gene. The positions of the major single nucleotide polymorphisms are shown. The mutation at +6442 leads to a deletion (fs = frameshift mutation).

Table 2: Potentially important polymorphisms in the *FCN2* gene.

SNP no.	Region and position	Base substitution	Amino acid substitution
rs3124952	promoter −986	A>G	—
rs3124953	promoter −602	G>A	—
rs3811140	promoter −557	A>G	—
rs28969369	promoter −64	A>C	—
rs17514136	promoter −4	A>G	—
rs17549193	exon 8 +6359	C>T	Thr236Met
rs7851696	exon 8 +6424	G>T	Ala258Ser

genotype has little value in predicting protein concentration in individuals. It has been suggested that the huge difference in MBL concentration in general between groups with different *MBL2* genotypes is woefully inadequate for predicting serum MBL levels in individuals and that using that approach is unreliable for inferring differences in disease cohorts [27]. That limitation is even more true of *FCN2* genotyping, although immunogenetic studies may provide interesting additional information to L-ficolin protein measurement.

3. Structure

The primary structure is composed of 288 amino acids forming a gene product of apparent Mr 35 000 after glycosylation [2]. A short N-terminal region implicated in multimer formation is followed by a series of 19 (Gly-X-Y) repeats forming the collagen-like region or domain. This is attached via a short linking sequence to a large globular domain with a distinctive fold, homologous to the C-terminal domains found in fibrinogen chains. This fibrinogen-like domain occurs in several apparently unrelated proteins, including tenascins, the acetylated sugar-binding tachylectins from a horseshoe crab (*Tachypleus tridentatus*) and the sialic acid-binding lectin from the slug, *Limax flavus* [28].

The combination of fibrinogen-like domain and collagen-like region in L-ficolin (and other ficolins) permits the gene product to form a basic subunit consisting a triple helical tail and a trio of globular heads. This 3-dimensional structure is often likened to a bowl of tulips and resembles the shape of the complement component C1q and the collectin family despite those other molecules not having primary sequence homology with ficolins or with each other. The triplet subunits can then associate to form higher multimers (Figure 2). The major form in plasma is believed to be a tetramer of subunits (12-mer) with an apparent Mr of approximately 400 000 [10, 29].

L-ficolin uniquely possesses a complex set of binding sites constituting a recognition surface that can detect various acetylated structures and neutral sugars in the context of extended polysaccharides. This conclusion is based on studies of its trimeric recombinant recognition domains solved by X-ray crystallography [19, 30]. There is an outer binding site (S1) close to the only calcium binding site. This could be considered the ancestral binding site, as it is homologous to that of the horseshoe crab tachylectin 5A as well as that found in human H- and M-ficolins. Surprisingly, S1 is not responsible for recognition of acetylated sugars unlike its counterpart in tachylectin 5A. Instead, L-ficolin

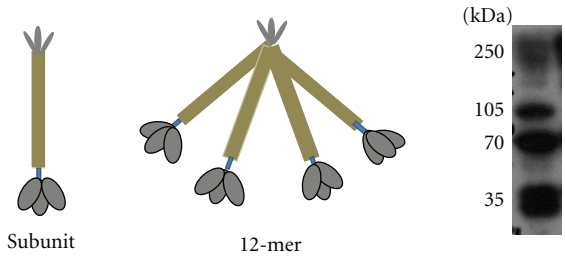

Figure 2: L-ficolin structure. The glycosylated gene product of Mr 35 K forms a basic triplet subunit. The subunit can form higher multimers, of which the 12-mer (4×3) is thought to be the most abundant in serum. The inset (right) shows a typical western blot after electrophoresis of L-ficolin under nonreducing conditions.

possesses three inner binding sites (S2, S3, S4) that are located on both sides of a cleft between the upper parts of the protomers. It is S3 that is mainly responsible for binding acetylated structures and also binds 1,3-β-glucans with assistance from the minor site, S4. The innermost S2 has affinity for galactose. The L-ficolin recognition groove with its contiguous subsites (S2–S4) is reminiscent of the peptidoglycan binding proteins of invertebrates [19].

The ficolins, collectins, and complement component C1q all have a similar 3-dimensional shape suited to function as multivalent recognition molecules with increased affinity for ligands achieved by multiple protein-oligosaccharide interactions. It is noteworthy that L-ficolin possesses a semi-open structure intermediate between the compact assembly of C1q and the wide open arrangement of MBL which has little interaction between the lectin domains and a buried surface 8% the size of that of C1q [19].

The collagen-like region is responsible for the formation of the helical tails of the molecule and also for functional signalling. In particular, the lysine-57 residue is a key component of the binding site for MBL-associated serine proteases and also for calreticulin, a putative complement/collectin/ficolin receptor on phagocytes [31].

4. Biological Properties

4.1. Biochemical Specificity. L-ficolin was reported to bind to mannan, mannan-Sepharose, and GlcNAc-BSA in a calcium-dependent manner [2], whereas other workers described calcium-independent binding to GlcNAc-Sepharose and elution with GlcNAc [3]. L-ficolin also bound to CNBr-activated (but underivatized) Sepharose blocked with Tris. Since L-ficolin did not bind to mannan coupled to "Emphase" (a polyacrylamide derivative) [32], it seems likely the affinity chromatography was achieved with Sepharose (i.e., polygalactose) not mannan; this interaction was possibly mediated by binding site S2. The inconsistency regarding calcium is to some extent explained by the observation that L-ficolin bound to GlcNAc-Sepharose or CysNAc-Sepharose beads not only in the presence of calcium but also in the absence of calcium at high NaCl concentrations [33]. Given that X-ray crystallography has shown that the ancestral (S1) calcium-associated binding site does not bind galactose or acetylated sugars, it appears that the other binding sites are influenced indirectly by their ionic environment.

It is clear that L-ficolin binds not just to acetylated sugars but also to nonsaccharide acetylated compounds [33]. Nevertheless, glycan array studies have established that L-ficolin does not bind to most acetylated oligosaccharides found on mammalian glycoconjugates [34]. Rather, L-ficolin has complex recognition requirements and binding probably requires the presence of two or more acetylated sugar groups presented in an appropriate conformation. In another glycan array study, L-ficolin preferentially recognised disulphated N-acetyllactosamine and tri- and tetrasaccharides containing terminal galactose or N-acetylglucosamine and binding was sensitive to the orientation of the bond between N-acetyllactosamine and the adjacent saccharide [35].

L-ficolin is the major 1,3-β-glucan-binding protein in human plasma [18] and can bind to lipoteichoic acid [36]. Potentially, therefore, L-ficolin could bind to a wide variety of fungi and Gram-positive bacteria. L-ficolin recognises and binds to the viral envelope glycoproteins (E1 and E2) of the hepatitis C virus and triggers the lectin pathway of complement by binding to a virally infected human hepatoma cell line [37].

L-ficolin also appears to bind human DNA, suggesting a mechanism for attaching to apoptotic or necrotic autologous cells and thus promoting the removal of dead and moribund cells and tissues [38]. It has already been mentioned that L-ficolin can bind to a protein, elastin [4], and a lipid [5]. Most significantly, it can bind to the pentraxins, C-reactive protein [39], pentraxin 3 (PTX3) [40], and serum amyloid P component (J. D. Chalmers, unpublished).

4.2. Microbial Specificity. L-ficolin has been found to bind to the Gram-negative bacteria, *Salmonella typhimurium* (Ra strain) [2], *Escherichia coli* [41], and *Pseudomonas aeruginosa* [42], as well as the Gram-positive species, (capsulated) *Staphylococcus aureus* and streptococci including the pneumococcus [33, 43, 44]. All interactions were partially sensitive to GlcNAc. L-ficolin binding to the intracellular bacterium *Mycobacterium bovis* has also been reported [45]. Additionally, binding to *Haemophilus influenzae* and *Moraxella catarrhalis* has been observed (J. D. Chalmers, unpublished).

Binding of L-ficolin to the protozoan causing Chagas' disease, *Trypanosoma cruzi*, has been demonstrated [46] and to the intestinal protozoan *Giardia lamblia* [47]. It also binds the opportunistic fungal pathogen, *Aspergillus fumigatus* [40]. This last interaction can be partially inhibited by GlcNAc or Curdlan (a β-1,3-glucose polymer).

The L-ficolin-mediated response to *A. fumigatus* is enhanced by calcium-independent binding to PTX3 [40]. Similar synergy was observed between L-ficolin and C-reactive protein in response to *P. aeruginosa* [42]. These appear to be impressive examples of how L-ficolin can combine with pentraxins to amplify antimicrobial recognition and effector mechanisms.

4.3. Complement Activation. L-ficolin is one of the few molecules known to activate the lectin pathway of complement

activation [48]. This arises after forming a complex with MBL-associated serine proteases (MASP)-1, -2, and -3, of which MASP-2 is crucial for complement activation. MASP-2 binding takes place at a site on the collagen-like region [31]. L-ficolin-MASP-2 interaction leads to activation of the latter, enabling it to cleave complement components C2 and C4 in a manner similar to the C1q, r, s complex of the classical pathway initiated by antigen-antibody formation. The roles of MASP-1 and its alternatively spliced gene product MASP-3 are less clear, but evidence is accumulating that MASP-1 may link complement to the coagulation system [49–53] as well as collaborating with MASP-2 in the generation of the C3 convertase [54, 55]. MASP-3 is primarily found complexed to H-ficolin and appears to regulate complement activation mediated by the latter [56].

The other activators of MASP-2 and hence the lectin pathway are the other human ficolins (M- and H-ficolin) and the collectins, MBL and CL-11/CL-K1 [16, 17]. In so far as they have been directly compared, H-ficolin emerged as the most potent complement activator, at least as measured in vitro by a particular C4 deposition assay [15]. L-ficolin and MBL were similar, but had only half the complement activation capacity of H-ficolin.

Unsurprisingly, L-ficolin has been shown to possess opsonic activity by enhancing phagocytosis of *Salmonella* by human neutrophils [2]. It can also promote the phagocytosis of *Pseudomonas aeruginosa* [42] and *Streptococcus pneumoniae* (J. D. Chalmers, unpublished). Although such opsonisation may be mediated by complement activation, it has also been suggested that L-ficolin can opsonise bacteria by binding calreticulin on phagocytes via its collagen-like domain [31].

L-ficolin can cooperate with pentraxins to opsonise bacteria and initiate the lectin pathway, as was mentioned earlier.

Two reports support the view that L-ficolin can opsonise autologous dead or dying cells and cellular debris [38, 57]. Kuraya et al. [57] concluded that L-ficolin binds to apoptotic cells and activates complement via the lectin pathway. Jensen et al. [38], however, observed binding to necrotic but not apoptotic cells, and only at supraphysiological concentrations of $\geq 20\,\mu$g/mL. High concentrations of L-ficolin also promoted the uptake of necrotic Jurkat cells by monocyte-derived macrophages in a phagocytosis assay [38]. Many molecules have been described as binding to apoptotic cells, however, and we have no idea of their relative physiological significance in vivo.

5. L-Ficolin in Health and Disease

5.1. L-Ficolin in Healthy Subjects. In healthy adult individuals, the distribution of serum L-ficolin is perfectly Gaussian, so the mean and median are exactly the same. That value has generally been reported to be between 3 and 4 μg/mL [58] although more recently it has been determined at 5.4 μg/mL [25]. A complicating consideration is that purified L-ficolin is very labile (unlike in serum); if anything other than a recently isolated preparation is used as a standard, the values obtained from the calibration curve will be higher than the true values. There is also scope for discrepancy between immunoassayed protein and a measurement based on activity. However, in general, we have found broadly similar values to be obtained with either acetylated BSA or anti-L-ficolin antibody (clone GN4) as solid-phase capture agent combined with the same labelled detection antibody (clone GN5).

Most normal values fall within the range 1000 to 6000 ng/mL, although occasionally much higher values are detected. No value much below 1000 ng/mL has been detected in a healthy adult; therefore, absolute L-ficolin deficiency has not been shown to exist. From genetic studies [21], a homozygous frameshift mutation in exon 8 (rs28357091; Ala264fs; FCN2-D) would be expected to give total deficiency, but this has not yet been found.

Normal values are generally lower in antenatal (cord blood) sera [3, 59–61] and correlate with gestational age, at least until shortly before term [60]. Only one study has investigated serum L-ficolin throughout life [61]. The highest values were found between 1 and 4 years of life (median 11300 ng/mL), then dropped during later childhood (4–16 years, median 8660 ng/mL) before reaching a stable adult level (median 3370) after 16 years of age. Although Sallenbach et al.'s values [61] were in good agreement with our and others' adult data and reasonably close agreement for antenatal data, their elevated values for later childhood were at variance with our finding of similar-to-adult values for such children [62].

Although no absolute deficiency of L-ficolin has yet been discovered, it seems possible that relative deficiency ("insufficiency") defined by low serum L-ficolin ± immunogenetics could contribute to disease susceptibility. Unfortunately, the disease process itself (or treatment) might affect circulating L-ficolin, as has been found for patients with haematological malignancies [26], preeclampsia [63], and sepsis (D. C. Kilpatrick, unpublished).

5.2. General Infections. L-ficolin insufficiency was associated with perinatal infections in preterm Polish babies [60]. In a cohort of over 1800 consecutive deliveries, the rate of perinatal infections in babies with cord blood L-ficolin <1000 ng/mL (the lowest 9%) was twice that of babies with higher concentrations (13.7% versus 7.7%; $P < 0.01$). This relationship was not independent of gestational age and birthweight, but suggests that L-ficolin insufficiency could be one of several factors that contribute to the adverse consequences of prematurity and low birthweight [60].

Schlapback et al. [64], however, found no significant relationship between low cord blood L-ficolin and sepsis in 47 premature infants. This was despite finding significant relationships between low H-ficolin and Gram-positive sepsis and between low MBL and Gram-negative sepsis. Incidentally, that study did confirm correlations between L-ficolin concentration and both gestational age and birthweight. It is possible that study was underpowered for L-ficolin: for Gram-negative sepsis, 47% (7/15) of the patients had low (<1000 ng/mL) L-ficolin compared with 23% (22/94) of infection-free, matched controls ($P < 0.07$) [64].

Uraemic patients have an increased susceptibility to infection, and peritonitis is a common complication in patients on continuous ambulatory peritoneal dialysis (CAPD). The +6359 C>T variant in the *FCN2* gene, causing a Thr ► Met alteration and a concomitant decrease in lectin activity, was found to be commoner in CAPD patients with a history of staphylococcal peritonitis compared with CAPD patients without such a history [65]. In the former, exit site *Staphylococcus aureus* was also more prevalent [65].

Another clinical context of interest is that of chemotherapy ± transplantation. MBL has been intensively studied in this context with wildly differing results [66, 67]. In our series of haematological malignancy patients who were rendered severely neutropenic, entirely negative results were obtained for both L-ficolin and H-ficolin [26].

However, the situation appears to be very different in the context of liver transplantation, where the recipient assumes the phenotype of the donor. The variant *FCN2* +6359 allele was associated with a doubling of the bacterial infection risk within the first year following transplant, and the risk was enhanced by coinheritance of *MBL2* variant alleles [68]. Patients with one or more lectin pathway genetic variants and infection had a much increased mortality rate which was highly statistically significant [68]. As well as those bacterial infections causing sepsis and pneumonia, the normal (high lectin activity) *FCN2* +6359 was associated with protection from cytomegalovirus after liver transplantation [69]. Again, the combination of *FCN2* and *MBL2* risk alleles conferred a particularly high degree of susceptibility.

5.3. Respiratory Infections. There is a strong theoretical basis to believe L-ficolin may be important in respiratory infections. As discussed previously, L-ficolin binds to and opsonises a wide variety of important respiratory pathogens (including capsulated *S. pneumoniae*, *S. aureus*, *P. aeruginosa*, *H. influenzae* and others). L-ficolin, like MBL, has been found in the inflamed lung (induced sputum and bronchoalveolar lavage fluid) in concentrations sufficient to cause complement activation (J. D. Chalmers, unpublished).

In perhaps the first disease association study involving L-ficolin, an association between L-ficolin insufficiency and recurrent respiratory infections in children was reported. This relationship was particularly marked for patients with coexisting allergic disorders (mostly rhinitis and/or asthma with high IgE) [70]. This preliminary observation prompted a fresh, prospective study on children aged 1 to 16 years (mean 8.9) to confirm or refute the previous retrospective findings and to distinguish between infection and allergy [62]. L-ficolin insufficiency was indeed significantly associated with asthma and/or allergic rhinitis in the context of recurrent respiratory infections, but not with those allergic disorders in the absence of infection or with recurrent respiratory infections in the absence of allergy [62]. The reason for this relationship is not clear, but it is possible that L-ficolin confers some protection from microorganisms that exacerbate allergic inflammation in the lung.

Another research group examined *FCN2* variants in a birth cohort followed up for 4 years. Analysis based on constructed haplotypes yielded no relationship with recurrent respiratory infection. No serum L-ficolin measurements were made [71].

Similarly negative immunogenetic results were obtained in a study of invasive pneumococcal disease [72], but again no serum L-ficolin data were obtained.

There was a clear association between low serum L-ficolin and idiopathic bronchiectasis [73]. This has since been confirmed in a much larger series, and there is evidence to suggest the basis could lie in protection from colonisation with *Pseudomonas aeruginosa* [74]. Certainly, low serum L-ficolin in those patients identifies a clinical phenotype associated with more severe disease and therefore with poorer quality of life.

5.4. Other Infectious Diseases. The distribution of *FCN2* haplotypes in leprosy patients differed significantly from healthy controls [75]. The authors interpreted their findings as an indication that normal (relatively high) L-ficolin concentrations protect against *M. leprae* infections, but serum L-ficolin was not actually measured.

A comparison of mild and severe *Plasmodium falciparum* malaria revealed that serum L-ficolin concentration is highest during acute severe disease, but this difference was not reflected in the distribution of the *FCN2* haplotypes [76].

In contrast, the distribution of *FCN2* haplotypes was altered in cutaneous leishmaniasis patients when compared with healthy controls [77]. Haplotypes expected to confer normal concentrations of L-ficolin were commoner in the controls, but again serum L-ficolin was not actually measured.

5.5. Pregnancy Disorders. A small proportion of women who had experienced recurrent miscarriage had serum L-ficolin values below the lower limit of normal [59]. These patients were sampled at a single point in time, and a longitudinal study would be required to determine if those low values were stable. It would also be instructive to learn if and how L-ficolin varied during pregnancy in such patients.

According to Wang and coworkers [63], serum L-ficolin increases several-fold during normal pregnancy, although it is not clear whether a direct comparison was made between pregnant and nonpregnant subjects. (Moreover, van de Geijn et al. [78] reported that serum L-ficolin was not influenced by pregnancy.) Certainly, Wang et al. found L-ficolin levels to be significantly lower in preeclamptic pregnancies compared with uncomplicated pregnancies of similar gestational age. Postnatal placentae from preeclamptic pregnancies contained higher concentrations of L-ficolin in lysates, and more intense immunohistochemical staining was observed in syncytiotrophoblast. The co-expression of L-ficolin and Ras protein in preeclamptic syncytiotrophoblast was noted. The authors interpret those observations as evidence of consumption of L-ficolin by apoptotic trophoblast causing depletion in the circulation.

5.6. Miscellaneous Disorders. The distribution of *FCN2* variants was found to be altered in poststreptococcal disease [79]. A haplotype associated with low levels of L-ficolin

Table 3: Some disease associations of L-ficolin insufficiency.

Disease	Associated with	Reference
Perinatal infections	Low serum L-ficolin	[60]
Staphylococcal peritonitis	*FCN2* +6359 variant allele (*FCN2*-B)	[65]
Bacterial infections following liver transplantation	*FCN2* +6359 variant allele (*FCN2*-B)	[68]
Childhood infections combined with allergic diseases	Low serum L-ficolin	[62, 70]
Idiopathic bronchiectasis	Low serum L-ficolin	[73, 74]
Preeclampsia	Low serum L-ficolin	[63]
Chronic rheumatic heart disease	Promoter haplotype GGA	[79]

was slightly but significantly more frequent in patients with chronic rheumatic heart disease (CRHD) compared with healthy controls. Conversely, another haplotype was commoner in controls than in either CRHD or rheumatic fever patients. Since L-ficolin can readily bind to *Streptococcus pyogenes* and thereby activate complement [44], it is conceivable that these immunogenetic differences are related to an altered innate response.

A similar *FCN2* investigation in Behcet's disease was essentially negative [80] as was the outcome of serum L-ficolin measurements in sarcoidosis [81]. Nevertheless there was a trend towards lower circulating L-ficolin in sarcoid patients, in contrast to an increase noted for MBL.

Immunohistochemical evidence for L-ficolin involvement in IgA nephropathy has been found [82], but not in renal allograft rejection [83].

The clearest associations of L-ficolin insufficiency with disease susceptibility are summarised in Table 3.

6. Animal Studies

Animal work, though of indirect relevance to clinical situations, often provides valuable supplementary evidence concerning the function of human molecules. For example, native (plasma-derived) and recombinant porcine ficolin-α was found to neutralise porcine reproductive and respiratory virus (a major pathogen of swine) in vitro in a GlcNAc-dependent manner [84]. However, variant alleles of ficolin-α were not associated with common infectious diseases (pneumonia, enteritis, serositis, septicemia) at necropsy, despite significant associations with MBL-A, MBL-C, and surfactant protein A [85]. Of more tenuous significance was the finding that a chimeric molecule combining the lectin domain of MBL and the collagen-like region of L-ficolin had enhanced protective activity towards Ebola virus than either of the parent molecules [86].

Ficolin-A-deficient knockout mice have been established. Survival of these mice after infection with *Streptococcus pneumoniae* was reduced compared with that of wild-type mice [87], but the phenotype of the knockout mice has not been described in detail.

Human L-ficolin cDNA has been cloned into an expression plasmid and used in a murine model of *Salmonella typhimurium* infection [88]. Administration of L-ficolin in that form protected mice from a potentially lethal challenge with *Salmonella*, with bacterial counts dramatically reduced a week after infection compared with empty-vector-treated controls. The recombinant L-ficolin enhanced monocyte phagocytosis of *Salmonella* in a dose-dependent manner [88].

7. Conclusions

Human L-ficolin is a unique plasma recognition molecule with a broad specificity for microorganisms. It is capable of complementing collectins (such as MBL) and pentraxins in forming a battery of protective molecules constituting the first line of defence. Its functional activities are likely to be mediated through the lectin pathway of complement activation. Although clinical research involving L-ficolin is still in its infancy, evidence is emerging that insufficiency of L-ficolin might increase susceptibility to respiratory infections. In particular, the possibility that L-ficolin is a key factor in protection from *Pseudomonas aeruginosa* warrants further investigation.

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