The Genetics of Ficolins

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Abstract

Ficolins constitute a family of proteins whose biological role has been an enigma for many years. Over the past few years it has become evident that ficolins are part of the innate immune system and function as recognition molecules in the complement system. The 3 human ficolins, ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin or Hakata antigen) are encoded by the *FCN1*, *FCN2* and *FCN3* genes, respectively. Phylogenetic studies suggest that ficolins are of ancient origin. Ficolin-3 seems to be the most ancient molecule, from a phylogenetic perspective. Searches in databases and phylogenetic tree analysis demonstrate that the ficolin precursor has gone through an expansion involving independent duplication events in the different branches of the evolutionary tree. Of particular interest is the prediction that ficolin-1 appears to be present as an ortholog molecule. All human *FCN* genes are polymorphic. The *FCN2* gene encoding ficolin-2, contains polymorphisms that affect ligand binding, while differences in the serum levels are associated with promoter polymorphisms. Recently, a frame-shift variation in the *FCN3* gene was described, leading to ficolin-3 deficiency and defective complement activation. This *FCN3* variation was also shown to be associated with immunode-

ficiency. This survey summarizes the current phylogenetic and inter-individual molecular understanding of the *FCN* genes.

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Important innate immune defence mechanisms rely on evolutionary conserved pattern-recognition molecules, which provide first-line assistance in clearance of pathogens and endogenous debris. These molecules exist both as cell-associated receptors and as soluble molecules. Examples of cell-associated pattern recognition molecules are Toll-like receptors (TLRs) [1] and intracellular NOD-like receptors [2], while soluble pattern-recognition molecules are exemplified by the pentraxin family of proteins [3], the lung surfactant proteins [4] and the initiator molecules of the complement cascade [5].

The complement system can be activated through 3 pathways: the classical pathway initiated by C1q; the alternative pathway initiated by spontaneous hydrolysis of complement factor C3 or direct pattern recognition of properdin, and the lectin complement pathway. Mannose-binding lectin (MBL) was regarded for many years as the prototypic initiator of the lectin pathway. However, due to the pioneering work of Matsushita, Fujita and colleagues [6–8], it is now established that the ficolin family of proteins utilizes the same serine proteases (MBL-as-

sociated serine proteases) to activate the complement system as MBL, although with varying efficiency.

Ficolins have been identified in a range of different species, such as humans [9–13], mice [14], chickens [15] hedgehogs [16], frogs [17] and ascidians [18]. In humans, 3 different ficolins have been described: ficolin-1 (M-ficolin, ficolin/P35-related protein), ficolin-2 (L-ficolin, ficolin/P35, Hucolin, EBP-37) and ficolin 3 (H-ficolin, Hakata antigen, thermolabile β 2-macroglycoprotein). In rodents and pigs only 2 ficolins, ficolin-A (or α) and ficolin-B (or β) have been described.

Ficolin-1 was primarily regarded solely as a cellular associated molecule found on the surface of circulating monocytes and in their cytoplasm [19]. Its mRNA expression is down-regulated and eventually terminated during differentiation of monocytes into macrophages and immature dendritic cells [20–22]. However, the expression may be re-induced in mature macrophages when cells are treated with the TLR-4 ligand LPS or the TLR-2 ligand Pam3Cys [23]. Phagocytosis of *Escherichia coli* by cells of the pro-monocytic cell line U937 is inhibited by an antibody against recombinant ficolin-1, suggesting that surface ficolin-1 acts as a phagocytic receptor [19]. However, ficolin-1 does not contain a typical transmembrane domain and whether the protein is tethered to the monocytic membrane directly or through other molecules remains to be elucidated. The function of cytoplasmic ficolin-1 in monocytes has not been studied, but based on its binding properties, ficolin-1 could act as an intracellular recognition molecule analogous to some of the TLRs and NOD molecules [24]. Ficolin-1 is also detected in secretory granules in the cytoplasm of neutrophils and type II alveolar epithelial cells in the lung, but the function in these cells remains to be defined [8, 25]. Recombinant ficolin-1 has been shown to activate the complement system [8, 25]. However, the complement activation potential of ficolin-1 appears to be less than for ficolin-2 and ficolin-3 [26]. Recently, it has been demonstrated that ficolin-1 indeed is secreted into the extracellular environment from monocytes and macrophages [22].

Ficolin-2 is predominantly expressed by hepatocytes and secreted into the blood stream [27–31]. Upon binding to microorganisms, ficolin-2 acts as an opsonin and enhances phagocytosis [10]. It has been suggested that receptors for ficolins may be present on phagocytic cells [32] but such receptors have not yet been found.

Ficolin-3 was initially identified independently as the Hakata auto-antigen recognised by autoantibodies in the sera of systemic lupus erythematosus patients, and as a

thermo-labile serum macro-protein [33, 34]. It appears that the presence of these auto-antibodies is associated with disease activity, low levels of ficolin-3, and hypocomplementaemia. Ficolin-3 is synthesised in the liver by hepatocytes and bile duct epithelial cells and it is secreted into both the bile duct and the circulation [35]. Ficolin-3 is also produced by ciliated bronchial epithelial cells and by type II alveolar epithelial cells in the lung and is secreted into the bronchial lumen and into the alveolar space.

Ficolin Structure

Ficolins exist as multimeric proteins consisting of 34–35 kDa subunits [26, 36]. Human ficolin-1 and ficolin-2 are 80% homologous at the amino acid level, whereas ficolin-3 is only 48% homologous with ficolins 1 and 2. The polypeptide chains of ficolins 1, 2 and 3 contain 326, 313 and 299 amino acids, respectively. The proteins share certain structural elements, including an N-terminal domain that contains cysteine residues, a collagen-like domain with typical Gly-Xaa-Yaa repeats of varying length, and a C-terminal carbohydrate recognition domain called the fibrinogen-like binding (FBG) domain. In contrast to other collagen-like defence molecules, such as MBL, pulmonary surfactant-associated protein (SP)-A and SP-D, the ficolins do not contain the typical α -helical neck region between the collagen-like region and the recognition domain. Nevertheless, the overall structure of the ficolins is similar to the structure of MBL, SP-A and SP-D. They all have a collagen-like sequence, which enables the assembly of trimeric structures composed of 3 identical polypeptide chains, or 3 almost identical chains for SP-A, which coil into triplets. Based on these trimers, the molecules are further assembled into higher-order oligomers.

The ficolins are composed of 4 or more trimers linked together by disulfide bonds and are thus very similar to MBL (fig. 1). The collagen-like domain interacts with the MBL-associated serine proteases, thereby enabling activation of the complement system [6, 8, 37]. The polar arrangement of the FBG domains enables the proteins to bind negatively charged carbohydrates such as N-acetylglucosamine (GlcNAc) and other acetylated compounds [38].

The FBG domain is similar to the C-terminal region of fibrinogen β and γ chains [39, 40], but the FBG domain is also found in tenascins [41] and in the microfibril-associated protein [42]. The horseshoe crab *Tachypleus tri-*

Fig. 1. Structural organisation of MBL and ficolins. MBL and the ficolins contain a short N-terminal cysteine-rich region followed by a collagen-like sequence, a divergent C-terminal carbohydrate binding domain called carbohydrate-recognition binding domain (CRD) for MBL and fibrinogen-like domain (FBG) for the ficolins. The polypeptides form triple helices in the collagen-like region, which further associate into higher oligomeric forms. The C-terminal enables the proteins to bind to ligands and the collagen-like domains associate with the MBL-associated serine proteases, thereby activating the lectin pathway of complement.

mentatus contains lectins called tachylectins 5A and 5B [43], which have a C-terminal fibrinogen-like domain that is highly homologous to the ficolins. The tachylectins bind and agglutinate Gram-negative and Gram-positive bacteria indicating the important role of the FBG domain in innate immunity.

The details of the recognition domains of the ficolins have recently been resolved based on the crystal structures of the FBG domains [44, 45; see also review by Garlatti et al. in this issue, pp 17–23]. Surprisingly, ficolin-2 has 4 distinct binding sites whereas only 1 domain is detected in ficolins 1 and 3. Ficolin-2 may therefore have a broader binding specificity than the other ficolins.

Genetics of the Ficolins

Three ficolin genes have been identified in humans: *FCN1*, *FCN2* and *FCN3*, which encode ficolin-1, ficolin-2 and ficolin-3, respectively. *FCN1* and *FCN2* are arranged back-to-back on chromosome 9q34. The exon organisations of *FCN1* and *FCN2* are similar although *FCN1* contains 9 exons instead of 8, as found in *FCN2* [12]. The extra exon encodes an additional segment of 4 Gly-Xaa-Yaa repeats. Exon 1 of the *FCN2* encodes the 5' UTR, the

leading peptide and a small segment of N-terminal amino acids. Exons 2 and 3 encode the collagen-like domain. The fourth exon encodes the linker region and exons 5–8 encode the FBG domain. The last exon also encodes the 3' UTR [12]. *FCN3* has been mapped to chromosome 1p36.11. The *FCN3* gene locus is composed of 8 exons and shares the same building structure as seen for *FCN1* and *FCN2*.

To get further insight into evolutionary relationships of the ficolin genes we have generated a neighbour-joining phylogenetic tree using sequences from the National Center for Biotechnology Information Entrez databases. Additional ficolin sequences were identified using *Homo sapiens* as reference sequence for Basic Local Alignment Search Tool (BLAST) searches of the Ensembl Genomic Database Browser. The entire amino acid sequences were aligned in the program BioEdit using ClustalW with default settings [46]. The alignments were thereafter edited manually in order to remove poorly aligned residue ranges and sequences. The concatenated ficolin alignments were put into the Phylogenetic Web Repeater (POWER) to generate a neighbour-joining tree. The neighbour joining was bootstrapped 1,000 times. Based on several output files, a representative phylogenetic tree was constructed (fig. 2). Additionally, the homology between the ficolins in different species was investigated by pairwise

Fig. 2. Phylogenetic neighbour-joining tree of the ficolin protein family. The tree was constructed based on the alignments of the entire amino acid sequence of the ficolins using the ClustalW programme. Sequences were obtained from the NCBI Entrez databases and Ensembl Genome Database.

alignment of the amino acid sequences without signal peptide (fig. 3).

The phylogenetic analysis suggests that the mammal *FCN3* gene branched out by gene duplication in early vertebrate evolution. The same view has previously been suggested by Kakinuma et al. [17]. Based on this tree, the origin of ficolin-3 goes back to the evolutionary stage before the divergence of frogs. The frog and invertebrate ficolins each form tight clusters of several ficolins. Based on pairwise amino acid homology-analyses, none of the

ficolins in either frog or invertebrates can be directly related to any of the human ficolins. Based on sequences in the databases, orthologs of human ficolin-3 were found in both higher and lower primates, as well as species such as the shrew, squirrel, bat and cat (fig. 2). Nevertheless, in both mouse and rat, *FCN3* (*fcn3*) has been identified as a pseudogene [47]. Therefore, it is reasonable to assume that the inactivation of the gene has arisen after branching of the rodent lineages, since both higher and lower primates relative to the mice and rat species have an in-

	huFCN1	huFCN2	huFCN3	chFCN1	chFCN2	chFCN3	rmFCN1	rmFCN2	rmFCN3	piFCNA	piFCNB	hoFCN1	hoFCN3	raFCNA	raFCNB	moFCNA	moFCNB	shFCN3	krFCN3
huFCN1		81	47	98	80	46	91	81	39	77	81	81	46	60	79	60	76	46	44
huFCN2			49	81	98	48	81	94	41	78	77	75	51	60	72	59	70	48	47
huFCN3				48	50	98	44	46	81	47	48	46	87	45	47	47	47	76	82
chFCN1					80	46	91	81	39	77	82	81	47	61	79	62	76	46	44
chFCN2						48	80	93	40	77	76	74	51	59	71	59	69	47	46
chFCN3							43	46	80	45	44	46	83	42	44	43	44	76	82
rmFCN1								81	38	77	80	78	44	59	77	59	74	45	44
rmFCN2									40	79	78	74	47	59	73	59	71	48	46
rmFCN3										40	39	41	78	38	40	39	41	67	75
piFCNA											82	75	48	59	74	59	72	47	45
piFCNB												80	49	61	79	60	77	47	45
hoFCN1													46	59	76	59	75	47	46
hoFCN3														45	48	46	47	78	84
raFCNA															61	89	60	41	43
raFCNB																61	89	47	45
moFCNA																	60	43	43
moFCNB																		45	44
shFCN3																			74
krFCN3																			

Fig. 3. Pairwise identity alignment of the ficolins. The homology among the ficolins in different species was investigated by pairwise alignment of the amino acid sequences without signal peptides. hu = Human; ch = chimpanzee; rm = rhesus monkey; pi = pig; ho = horse; ra = rat; mo = mouse; sh = shrew; kr = kangaroo rat.

tact *FCN3* gene. However, it is at present only in the human situation that ficolin-3 has been purified and characterised. Whether differences between *FCN3* promoter sequences and differences in regulatory mechanisms between species may affect relative expression and protein translation of ficolin-3 remains to be elucidated.

The phylogenetic tree demonstrates that the ficolin precursor has gone through an expansion involving additional duplication events during evolution. Several species seem to have duplicated the ficolin precursor gene independently. The pairwise alignment of the amino acid sequence suggest that the mouse and rat ficolin-B is the orthologue of the human ficolin-1, with identities of 76 and 79%, respectively. Alignment of the human ficolin-1 protein with ficolins from other species shows that ficolin-1 is present in several species such as primates (ficolin-1), pig (ficolin-β), horse (ficolin-2), dog (ficolin-1), mouse (ficolin-B) and rat (ficolin-B). All of these ficolins show high amino acid homology (>76%). In addition, humans and pigs have an extra ficolin protein that is also homologous to the common 'ficolin-1'. These proteins are called ficolin-2 or L-ficolin and ficolin-α, respective-

ly. The ortholog of the human ficolin-2 was only observed in higher and lower primates, suggesting that the ficolin-2 gene diverged after branching of the mouse and primate lineages. Also, pigs seem to have duplicated their genes independently, since they cluster within the species. In general, the exon organisation of the different ficolins shows highly homologous structures. Most of the primary amino acid sequences in all the mammal ficolins align well. However, the amino acid sequence located in exons 4–6 (relative to the mouse ficolin-A) varies greatly among the ficolins (fig. 4). This region corresponds to exons 4 and 5 in mouse ficolin-B and human ficolin-1, and exon 4 of the human ficolin-2. When this stretch of 36 amino acids of the mouse ficolin-A is aligned to all other ficolins, it is clear that the human ficolin-1 corresponds to the mouse ficolin-B. Furthermore, this alignment illustrates that the mouse and rat ficolin-A constitute a separate group of proteins. Additionally, the primate ficolin-2 also seems to form its own ficolin group, supporting the analysis above. Based on these observations, it is therefore likely that 'ficolin-1' is a true orthologue that is present in all higher mammals, whereas the primate ficolin-2 and pig ficolin-α, as well as mouse and rat ficolin-A are the result of independent gene duplications in the different species. This implies that some mammals or mammal families have independently duplicated their 'ficolin-1' (*FCN1*) gene probably due to selection forces operating at different time periods. It remains to be established how pronounced this form for duplication of the 'ficolin-1' gene is in different species.

Tissue Expression

The *FCN1* gene is primarily expressed in peripheral blood leukocytes and in bone marrow-derived cells although minor expression is also observed in the spleen and the lung [26]. This is in agreement with studies showing that ficolin-1 is present in monocytes and weakly present in the spleen and type II alveolar cells in the lung [8, 19, 22, 25, 48]. It has been shown by real-time PCR examination of normal human bone marrow that *FCN1* is expressed largely in myelocytes, metamyelocytes and band cells with a profile quite similar to that of gelatinase [49]. In accordance with this, confocal microscopy and subcellular fractionation demonstrated that ficolin-1 is primarily localized in gelatinase granules of neutrophils, but also in highly exocytosable gelatinase poor granules not previously described [49]. The protein is released from neutrophil granules by stimulation with fMLP or

Fig. 4. Alignment of the mouse ficolin-A exons 4–6 with other ficolins. The amino acid sequence of exons 4–6 (relative to the mouse ficolin-A) in different species was aligned with mouse ficolin-A as reference sequence. This alignment shows that mouse and rat ficolin-A are different from the other ficolins. Furthermore, many of the known mammalian ficolins (except for ficolin-

3) must be considered as ‘ficolin-1’ related proteins. The primate ficolin-2 seems to represent a separate group of ficolins. mo = Mouse; ra = rat; hu = human; ch = chimpanzee; rm = rhesus monkey; gu = guinea pig; do = dog; pi = pig; ho = horse; jm = java macaque monkey.

PMA, but the majority (90%) becomes associated with the surface membrane of the cells. Altogether the neutrophils appear to be a major source and reservoir of ficolin-1. Ficolin-1 is also found in human serum and plasma, but the real concentration in healthy individuals is still a matter of debate, since we found a median concentration of 0.06 µg/ml (range 0.04–0.1 µg/ml) in a limited number of healthy donors [22], while a more recent study showed that the median concentration in a larger group of donors was around 1 µg/ml (range 0.28–4 µg/ml) [50]. Whether this reflects true biological differences or assay or sampling differences remains to be elucidated. Nevertheless, both studies suggest that ficolin-1 may play a role

as a secreted molecule of importance for local and systemic immune defence.

FCN2 is expressed mainly in the liver [10, 26]. Very low mRNA *FCN2* levels may also be detected in the bone marrow, in tonsils, in the intestine and in the foetal lung [26]. It is, however, unknown whether these expression patterns are also translated into ficolin-2 synthesis. Ficolin-2 is found with a median serum/plasma concentration in Caucasians of around 5 µg/ml, but with inter-individual variations ranging from 1–12 µg/ml [27–31].

FCN3 is expressed mainly in the liver and the lung, but lower expression is also observed in the heart, kidney, spleen, pancreas and placenta [26]. Ficolin-3 is the most

abundant of the 3 ficolins in serum, where it is found with a median concentration of 25 µg/ml (range 3–54 µg/ml) [38, 51, 52]. Of particular interest is the observation that the expression of *FCN3* in the lung exceeds that in the liver, suggesting a role of this protein in lung defence and tissue homeostasis along with the classical collagen-like lung defence proteins SP-A and SP-D [26]. Whether ficolin-3 also has a function in the pulmonary surfactant system remains to be elucidated. A low mRNA level was also detected in brain tissue, which is in agreement with a study showing *FCN3* expression in a human glioma cell line [53]. Thus, the 3 human *FCNs* seem to differ also with respect to their pattern of expression in various tissues, suggesting that these molecules play distinct physiological roles in different organs [35].

Variation in *FCN* Genes

Common genetic variations in the MBL gene (*MBL2*) affecting both the expression and structure of MBL have been studied extensively [54]. These genetic variations have in numerous studies been reported to be associated with different diseases [55]. Although the penetrance of *MBL2* genetic variation as a single parameter is debated, it is generally accepted that the presence of *MBL2* variant alleles is associated with increased risk of infections in individuals with accompanying comorbidity. More recently it has also been documented that highly expressing *MBL2* alleles and high MBL serum concentrations may be associated with adverse effects [56–58]. Thus, because of the similarities between MBL and the ficolins, systematic sequencing studies of the variation in the *FCN* genes in different ethnic groups have been performed [28, 59, 60].

The *FCN1* Gene

A large number of genetic variants with large race-related differences have been observed in the *FCN1* gene (table 1) [60]. Four *FCN1* exon variations result in amino acid substitutions (Gly43Asp, Arg93Gln, Trp279Stop, and Gly303Ser). Only 1 association study of *FCN1* polymorphisms has been published so far. This shows that 2 SNPs, which are in complete linkage disequilibrium – 1 in the promoter region and 1 synonymous SNP in exon 9 – are found with increased frequency in 2 different rheumatoid arthritis cohorts compared with controls [61]. The SNPs were not associated with the well-established HLA shared epitopes, rheumatoid factor or the presence of anti-citrullinated IgG antibodies. Still, we lack informa-

tion about the putative functional importance of *FCN1* polymorphisms. Recently, an interesting study was published showing that premature children with adverse outcome of necrotising enterocolitis had significantly lower levels of ficolin-1 than survivors [62]. Moreover, infants with low ficolin-1 required more frequent assisted ventilation than those with high ficolin-1 levels. Whether these observations can be attributed to inter-individual genetic differences remains to be established.

The *FCN2* Gene

A number of DNA variations have been observed in the *FCN2* gene (table 2) [60]. Three single base substitution polymorphisms in positions –986, –602 and –4, respectively, are associated with variation serum concentration of ficolin-2, while 2 other polymorphisms situated in positions –557 and –64 appear not to have a significant impact on the serum concentration of ficolin-2 [28, 31, 63]. Nevertheless, data showing the functional impact of these polymorphisms are lacking. In addition, several polymorphisms resulting in amino acid changes have been observed in the coding part of the *FCN2* gene (table 2). Some of these polymorphisms are specific for different ethnic groups while others are broadly distributed [60]. After the original report of the *FCN2* polymorphisms [28], some of the *FCN2* variants have also been demonstrated in Dutch, Japanese, Polish, English and Brazilian populations [59, 63–66]. Of particular interest are two *FCN2* amino acid substituting SNPs located in exon 8 encoding the FBG domain (Thr236Met and Ala258Ser) [28]. When serum from individuals homozygous for the wild type and the Thr236Met and Ala258Ser variants, was incubated with solid phase or soluble GlcNAc, great differences in their ability to bind to GlcNAc were observed [28] (fig. 5). The Ala258Ser variant showed elevated binding capacity compared to wild type, whereas variant Thr236Met exhibited reduced binding capacity. Similar results were observed in follow-up studies when wild type ficolin-2 and the Thr236Met variant produced in CHO cells were employed [67]. The observed SNPs are likely to cause specific alterations of the FBG domain, thus affecting the binding to ligands, but they are not located in the observed FBG binding sites that have been deduced based on crystallographic observations [44]. Two well-conducted studies have explored the putative importance of the *FCN2* polymorphisms and the possible importance in invasive pneumococcal disease in adults and self reported respiratory infections in children [65, 68], but no significant associations were observed. Rheumatic fever and chronic rheumatic heart

Table 1. Polymorphisms identified in the *FCN1* gene. Positions of polymorphisms are counted with respect to the *FCN1* translation start site with the A of ATG being +1 and the 5'-following base being -1

Base position	Major allele	Minor allele	dbSNP	Region	Amino acid change	Minor allele frequency			
						DNK (n = 60)	MOZ (n = 50)	GHA (n = 50)	JPN (n = 50)
-1233	A	C	rs56092718	promoter	–	0.00	0.00	0.05	0.00
-1180	G	T	rs7022634	promoter	–	0.00	0.26	0.24	0.00
-881	G	A	rs56402344	promoter	–	0.00	0.06	0.03	0.00
-807	G	A	rs56246087	promoter	–	0.00	0.00	0.01	0.00
-791	A	G	rs28909068	promoter	–	0.00	0.04	0.04	0.09
-756	C	T	rs3762083	promoter	–	0.00	0.00	0.00	0.01
-755	G	A	rs56225434	promoter	–	0.00	0.18	0.22	0.00
-631	G	A	rs55889197	promoter	–	0.00	0.00	0.01	0.00
-582	C	T	rs17039501	promoter	–	0.00	0.15	0.20	0.00
-542	G	A	rs10120023	promoter	–	0.31	0.14	0.06	0.06
-523	T	C	rs56005457	promoter	–	0.00	0.01	0.01	0.00
-472	C	T	rs56087167	promoter	–	0.00	0.04	0.00	0.00
-399	G	A	rs17039495	promoter	–	0.00	0.18	0.22	0.00
-271	T	dT	rs28909976	promoter	–	0.38	0.33	0.25	0.34
-210	G	Dg	rs56036264	promoter	–	0.00	0.00	0.01	0.00
-205	G	C	rs28909977	promoter	–	0.00	0.00	0.00	0.00
-160	C	G	rs56077839	promoter	–	0.00	0.04	0.02	0.00
-144	C	A	rs10117466	promoter	–	0.34	0.12	0.06	0.00
-64	C	T	rs55712611	promoter	–	0.00	0.01	0.00	0.00
+33	G	T	rs10858293	exon 1	Gly11Gly	0.33	0.27	0.28	0.06
+116	T	G	rs56330318	intron 1	–	0.00	0.00	0.01	0.00
+184	C	T	rs56045618	intron 1	–	0.00	0.01	0.00	0.00
+1435	G	A	rs10441778	exon 2	Gly43Asp	0.00	0.07	0.02	0.00
+1538	G	A	rs55871507	intron 2	–	0.00	0.01	0.00	0.00
+3144	C	T	rs56329856	intron 3	–	0.00	0.01	0.01	0.00
+3164	G	A	rs7863062	intron 3	–	0.00	0.17	0.20	0.00
+3231	C	T	rs2989722	intron 3	–	0.65	0.31	0.23	0.34
+3235	A	G	rs7042453	intron 3	–	0.00	0.44	0.45	0.09
+3292	G	A	rs56163380	intron 3	–	0.00	0.15	0.14	0.00
+3374	A	G	rs3012788	intron 3	–	0.35	0.25	0.34	0.57
+3384	C	T	rs2989721	intron 3	–	0.35	0.25	0.34	0.57
+3387	G	dG	rs11421281	intron 3	–	0.35	0.25	0.34	0.57
+3458	G	A	rs56345770	exon 4	Arg93Gln	0.00	0.07	0.09	0.01
+3512	G	A	rs56380216	intron 4	–	0.00	0.00	0.00	0.01
+4356	C	T	rs55862654	intron 5	–	0.00	0.17	0.22	0.00
+4408	C	A	rs55894555	intron 5	–	0.00	0.17	0.13	0.00
+4410	C	dC	rs11297508	intron 5	–	0.00	0.33	0.24	0.00
+4473	T	C	rs55743915	intron 5	–	0.00	0.00	0.03	0.00
+4701	G	A	rs55806386	intron 5	–	0.00	0.01	0.00	0.00
+4888	G	C	rs2070622	intron 6	–	0.34	0.06	0.12	0.58
+5358	C	T	rs2274845	exon 7	Asn190Asn	0.37	0.06	0.11	0.57
+5370	C	T	rs56308117	exon 7	His194His	0.00	0.01	0.00	0.00
+7918	G	A	rs1071583	exon 9	Gln275Gln	0.37	0.01	0.06	0.56
+7929	G	A	rs56094122	exon 9	Trp279Stop	0.00	0.00	0.02	0.00
+8000	G	A	rs56151153	exon 9	Gly303Ser	0.00	0.01	0.00	0.00

DNK = Denmark; MOZ = Mozambique; GHA = Ghana; JPN = Japan; n = number of individuals analysed.

Table 2. Polymorphisms identified in the *FCN2* gene. Positions of polymorphisms are counted with respect to the *FCN2* translation start site with the A of ATG being +1 and the 5'-following base being -1

Base position	Major allele	Minor allele	dbSNP	Region	Amino acid change	Minor allele frequency				
						DNK (n = 60)	MOZ (n = 50)	GHA (n = 50)	JPN (n = 50)	ARG (n = 50)
-1249	C	A	rs55848654	promoter	-	0.00	0.13	0.13	0.00	0.00
-1232	T	C	rs55669323	promoter	-	0.00	0.01	0.00	0.00	0.02
-1012	A	G	rs56014433	promoter	-	0.00	0.01	0.01	0.00	0.00
-1004	C	A	rs55690104	promoter	-	0.00	0.00	0.01	0.00	0.00
-986	G	A	rs3124952	promoter	-	0.49	0.24	0.20	0.12	0.45
-959	G	A	rs55726384	promoter	-	0.00	0.05	0.00	0.00	0.00
-902	C	A	rs3811143	promoter	-	0.00	0.02	0.00	0.12	0.02
-602	G	A	rs3124953	promoter	-	0.19	0.02	0.00	0.10	0.22
-557	A	G	rs3811140	promoter	-	0.12	0.18	0.31	0.14	0.17
-251	G	A	rs55809110	promoter	-	0.00	0.13	0.12	0.00	0.00
-214	G	A	rs12344051	promoter	-	0.00	0.05	0.06	0.00	0.00
-171	C	T	rs3811139	promoter	-	0.00	0.00	0.00	0.06	0.00
-64	A	C	rs28969369	promoter	-	0.02	0.22	0.32	0.15	0.16
-4	A	G	rs17514136	promoter	-	0.24	0.22	0.20	0.04	0.20
+33	C	T	rs55797213	exon 1	Gly11Gly	0.00	0.10	0.08	0.00	0.00
+125	T	C	rs3128627	intron 1	-	0.08	0.00	0.00	0.01	0.00
+1766	G	A	rs55865317	exon 2	Gly54Gly	0.00	0.07	0.04	0.00	0.00
+1878	T	C	rs3124955	intron 2	-	0.33	0.18	0.17	0.45	0.21
+2472	A	G	rs3128624	intron 2	-	0.31	0.30	0.31	0.46	0.21
+2488	T	C	rs4520243	exon 3	Arg74Arg	0.34	0.30	0.31	0.47	0.21
+2545	G	A	rs7037264	intron 3	-	0.38	0.71	0.74	0.45	0.57
+3980	C	T	rs55985459	intron 4	-	0.00	0.00	0.02	0.00	0.00
+4423	C	T	rs55895215	exon 5	Arg103Cys	0.00	0.01	0.00	0.00	0.00
+4466	G	A	rs12684476	exon 5	Gly117Ser	0.00	0.00	0.00	0.08	0.00
+4526	C	T	rs56084543	exon 5	Thr137Met	0.00	0.01	0.01	0.00	0.00
+4588	G	A	rs55834586	intron 5	-	0.00	0.06	0.04	0.00	0.00
+4888	C	T	rs56200327	intron 5	-	0.00	0.01	0.01	0.00	0.00
+4957	G	A	rs56235352	exon 6	Arg147Gln	0.00	0.01	0.00	0.00	0.00
+4987	G	A	rs56281005	exon 6	Arg157Gln	0.00	0.00	0.00	0.01	0.00
+5060	C	T	rs34789496	exon 6	His181His	0.03	0.00	0.01	0.14	0.17
+5070	G	A	rs55860122	exon 6	Ala185Thr	0.00	0.00	0.00	0.01	0.00
+5121	G	A	rs12684723	intron 6	-	0.13	0.16	0.24	0.15	0.17
+6359	C	T	rs17549193	exon 8	Thr236Met	0.25	0.45	0.37	0.04	0.22
+6424	G	T	rs7851696	exon 8	Ala258Ser	0.10	0.17	0.25	0.14	0.18
+6459	C	T	rs55874707	exon 8	Asn269Asn	0.00	0.02	0.02	0.00	0.00
+6443_44	CT	A	rs28357091	exon 8	Ala264fs	0.01	0.00	0.00	0.00	0.00

DNK = Denmark; MOZ = Mozambique; GHA = Ghana; JPN = Japan; ARG = Argentina; n = number of individuals analysed.

disease has been investigated for association with promoter polymorphisms in the *FCN2* gene [69]. These latter results show that the *FCN2* haplotype (-986/-602 and -4 G/G/A) that is associated with the lowest ficolin-2 serum [31] is also associated with chronic rheumatic heart disease. Conversely, a protective effect of an *FCN2* haplotype associated with higher ficolin-2 serum levels has been observed in relation to clinical leprosy [66]. Although, these findings are very interesting, they need to be confirmed

in independent studies and the possible biological mechanisms remain to be elucidated.

The *FCN3* Gene

Only few DNA variations have been detected in *FCN3* including 5 amino acid exchanging variants (table 3) [60]. All of the variations are present in very low frequencies.

Investigations of serum from several hundred thousand individuals show that ficolin-3 deficiency is a rare

Fig. 5. Binding of ficolin-2 variants to GlcNAc. **a** Dilutions of human serum representing ficolin-2 variants were added to GlcNAc-coated ELISA wells and bound ficolin-2 was measured. GlcNAc-binding is given as index values based on OD values (490 nm) from the GlcNAc-ELISA assay divided with the ficolin-2 concentration in the full serum samples (mg/l) in order to compensate for sample differences in ficolin-2 concentrations. A difference in binding profile towards GlcNAc was observed for the investigated ficolin-2 variants wild type, Thr236Met and Ala258Ser, respectively, indicating changed functionality due to the polymorphisms. Each graph represents the average of 3 individuals with

identical genotype. Standard error of the mean is indicated. No binding was observed to BSA (negative control). **b** Human serum variants were incubated with GlcNAc-agarose beads and ficolin-2 was eluted with GlcNAc. Ficolin-2 concentrations were measured in ELISA in full serum and eluates from ficolin-2-purified samples, and the purification yield was subsequently determined. The Ala258Ser variant showed a marked increase in binding capacity towards GlcNAc compared to the wild type and Thr236Met variants. Each bar represents 3 individuals. Standard error of the mean is indicated. Adapted from [28].

condition. Ficolin-3 was found in all of the sera from more than 10,000 Japanese blood donors and in virtually all sera from several 100,000 Japanese patients [33]. However, a frame-shift variation Leu117fs in exon 5 of the *FCN3* gene in position *FCN3*+1637 (rs28357092) has been observed [28]. This variant may be found in healthy Caucasian and African populations, with an allele frequency of 0.01 and 0.04, respectively [52]. The variant causes a reading frame shift and an altered amino acid composition of the C-terminal end of ficolin-3, spanning from amino acid position 117 to position 180, where the non-sense protein sequence is terminated due to an early stop codon (fig. 6). Theoretically, this causes a truncated ficolin-3 variant (Leu117fs) lacking 183 amino acids of the sequence corresponding to the entire FBG domain. Nevertheless, experiments with recombinant ficolin-3 containing the Leu117fs variant show that the variant protein cannot be expressed and would therefore most likely lead

to complete ficolin-3 deficiency in vivo [52]. To further substantiate these seminal findings, we screened 1,282 patients with suspected primary immunodeficiency for the *FCN3delC+1637* Leu117fs variant [70]. We found that 1.8% were heterozygous, which is the same frequency as in the healthy population, while 1 patient was homozygous. The index patient suffered from severe unexplained recurrent bacterial lung infections, bronchiectasis, lung fibrosis and obstructive lung disease and had experienced cerebral abscesses. In addition, the patient had undergone splenectomy due to unexplained thrombocytopenia. He also had large warts on his fingers. In the patients investigated, the median ficolin-3 serum concentration in wild types was 27.5 µg/ml (range 3.5–42.6 µg/ml) while it was 14.1 µg/ml (range 8.0–31.4 µg/ml) in *FCN3delC+1637* heterozygotes, showing that heterozygous carriers may in general have a 50% reduction in ficolin-3 serum concentration. No ficolin-3 antigen could

Fig. 6. Illustration of ficolin-3 structure and the effect of *FCN3*+1637delC frame-shift variation. The upper panel shows a model of the oligomerisation into higher orders and the domains of the ficolin-3 monomer structure in wild type and the mutated polypeptide. Exon regions of *FCN3* are marked with black vertical lines. The *FCN3*+1637delC_(L117fs) frame shift (fs) variant (mutation) is represented by a red dot. Adapted from [70].

Table 3. Polymorphisms identified in the *FCN3* gene. Positions of polymorphisms are counted with respect to the *FCN3* translation start site with the A of ATG being +1 and the 5'-following base being -1

Base position	Major allele	Minor allele	dbSNP	Region	Amino acid change	Minor allele frequency			
						DNK (n = 60)	MOZ (n = 50)	GHA (n = 50)	JPN (n = 50)
-954	C	T	rs41465749	promoter	-	0.01	0.02	0.00	0.00
-813	G	A	rs56174383	promoter	-	0.00	0.01	0.00	0.00
-608	G	A	rs28385648	promoter	-	0.02	0.00	0.00	0.00
-325	G	A	rs41415450	promoter	-	0.11	0.00	0.00	0.00
+34	C	G	rs41366544	exon 1	Leu12Val	0.01	0.00	0.00	0.00
+51	G	A	rs56088921	exon 1	Gly17Gly	0.00	0.00	0.01	0.00
+1402	T	C	rs56088921	intron 4	-	0.00	0.00	0.02	0.00
+1549	T	C	rs28385723	intron 4	-	0.00	0.01	0.01	0.00
+1637	C	dC	rs28357092	exon 5	Leu117fs	0.01	0.04	0.00	0.00
+1663	A	G	rs56407240	exon 5	Thr125Ala	0.00	0.04	0.00	0.00
+3836G	C	G	rs3813800	intron 5	-	0.00	0.06	0.06	0.05
+3836dC	C	dC	rs3813800	intron 5	-	0.01	0.00	0.00	0.00
+3836A	C	A	rs3813800	intron 5	-	0.00	0.01	0.00	0.00
+3951	G	C	rs56405086	exon 6	Glu166Asp	0.00	0.00	0.00	0.04
+5543	T	C	rs55863154	exon 8	Val287Ala	0.00	0.01	0.00	0.00

DNK = Denmark; MOZ = Mozambique; GHA = Ghana; JPN = Japan; n = number of individuals analysed.

be detected in the *FCN3+1637delC* homozygous patient. The structure based on SDS-PAGE and Western blotting appeared the same in wild type and in heterozygotes. Complement deposition on an acetylated compound was absent in ficolin-3 deficient serum, but could be reconstituted with recombinant ficolin-3.

Complement deficiencies are usually associated with increased risk for invasive bacterial infections or for developing autoimmune diseases. The index patient suffered from severe disabling respiratory and intracerebral abscesses. This is in agreement with the high expression of ficolin-3 in the lungs as well as the expression by glioma cells as previously mentioned [26, 35, 53]. The thrombocytopenia may have had an autoimmune origin, but this awaits further characterization. Ficolin-3 has also been shown to be important in sequestration of dying host cells [71, 72]. It is therefore tempting to speculate that deficiency of ficolin-3 hampers the removal of cellular debris and endogenous waste material, thus aggravating and perpetuating ongoing infectious events and inflammatory processes without being associated with a particular infection per se. Taken together these findings show that genetically determined deficiency of ficolin-3 might cause a novel complement deficiency syndrome. Recently, it has been reported that children with malignancies treated with chemotherapy who had low ficolin-3 serum levels (<14 µg/ml) had more episodes of fever and were more neutropenic than children with higher ficolin-3 concentrations [73]. Whether these findings may be linked to heterozygosity for the *FCN3+1637delC* Leu117fs

variant remains to be established. It is, however, striking that the median serum concentration in *FCN3+1637delC* Leu117fs variant heterozygotes was 14.1 µg/ml, which is the ficolin-3 cut off value reported in the chemotherapy study [73].

Concluding Remarks

It appears from database search and phylogenetic tree analysis that the most ancient ficolin molecule is ficolin-3, which then evolved to the other ficolins. However, each branch of the phylogenetic tree creates new ficolins. Nevertheless, based on in silico analysis it is likely that 'ficolin-1' is a true ortholog protein present in all higher mammals, whereas for instance primate ficolin-2 and pig ficolin-α are the results of independent gene duplications in the different species. The ficolins may be assumed to be involved in the pathophysiology of different infectious and inflammatory diseases. Although the literature in this field is rudimentary, several lines of evidence suggest that polymorphisms or haplotypes in the *FCN1* and *FCN2* genes may be associated with different diseases. A striking example is the observation that genetically determined deficiency of ficolin-3 might be associated with immunodeficiency in the humans. Identification of *FCN* variants associated with clinical outcomes is a new and blooming field, which should be thoroughly investigated in the years to come.

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