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MASP1 and *MASP2*

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Synonyms

MASP1: Mannan-binding lectin serine protease 1; Complement-activating component of Ra-reactive factor; CRARF; Crarf; MASP; PR555; P100; *MASP2*: Mannan-binding lectin serine protease 2

Historical Background

Ikeda and colleagues were the first to refer to MASPs, in 1987. They described MASP-1 as the activating component of the mouse bactericidal Ra-reactive factor (RaRF). RaRF circulates in plasma as a complex of mannose-binding lectin (MBL) and serine proteases, activating the complement factors C4 and C2 upon binding to lipopolysaccharides in Ra chemotype Salmonella (Gram-negative) bacteria (Ikeda et al. 1987). Five years later, Matsushita and Fujita identified MASP-1 in serum, complexed with MBL (Matsushita and Fujita 1992). Thiel et al. identified a second MASP in 1997, named MASP-2 (Thiel et al. 1997). In 2001, Dahl and colleagues designated another protease as MASP-3, due to its homology to the other MASPs. The *MASP1* gene encodes MASP-1, MASP-3, and a truncated protein of 44 kDa called MAp44, by alternative splicing of the primary mRNA transcript (MAp44 was independently described by Degn et al. in 2009 and Skjoedt et al. in 2010 – they called it MAP-1) (Degn et al. 2009; Skjoedt et al. 2010a). By the same process, *MASP2* encodes MASP-2 and the truncated protein of 19 kDa, MAp19 (independently described by Stover et al. in 1999 and by Takahashi et al. in 1999, who also called it sMAp or small MAp)(Stover et al. 1999b; Takahashi et al. 1999).

Introduction

MASP1 and *MASP2* are two genes encoding mannose-binding lectin-associated serine proteases (or MASPs) and truncated mannose-binding lectin-associated proteins (MAps). These gene products are part of the complement system, which comprises more than 50 proteins acting in the first line of host defense against infectious organisms and linking innate and adaptive immunity. Three different pathways activate the complement system: the classical, the alternative, and

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the lectin pathway. All three converge in a proteolytic cascade that culminates in opsonization and phagocytosis, inflammation and apoptotic cell removal, formation of the membrane attack complex (MAC), and cell lysis (Walport 2001; Ricklin et al. 2010). *MASP1* and *MASP2* products activate (MASP-1 and MASP-2) or regulate (MASP-3 and MAp44) the lectin pathway of complement (the function of another MAp - MAp19 is still unclear, and MASP-3 also activates the alternative pathway) (Dunkelberger and Song 2010; Kjaer et al. 2013; Dobó et al. 2016).

Structure of MASPs

MASP-1, -2, and -3 are secreted as single-chain proenzymes (zymogens). They become activated through cleavage of a single Arg-Ile bond (the "activation peptide"), resulting in conformational changes and generation of two polypeptide chains A (heavy chain) and B (light chain), linked by a disulfide bond (Thiel 2007). The heavy chain comprises five domains. The N-terminal CUB1 (C1r/C1s, urchin-EGF, and bone morphogenetic protein-1) domain and the epidermal growth factor Ca2+-binding (EGF)-like domain are responsible for Ca2+-dependent opposite dimerization of mature MASP monomers. A second CUB domain (CUB2) and two contiguous complement control protein modules (CCP1 and CCP2) complete the highly conserved heavy chain structure. A short linker unites the A chain with a chymotrypsin-like serine protease (SP) domain, which constitutes the B chain. The two SP domains of a MASP zymogen homodimer are complexed with MBL and protrude from the cone defined by its collagenous stalks. This turns them accessible to any adequate substrate, including SP domains of neighbor zymogens complexed with other pattern recognition molecules (PRMs) (Kjaer et al. 2013; Kjaer et al. 2015). MASP-1 and MASP-3 differ only in their SP domains (Dahl et al. 2001). MAps have no SP domain and thus lack enzymatic activity (Stover et al. 1999a; Takahashi et al. 1999; Degn et al. 2009; Skjoedt et al. 2010a).

MASP Evolution

There are many similarities between MASPs and the serine proteases of the classical pathway, C1r and C1s (Takada et al. 1993; Thiel et al. 1997). In fact, they share the same domain organization with C1r and C1s, but have different substrate specificities and different physiological functions (Gál et al. 2009). The coding sequence of the serine protease domain in MASPs clearly diverge by the presence of introns and by the codon for the active center serine residue. There are introns splitting the coding gene sequence of MASP-1 and chymotripsin. In these genes, the active serine residue is encoded by a TCN codon (where "N" means any base). Retrotransposition of the MASP-3-like factor B coding sequence in ascidians, followed by gene duplication events, might have originated the intron less exon coding for the serine protease domain of MASP-2, MASP-3, C1r, and C1s, where the active serine residue is encoded by AGY (where "Y" means C or T). Furthermore, MASP-1 has a "histidine loop" structure typical of trypsin serine proteases, which is absent in MASP-2. The MASP-1 serine residue and the "histidine loop" can also be observed in MASPs from ascidians, suggesting that "TCN type" proteases (so as human MASP-1) are ancestral to "AGY type" proteases (including MASP-2), during the evolution of the MASP/C1r/C1s family (Ji et al. 1997; Endo et al. 1998). Thus, MASP-1 most probably represents the ancestral protease of the lectin pathway, which emerged in Cnidaria (Nonaka and Miyazawa 2002; Kimura et al. 2009).

Expression of MASPs/MAps

MASP1 encodes MASP-1 and MASP-3 and the nonenzymatic MBL-associated protein MAp44, whereas *MASP2* encodes MASP-2 and the nonenzymatic MBL-associated protein MAp19. Whereas liver hepatocytes mainly express MASP-1 and MASP-2, Kupffer cells express MAp19. High levels of MAp19 were also detected in urine (Degn et al. 2011a). On its turn, the female reproductive tract, colon, and prostate express MASP-3 mRNA, whereas heart cells

(left ventricle) and skeletal muscle cells express MAp44 (data of the GTEX Portal database and (Thiel et al. 1997; Degn et al. 2009; Skjoedt et al. 2010b)).

Complement and MASPs/MAps

All proteins, including MAp19 and MAp44, form complexes with the pattern recognition molecules (PRMs) of the lectin pathway: MBL, the ficolins (H-, L-, and M-ficolin), and collectin-11. The complexes most often constitute of one MASP homodimer with one PRM, but a PRM may also include different homodimers or a heterodimer (Degn et al. 2013b; Rosbjerg et al. 2014). Besides that, free circulating homo- or heterodimers of MASPs and MAps may occur (Rosbjerg et al. 2014). The PRMs bind to carbohydrates or acetylated residues on microorganism surfaces (pathogen-associated molecular patterns or PAMPs) or to aberrant glycocalyx patterns on apoptotic, necrotic, or malignant cells (damage-associated molecular patterns or DAMPs) (Ricklin et al. 2010). Upon binding, MASP-1 and MASP-2 convert from pro-enzymes to its active forms and activate the lectin pathway of the complement cascade (Fig. 1). In contrast, MASP-3 activates the alternative pathway through pro-factor D cleavage (Dobó et al. 2016) and inhibits the activation of the lectin pathway by competing with MASP-1 and MASP-2 for binding sites on PRMs (Degn et al. 2009). The truncated protein MAp44 inhibits complement activation by competing with binding sites, analogous to MASP-3 (Skjoedt et al. 2012). Although found in complex with PRMs, the physiological function of MAp19 is still unclear.

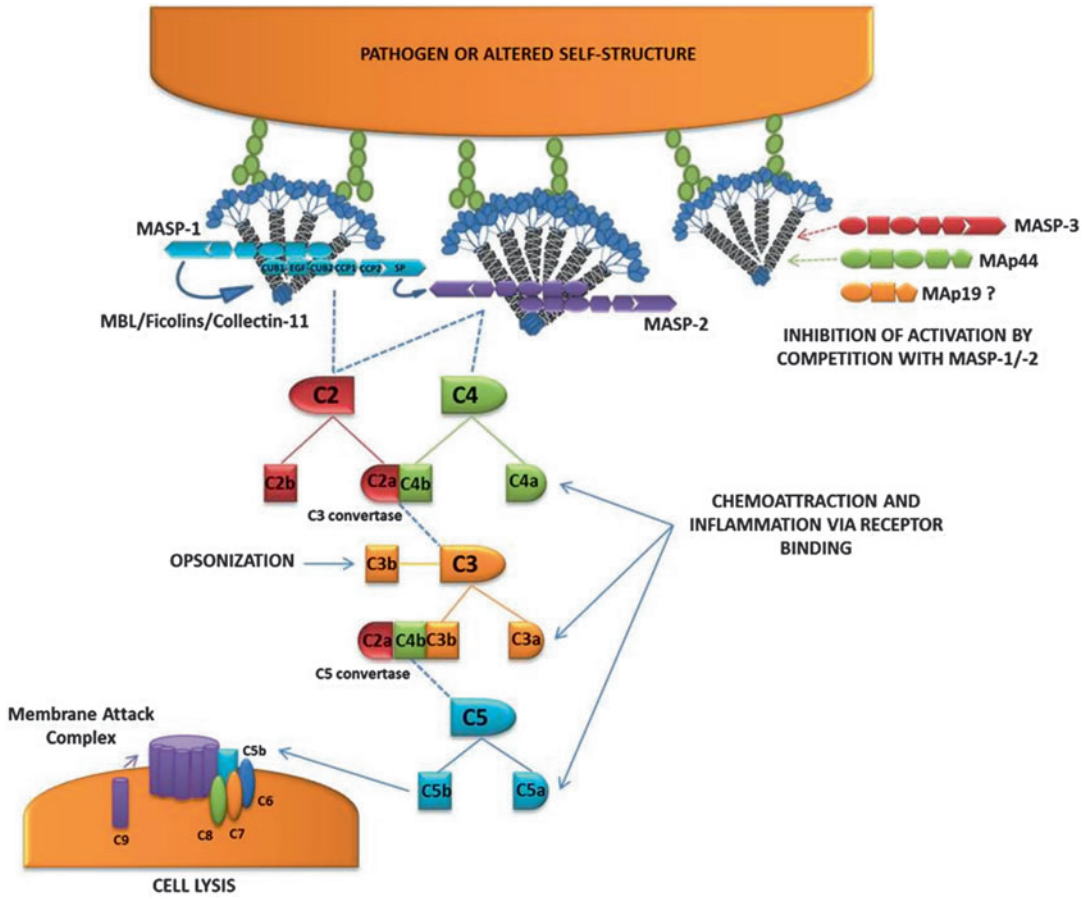
Protein Functions

MASP-1

MASP-1 protein has a leader peptide of 19 amino acids and 699 amino acid residues. The A-chain has 429 residues (66 kDa), whereas the B-chain with the SP domain has 251 residues (31 kDa). Normal levels of MASP-1 in serum/plasma are around 11 μg/mL (range 4–30 μg/mL), 20-fold higher than those of MASP-2. At one year of age, MASP-1 is already present at adult level, reaching 60% of this value at birth (Thiel et al. 2012).

The lectin pathway becomes activated through MASP-1 autoactivation. In MASP-1 homodimers complexed with PRMs juxtaposed on an appropriate binding surface; autoactivation occurs in two steps: first by zymogen autoactivation (one MASP-1 zymogen activates another one of the same kind) and second by autocatalytic activation (an already activated proteinase activates another one of the same kind). The activated MASP-1 is essential for the activation of MASP-2 and probably MASP-3 (Dobó et al. 2016). Compared with MASP-2, the rate of MASP-1 zymogen autoactivation is 3000-fold higher, and its rate of autocatalytic activation is about 140-fold faster (Megyeri et al. 2013). Activation seems to occur through intercomplex cross-activation, most probably by clustering and juxtaposition of PRM/MASP-1 and PRM/MASP-2 complexes on ligand surfaces. Therefore, deficiency of MASP-1 can preclude MASP-2 activation and, consequently, block activation of the lectin pathway (Degn et al. 2014). Activated MASP-1 cleaves C2, generating 60% of C2a components necessary for building C3 convertases. Nevertheless, it cannot cleave C4 and is thus not sufficient for activating the lectin pathway by itself (Møller-Kristensen et al. 2007; Héja et al. 2012). Although MASP-1 cleaves C3 in vitro, this is physiologically irrelevant (Ambrus et al. 2003).

MASP-1 broad substrate specificity relies on its open binding groove, which resembles thrombin and trypsin and is very atypical for complement serine proteases. Thus, in the light of the common evolutionary pathway of complement and coagulation cascades, it is not surprising that MASP-1 has thrombin-like activity, inducing clot formation by mediating the formation of cross-linked fibrin. Even more so, in the presence of glycosaminoglycans, antithrombin inhibits MASP-1 with higher efficiency than C1-inhibitor, which is a common inhibitor of complement proteases (Gál et al. 2009). Although with a lower catalytic efficiency and in a different



***MASP1* and *MASP2*, Fig. 1** Activation of the lectin pathway. The PRMs, MBL, ficolins (-1, -2 or -3), or collectins (-10, -11), complexed with MASP-1 or -2, bind to PAMPs or DAMPs on cell surfaces. Upon binding, pro-enzyme MASP-1 autoactivates and is converted to its active form, being able to activate MASP-2 and cleave the complement factor C2. MASP-2 subsequently cleaves C4. The activation of this pathway results in the formation of the membrane attack complex (MAC), opsonization, and chemoattraction of other immune cells and inflammation

manner than thrombin, MASP-1 preferentially cleaves the Val34 variant factor XIII and the α-chain of fibrinogen at a site generating only fibrinopeptide B (not fibrinopeptide A). Interestingly, this fibrinopeptide attracts neutrophils to coagulation sites, which sums up to the pro-inflammatory effects of MASP-1 activities (Krarup et al. 2008). MASP-1 also directly activates the carboxypeptidase thrombin-activatable fibrinolysis inhibitor (TAFI), preventing fibrinolysis (Hess et al. 2012). MASP-1 also cleaves prothrombin at three cleavage sites, giving rise to an alternative form of thrombin. Furthermore, MASP-1 and thrombin effects are synergistic on clot formation (Jenny et al. 2015b).

The thrombin-like activity also enables MASP-1 to cleave protease-activated receptor 4 (PAR4) in endothelial cells. This induces Ca2+ signaling and NFkappaB and p38 MAPK pathways (Megyeri et al. 2009), leading to the release of IL-6 and IL-8, and activating the chemotaxis of neutrophil granulocytes (Jani et al. 2014). MASP-1 is also able to modulate the immune response by the release of pro-inflammatory bradykinin, able to cause vasodilatation, and increase vascular permeability, from high-molecular weight kininogen (Dobó et al. 2011).

MASP-2

The amino acid sequence of MASP-2 protein is 52% similar to MASP-1. MASP-2 has a leader peptide of 15 amino acids and 686 amino acid residues. The A-chain has 429 residues (52 kDa), whereas the B-chain with the SP domain has 242 residues (31 kDa) (Thiel et al. 1997). MASP-2 levels in serum/plasma are around 400–500 ng/mL (range 70–1200 ng/mL) and stable over time in healthy individuals (Møller-Kristensen et al. 2003; Ytting et al. 2007).

Although MASP-2 is able to autoactivate in vitro, MASP-1 is essential for its activation under physiological conditions. Once activated, it becomes a potent activator of both MASP-2 and MASP-1 zymogens, through cross-activation of juxtaposed PRM-MASP complexes (Degn et al. 2012) (Héja et al. 2012). MASP-2 cleaves the complement components C4 and C2, which associate to form the C3 convertase C4bC2b, common to the lectin and classical pathways. Compared with C1s of the classical pathway, MASP-2 has a 1000-fold higher catalytic activity, and C1-inhibitor inhibited it 50-fold faster (Kerr et al. 2008).

In the coagulation cascade, MASP-2 cleaves prothrombin in a manner similar to factor Xa, coating pathogens recognized by MBL or ficolins with a covalently linked fibrin clot (Krarup et al. 2007). Nevertheless, its procoagulant activity is dependent on MASP-1, since its activation is MASP-1 dependent, and its plasma levels are much lower. As MASP-1, it generates fibrinopeptide fragments that help to initiate the innate immune response and limit dissemination of infection (Gulla et al. 2010). Inhibitors of the coagulation pathway like aprotinin and TFPI (tissue factor pathway inhibitor) do also inhibit MASP-2 activity in vitro (Petersen et al. 2000; Keizer et al. 2015).

MASP-3

Phylogenetically, MASP-3 is a highly conserved serine protease. Sequence identities of the human, shark, and carp MASP-3 A chains were higher than 60%, whereas those of the MASP-1 and MASP-2 chains are half of that, around 30%. Regarding B chains, these values are higher than 60% for shark and carp and reach more than 90% with pig and rat. MASP-3 is formed of 728 amino acids, has a regulatory domain (A chain) similar to MASP-1, containing the CUB1, EGF, CUB2, CCP1, and CCP2 domains. The SP domain (B-chain) is, however, unique to this protein and encoded by a single MASP1 exon (Dahl et al. 2001). MASP-3 is constantly expressed in serum with a medium concentration of 5.2–6.4 μg/mL, ranging from 1.8 to 12.9 μg/mL (Skjoedt et al. 2010b; Degn et al. 2010).

MASP-3 circulates complexed with PRMs, mostly with ficolin-3 (Skjoedt et al. 2010b). It is the only proteinase able to activate pro-factor D of the alternative complement pathway in "resting blood" (independent of activation of the coagulation or complement cascades) (Oroszlán et al. 2015; Dobó et al. 2016; Banda et al. 2016). It also cleaves pro-factor B in vitro (Iwaki et al. 2011) and peptide substrates with the sequence Lys/Arg-Ile/Leu-Phe/Tyr (Yongqing et al. 2013). However, otherwise than MASP-1 and MASP-2, MASP-3 competes with MASP-1 and MASP-2 for binding sites on the recognition molecules, which may result in decreased activation of the lectin pathway (Degn et al. 2009). Nevertheless, it seems that once MASP-1 and MASP-2 are associated with MBL, they cannot be replaced by MASP-3 (Laursen et al. 2012). In contrast to the other MASPs, MASP-3 is not inhibited by C1-inhibitor. Therefore, different mechanisms are probably involved in MASP-3 activation and control, than those of MASP-1 and -2 (Zundel et al. 2004).

MASP-3 is also able to cleave insulin-like growth factor binding protein 5 and may have a role in embryogenesis (Cortesio and Jiang 2006). In fact, rare mutations in exon 12 of *MASP1* (*p.H497Y*, *p.C630R*, *p.G666E*, *p.G687R*, and *p.W290X*), which apparently abrogate MASP-3 enzymatic activity, were found in four affected families with autosomal recessive 3MC syndrome. Since this syndrome implies in various developmental disorders, it seems that MASP-3, together with CL-K1, is involved in early embryonic development (Sirmaci et al. 2010; Rooryck et al. 2011).

MAp44

MAp44 (also called MAP-1 or MBL-associated protein 1) is a truncated protein of the *MASP1* gene and has a regulatory domain similar to MASP-1 and MASP-3, but without the CCP2 domain. It lacks the SP domain and has, instead, a C-terminal peptide. Normal serum levels of this protein are around 1.5 μg/mL, ranging from 0.3 to 3.2 μg/mL (Degn et al. 2009, 2010). A light decrease of its concentration in serum can be observed during the first 6 months after birth (Degn et al. 2010).

Similarly to MASP-3, MAp44 apparently downregulates complement activation by competition for PRM binding sites with the other MASPs (Degn et al. 2009, 2013a; Skjoedt et al. 2010b; Pavlov et al. 2012). Otherwise than MASP-3, MAp44 can displace MASP-1 and -2 that are associated with MBL (Degn et al. 2013a).

MAp19

MAp19 or sMAP, a truncated *MASP2* product of 19 kDa, contains the same CUB1 and EGF-like domains of MASP-2 and has additionally four unique amino acids at the C-terminal end, encoded by its specific *MASP2* exon 5. This protein lacks the catalytic domain and, thus, has no serine protease activity (Thiel et al. 1997; Yongqing et al. 2012). Median levels of MAp19 in plasma are 217 ng/mL, ranging from 26 to 675 ng/mL, and of 63 ng/ml in urine (Degn et al. 2011a).

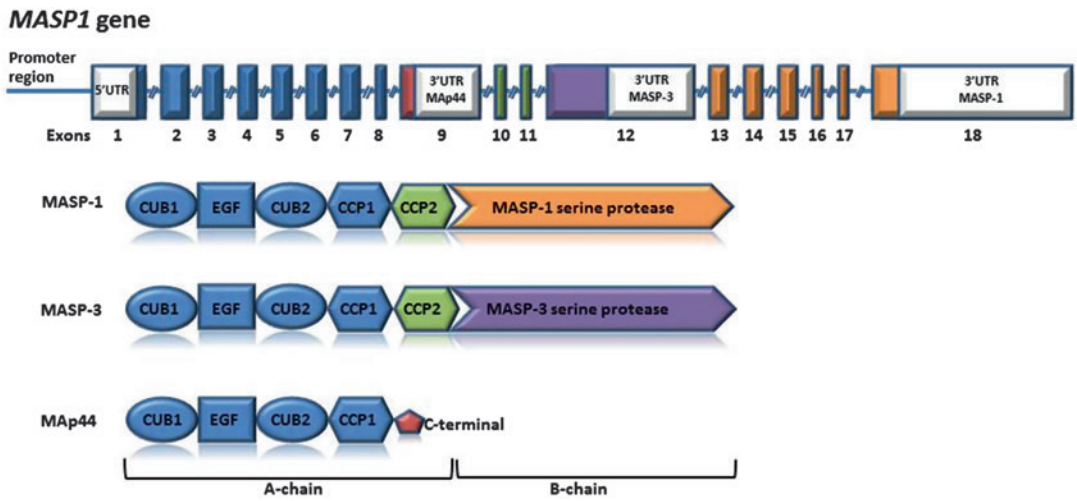
MAp19 forms homodimers via CUB1 and EGF domains with PRMs in a calcium-dependent manner, like MASP-2 (Gregory et al. 2004), but with 10 times lower affinity, being not able to compete with other MASPs for PRMs binding sites (Degn et al. 2011a). In human urine, MAp19 may prevent calcium oxalate renal stone formation (Kang et al. 1999; Degn et al. 2011a). MAp19 interacts with the nucleocapsid N protein of the severe acute respiratory syndrome coronavirus in vitro, but the functional significance of this remains unclear (Liu et al. 2009).

Gene Structure and Regulation

MASP1

The *MASP1* gene has a length of 5276 nucleotides and 18 exons (Fig. 2). It is located on the reverse strand of the long arm of chromosome 3, on 3q27-q28 (Sato et al. 1994; Takada et al. 1995) between the coordinates 3: 187,217,285 - 187,292,022 (human genome version GRCh38; http://www.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000127241;r=3:187217285-187292022).

MASP1 has a regulatory region with unmethylated CpG sites, extending from the promoter to intron 2, which harbors a strong enhancer with sites recognized by CEBP (CAAT-enhancer binding proteins) factors. This region also presents high levels of acetylated lysine 27 of histone 3 (H3K27), a histone modification typical of expressed genes. There are several CTCF (CCCTC-binding factor) binding sites distributed from exons 6–8 of the gene, which may regulate gene expression (data of the ENCODE project, available in https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr3%3A186951870-187009810&hgsid=568782047_CGqWyHye7aCjuQUmwibBtbC4JmP8). *MASP1* generates three different mRNAs by alternative splicing of a primary transcript in the mutually exclusive splice region located between exons 8 and 13, and encodes the proteins MASP-1, MASP-3, and MAp44 (Degn et al. 2009). Exon 1 harbors the untranslated sequence (5'UTR), but the last five nucleotides are translated to all three *MASP1* products. The five CUB1-EGF-CUB2-CCP1-CCP2 domains of MASP-1 and MASP-3 (forming the A-chain) are encoded by the exons 1–8, 10–11. The serine protease (SP) domains of MASP-1 and MASP-3 (B-chain) are unique to each one of them and encoded by exon 12 (MASP-3) and exons 13–18 (MASP-1). MAp44, otherwise than MASP-1 and MASP-3, does not present the CCP2 and SP domains, but has a unique C-terminal domain encoded by alternative exon 9 (Dahl et al. 2001; Degn et al. 2009; Skjoedt et al. 2010a).



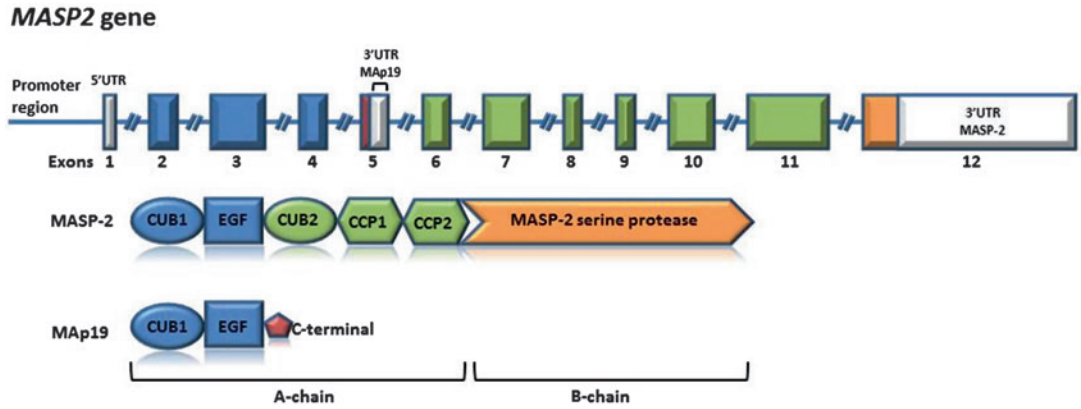
***MASP1* and *MASP2*, Fig. 2** *MASP1* gene and MASP-1, MASP-3, and Map44 protein structures. Exons encoding protein domains and corresponding regions in the protein structure are shown with the same color. The domains of the A-chain are indicated by *blue* and *green* colors. The MASP-1 and MASP-3 serine protease domain are in *orange* and *purple*, respectively, and the C-terminal domain of Map44 is in *red*. *MASP* mannose-binding lectin-associated serine protease, *MAp44* mannose-binding lectin-associated protein of 44 kDa, *CUB* C1r/C1s, Uegf, and bone morphogenetic protein, *EGF* epidermal growth factor, *CCP* complement control protein. Exons are drawn to scale and introns are truncated

MASP2

The *MASP2* gene, located on the reverse strand of chromosome 1p36.22, between the genomic coordinates 1: 11,026,523-11,047,233 (human genome version GRCh38), comprises 12 exons, including the alternative exon 5. It has an extension of less than 22 kb and generates two different proteins through alternative pre-mRNA splicing: the 2439 nucleotide long MASP-2 mRNA and the 746 nucleotide long MAp19 mRNA (http://www.ensembl.org/Homo_sapiens/Transcript/Summary?db=core;g=ENSG00000009724;r=1:11026523-11047233;t=ENST00000400897) (Fig. 3).

The upstream untranslated (5'UTR) sequence (exon 1) and the sequence encoding the signal peptide (exon 2), the CUB1 domain (exons 2 and 3), and the EGFR domain (exon 4) are common to both mRNAs. Exon 5 is an alternative splice/polyadenylation exon, encoding the four C-terminal amino acids and the stop codon of MAp19 protein. All other exons encode specific MASP-2 domains: CUB2 (exons 6 and 7), CCP1 (exons 8 and 9), CCP2 (exons 10 and 11), and the SP domain with the activating peptide (exon 12). The downstream 3'UTR region differs for both mRNAs (exons 5 and 12) and may have different effects on mRNA stability in the cytoplasm.

The *MASP2* gene is mostly expressed in liver hepatocytes (for MASP-2) and Kupffer cells (for MAp19) (Degn et al. 2011a), but the levels of MAp19 mRNA are higher than those of MASP-2 mRNA in the liver, based on results of RNA high throughput sequencing. Nevertheless, MAp19 mRNA synthesis is restricted to this tissue, whereas MASP-2 mRNA is also found in the nervous system and reproductive tract (http://www.gtexportal.org/home/gene/*MASP2*). Its promoter is regulated by STAT3, IL-1b and IL-6 (Unterberger et al. 2007), HNF4 (hepatocyte nuclear factor 4 alpha), and TCF7L2 (transcription factor 7-like 2) in cells of the mesodermal and ectodermal lineages, respectively. It is also regulated by the methylation of CpG sites, extending to exon 2. Introns 3 and 4 may function as weak enhancers in hepatocellular carcinoma, chronic myelogenous leukemia, and embryonal stem cells, which is in accordance with a possible role of this region in MAp19 expression. Exon 8 exhibits a strong regulatory region with unmethylated CpG sites and is recognized by



***MASP1* and *MASP2*, Fig. 3** *MASP2* gene and MASP-2 and MAp19 protein structures. Exons coding protein domains and these regions in the protein structure are shown with the same color. The regulatory domains are indicated by *blue* (CUB1 and EGF) and *green* colors (CUB2, CCP1, and CCP2). The MASP-2 serine protease region is in *orange* and the -terminal domain of MAp19 in *red*. *MASP* mannose-binding lectin-associated serine protease, *MAp19* mannose-binding lectin-associated protein of 19 kDa, *CUB* C1r/C1s, Uegf, and bone morphogenetic protein, *EGF* epidermal growth factor, *CCP* complement control protein. Exons are drawn to scale and introns are truncated

different proteins, but mostly by the chromatin-binding factor CTCF, the leucine zipper protein MAFK, the DNA repair proteins SMC3 and RAD21, and the tumor suppressor RUNX3. This region may work as an insulator and/or weak enhancer in most cell types. Alternative exon 5, exons 7 and 9 present other methylated CpG sites (data of the ENCODE project, available in https://genome.ucsc.edu/cgi-bin/hgTracks?db=hg19&lastVirtModeType=default&lastVirtModeExtraState=&virtModeType=default&virtMode=0&nonVirtPosition=&position=chr1%3A11087520-11108236&hgsid=512615401_Zyqq7OgOBAeKSAynyGyzYSfKoEP9).

MASP1 and *MASP2* Genetic Variations

MASP1

According to data of the 1000 Genomes Project, *MASP1* presents 4757 described variants. Of these, only 333 exhibit global minor allele frequency (MAF) $\geq$1% (mean frequency of all assessed population). Most of the polymorphisms, i.e., 265, occur in the intronic region. Of these, 13 are deletions, 21 insertions, and 231 nucleotide substitutions. Among the remaining polymorphisms, 25 occur in the upstream sequence, 13 in the 3′UTR region, 6 in the exons (1 missense and 5 synonymous variants), and 24 in the downstream region (Ensembl genome browser). Although the *MASP1* gene presents many frequent variants, the functional impact and association with diseases has not yet been investigated for most of them.

At least 13 *MASP1* polymorphisms have been associated with modulation of MASP-1, MASP-3, and Map44 serum levels (Table 1). Interestingly, some of these single nucleotide polymorphisms (SNPs), such as rs3774275:A>G, rs698090:C>T, and rs67143992:G>A, were associated with the serum levels of all three proteins, increasing MASP-1 and MAp44 and decreasing MASP-3 concentrations (Ammitzbøll et al. 2013). The *MASP1 TGAG* haplotype composed by SNPs present in the upstream regulatory region (rs35089177:T>A, rs62292785:G>A, rs7625133:A>C, and rs72549254:G>A) also seems to increase MASP-1 and MAp44 and decrease MASP-3 serum levels (Ammitzbøll et al. 2013).

The SNP rs850312:G>A (*p.L617=*) located in the coding region responsible for MASP-3 CCP2 was associated with earlier onset of *Pseudomonas aeruginosa* colonization in homozygous (A/A) or heterozygous (G/A) patients with cystic fibrosis (Haerynck et al. 2012) and higher on-admission MASP-3 levels in critically ill children (Ingels

***MASP1* and *MASP2*, Table 1** *MASP1* gene polymorphisms associated with MASP-1, MASP-3, and MAp44 serum levels

dbSNP	Allele	Gene region	Amino acid position	Protein region	Global MAF	Serum levels[a]	Reference
rs190590338	*G>A*	Promoter	n.a.	n.a.	<1%	Higher MASP-1 levels in G/A	Ammitzbøll et al. 2013
rs7625133	*A>C*	Promoter	n.a.	n.a.	3%	Lower MAp44 levels in A/C and C/C	Ammitzbøll et al. 2013
rs35089177	*T>A*	Promoter	n.a.	n.a.	28%	Lower MASP-1 and MAp44 levels in T/A and AA	Ammitzbøll et al. 2013
rs75284004	*A>G*	Promoter	n.a.	n.a.	1%	Lower MASP-3 levels in A/G	Ammitzbøll et al. 2013
rs62292785	*G>A*	Promoter	n.a.	n.a.	10%	Lower MASP-1 levels in G/A	Ammitzbøll et al. 2013
rs72549254	*G>A*	Intron 1	n.a.	n.a.	17%	Higher MASP-3 levels in A/G Lower MAp44 levels in AG and AA	Ammitzbøll et al. 2013
rs710469	*C>T*	Intron 2	n.a.	n.a.	49%	Higher on-admission MASP-3 levels in critically ill children with T/T	Ingels et al. 2014
rs3774275	*A>G*	Intron 8	n.a.	n.a.	24%	Higher MASP-1, Map44 and lower MASP-3 levels in A/G and G/G	Ammitzbøll et al. 2013
rs113938200	*C>T*	Exon 9	p. Asn368Asp	C-terminal MAp-44	<1%	Lower MAp44 levels in C/T	Ammitzbøll et al. 2013
rs698090	*C>T*	Exon 9	n.a.	3'UTR Map-44	46%	Higher MASP-1, Map44 and lower MASP-3 levels in C/C Higher MAp44 levels in C/T	Ammitzbøll et al. 2013
rs72549154	*G>T*	Exon 12	p. Arg576Met	SP MASP-3	7%	Lower MASP-1 levels in G/T	Ammitzbøll et al. 2013
rs850312	*G>A*	Exon 12	p. Leu617Leu	CCP2 MASP-3	21%	Higher on-admission MASP-3 levels in critically ill children with A/A, A/G	Ingels et al. 2014
rs67143992	*G>A*	Exon12	n.a.	3′ UTR MASP-3	9%	Higher MASP-1, MAp44 and lower MASP-3 levels in G/A Higher MAp44 and lower MASP-3 levels in A/A	Ammitzbøll et al. 2013

dbSNP single nucleotide polymorphism database, *n.a.* not applicable, *Global MAF* minor allele frequency of 1000 genomes project in all populations, *CCP* complement control protein, *SP* serine protease, *UTR* untranslated

[a]compared to the homozygote state of the major allele

SNPs in *bold* are considered expression quantitative trait loci (eQTLs) by influencing the expression level of mRNA (http://www.gtexportal.org/home/eqtls/byGene?geneId=*MASP1*&tissueName=All)

et al. 2014). As higher MASP-3 levels are related to a better outcome, A/A and G/A genotypes were considered exhibiting a protective effect in critically ill children (Ingels et al. 2014). On the other hand, the SNP rs710459:C>T was associated with a higher risk of bladder cancer in a population of Spanish patients (de Maturana et al. 2013) and the SNP rs3105782:A>G with male infertility in samples of northern Europe (Aston and Carrell 2009).

Rare *MASP1* mutations cause the monogenic autosomal recessive 3MC syndrome (Mingarelli, Malpuech, Michels and Carnevale) syndrome, by affecting the MASP-3 SP domain. Six different nonsynonymous, one nonsense, and two splice mutations disrupt the catalytic activity of the SP domain, by either changing amino acid or truncating the protein, respectively. Interestingly, homozygous splice mutations abolish lectin pathway activation and reduce 2.5-fold the activity of the alternative pathway, since they also interrupt viable MASP-1 production. Despite this, phenotypes did not differ according to the different mutations (Sirmaci et al. 2010; Rooryck et al. 2011; Degn et al. 2011b; Atik et al. 2015).

MASP2

MASP2 is a polymorphic gene with 2882 described variants spread across the gene; however, only 182 present global MAF (minor allele frequency) ≥1% (Ensembl genome browser). Among these, about 71% are located in the *MASP2* intronic region, 10.9% in the upstream sequence, 0.5% in the 3'untranslated, 8.8% in the exons (being 38% missense variants), and 8.8% in the downstream region. SNPs are the most common type of genetic variation (83%), followed by deletions (10%) and insertions (7%) (Ensembl genome browser).

Some of the *MASP2* polymorphisms have been described modulating MASP-2 and MAp19 serum levels (Table 2). The SNP rs72550870:T>C is responsible for the substitution of aspartic acid to glycine in residue 120 (*p.D120G*) in the CUB1 domain, turning the proteins unable to bind to MBL and ficolins, affecting complement activation (Stengaard-Pedersen et al. 2003; Sørensen et al. 2005). This substitution affects both MASP-2 and MAp19 serum levels leading to a reduced concentration (Sørensen et al. 2005). The *p.156_159dupCHNH* is a rare four amino acid tandem duplication in the EGF domain, found only in the Chinese population. It was associated with low MASP-2 levels, probably affecting the protein dimerization (Thiel et al. 2007; Thiel et al. 2009). The SNP rs12085877:G>A leads to arginine to histidine residue substitution (*p.R439H*) in the MASP-2 SP domain, which leads to decreased MASP-2 concentration and reduced enzymatic activity of MBL-MASP-2 complexes (Thiel et al. 2007; Thiel et al. 2009). At least 10 other *MASP2* gene variants modulating MASP-2 and MAp19 serum levels have been described; however, the reason for their impact on protein concentrations is not completely understood (Thiel et al. 2007; Thiel et al. 2009; Boldt et al. 2011a; Beltrame et al. 2015).

Complex Diseases Associated with *MASP1* and *MASP2*

MASPs serum levels may influence the immune host response against pathogens. In fact, SNPs modulating protein concentration have been associated with the etiology of viral, parasitic, and bacterial diseases (Table 3) (Beltrame et al. 2015). They seem to have a dual role in disease development, in the same manner as observed for other complement proteins. In general, MASP deficiency can lead to a compromised immune response against pathogens, thereby facilitating infection and disease progression. On the other hand, high levels may exacerbate inflammatory response and lead to tissue injuries (Boldt et al. 2016).

However, while lower serum MASP levels normally do not cause diseases, when combined with other immune suppressed states, they may have severe consequences (Yongqing et al. 2012). In 2003, inherited MASP-2 deficiency was reported for the first time in a patient with several recurrent infections and autoimmune disease. DNA sequence analysis revealed a homozygous point mutation in exon 3, causing substitution of glycine for aspartic acid at position 120 (p.D120G), leading

***MASP1* and *MASP2*, Table 2** *MASP2* gene polymorphisms associated with MASP-2 and MAp19 serum levels

dbSNP	Allele	Gene region	Amino acid position	Protein region	Global MAF	Serum levels[a]	Reference
rs7548659	*G>T*	Promoter	n.a.	n.a.	43%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013
rs61735600	*C>T*	Exon 3	*p.R99Q*	CUB1	2%	High MASP-2 concentration	Thiel et al. 2007
rs72550870	*T>C*	Exon 3	*p.D120G*	CUB1	1%	Low MASP-2 and MAp19 concentration	Sørensen et al. 2005, Thiel 2007, Kristian Stengaard-Pedersen et al. 2003, Boldt et al. 2013
rs56392418	*C>T*	Exon 3	*p.P126L*	CUB1	4%	Low MASP-2 concentration	Thiel et al. 2009
-	*c.466_477dupTGCCACAACCAC*	Exon 4	*p. 156_159dupCHNH*	EGF	0.26%	Low MASP-2 concentration	Thiel et al. 2007, 2009
rs2273343	*T>C*	Exon 4	*p.H155R*	EGF	1%	Low MASP-2 concentration	Thiel et al. 2009
rs2273344	*C>T*	Intron 4	n.a.	n.a.	16%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013
rs9430347	*G>A*	Intron 5	n.a.	n.a.	15%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013
rs17409276	*G>A*	Intron 9	n.a.	n.a.	16%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013
rs12711521	*C>A*	Exon 10	*p.D371Y*	CCP2	42%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013
rs2273346	*A>G*	Exon 10	*p.V377A*	CCP2	12%	Low MASP-2 concentration	Thiel et al. 2007, 2009
rs12085877	*G>A*	Exon 12	*p.R439H*	SP	3%	Low MASP-2 concentration	Thiel et al. 2009
rs1782455	*G>A*	Exon 12	*p.S493=*	SP	31%	High MASP-2 and low MAp19 concentration	Boldt et al. 2013

dbSNP single nucleotide polymorphism database, *n.a* not applicable, *Global MAF* minor allele frequency of 1000 genomes project in all populations, *CCP* complement control protein, *SP* serine protease, *EGF* epidermal growth factor

[a]Homozygote of the minor allele effect

SNPs in *bold* are considered expression quantitative trait loci (eQTLs) by influencing the expression level of mRNA (http://www.gtexportal.org/home/eqtls/byGene?geneId=*MASP2*&tissueName=All)

***MASP1* and *MASP2*, Table 3** Diseases associated with *MASP1* and *MASP2*

Associated disease	Plasma/serum levels	Polymorphisms	Effects	Reference
Subacute myocardial infarction	MASP-1 higher MASP-2 lower	-	MASP levels may be altered in vascular diseases	(Frauenknecht et al. 2013)
Acute ischemic stroke	MASP-1 lower MASP-2 lower	-	MASP levels may be altered in vascular diseases	(Frauenknecht et al. 2013)
Systemic lupus erythematosus (SLE)	MASP-1, MASP-3 and MAp44 higher	-	MASP levels may be a role in SLE pathogenesis	(Troldborg et al. 2015)
Multiple sclerosis	MASP-3 higher	-	Protective role against establishment of the disease	(Christensen et al. 2007)
Hereditary angioedema	MASP-1 lower	-	MASP-1 may be a role in the pathophysiology and severity of disease	(Hansen et al. 2015)
Diabetes mellitus type 1	MASP-1 and MASP-2 higher	-	Role in the enhanced thrombotic environment and vascular complications	(Jenny et al. 2015a)
Cystic fibrosis	-	rs850312 *MASP1 gene L617L*	Associated with earlier onset of chronic *Pseudomonas aeruginosa* colonization	(Haerynck et al. 2012)
Pneumococcal infection	MASP-2 deficiency	*p.D120G*	Higher susceptibility	(Stengaard-Pedersen et al. 2003; Ali et al. 2012)
Leprosy	MASP-2 lower	*p.P126L p.R439H*	Higher susceptibility and lepromatous form of leprosy	(Boldt et al. 2013)
Pulmonary tuberculosis	MASP-2 lower	*p.D120G p.V377A rs6695096 (intron 7)*	Higher susceptibility	(Sokolowska et al. 2015; Chen et al. 2015)
Critically ill children upon intensive care unit	MASP-2 lower	-	Higher susceptibility to new infections	(Ingels et al. 2014)
Pediatric cancer	MASP-2 lower	-	Increased risk of episodes of fever and severe chemotherapy-induced neutropenia	(Schlapbach et al. 2007)
Septic shock	MASP-2 lower	-	Acute decrease of MASP-2 in the early phase might correlate with mortality	(Charchaflieh et al. 2012)
Chagas disease	MASP-2 lower	*p.D371Y p.P126L p.V377A*	Higher risk of chagasic cardiomyopathy	(Boldt et al. 2011b)
Placental malaria	MASP-2 lower	*p.R439H*	Protective role	(Holmberg et al. 2012)
HIV	MASP-2 lower	*p.P126L*	Increased the susceptibility to HIV infection; protective effect against AIDS	(Boldt et al. 2016)
Hepatitis C	MASP-2 higher	*p.D371Y*	Susceptibility to HCV infection	(Tulio et al. 2011)
Human T-lymphotropic virus 1 infection	MASP-2 higher	*p.D371Y*	Susceptibility to infection	(Coelho et al. 2013)

(*continued*)

***MASP1* and *MASP2*, Table 3** (continued)

Associated disease	Plasma/serum levels	Polymorphisms	Effects	Reference
Severe infections after chemotherapy	MASP-2 higher	-	In adult patients with hematological cancer	(Ameye et al. 2012)
Pediatric cancers	MASP-2 higher	-	Acute lymphoblastic leukemia, non-Hodgkin lymphoma, central nervous system tumors	(Fisch et al. 2011)
Colorectal cancer	MASP-2 higher		Poor survival and recurrence after surgery	(Ytting et al. 2008)
Rheumatic fever	MASP-2 lower	*p.V377A* *p.R439H*	Protect against rheumatic fever and rheumatic heart disease	(Catarino et al. 2014)
Rheumatoid arthritis	MASP-2 lower	*p.D120G* *p.R439H*	Susceptibility to rheumatoid arthritis and articular symptoms	(Goeldner et al. 2014)
Panic and bipolar disorders; Schizophrenia	MASP-2 lower	-	Connection with autoimmunity	(Foldager et al. 2012; Foldager et al. 2014)

to profound effects on the function of the CUB1 domain. This turns the protein unable to form complexes with PRMs and thus to activate complement, resulting in very low MASP-2 serum levels and deficiency of lectin pathway activation in homozygote individuals. Although the mutation does also affect MAp19 levels, they were not as severely reduced (Stengaard-Pedersen et al. 2003). Variations in MASP-2 levels may be associated with other diseases, and levels lower than 100 ng/mL are considered as a threshold to consider MASP-2 deficiency (Thiel et al. 2007).

Components of the coagulation cascade amplify complement activation in such a manner that both complement and coagulation cascade are interconnected (Ricklin et al. 2010). MASP-1 plays an important role in thrombus formation in a murine model of occlusive thrombosis (Bonte et al. 2012). The expression of *MASP1* gene was observed to be upregulated in primary uterine leiomyosarcoma (Davidson et al. 2014) and in HCV infected-hepatocyte cell lines (Saeed et al. 2013). Activity of the MBL/MASP-1 complex has been associated with disease severity in poststreptococcal acute glomerulonephritis, through glomerular antibody deposition that may lead to glomerular fibrinogen deposits and sustained hematuria (Hisano et al. 2007). The same occurred with liver fibrosis, where serum levels of the MBL/MASP-1 complex were higher in patients and associated with severity of the disease (El Saadany et al. 2011).

MAp44 has been associated with cardioprotective effects, preserving cardiac function, decreasing infarct size, and preventing thrombogenesis in murine models of ischemia/reperfusion injury and arterial thrombosis by inhibiting MBL and C3 deposition (Pavlov et al. 2012).

Summary

MASP1 and *MASP2* are two genes encoding mannose-binding lectin-associated serine proteases and associated truncated proteins of the lectin pathway of complement cascade. The complement system comprises more than 50 proteins acting in the first line of host defense against infectious organisms and linking innate and adaptive immunity. *MASP1* encodes MASP-1 and MASP-3 and the nonenzymatic MAp44, whereas *MASP2* encodes MASP-2 and the nonenzymatic MAp19. Liver hepatocytes mainly express MASP-1 and MASP-2 and Kupffer cells MAp19, although high levels of MAp19 were

also detected in urine. The female reproductive tract, colon, and prostate express MASP-3 mRNA, whereas heart cells (left ventricle) and skeletal muscle cells express MAp44. All proteins form complexes with the pattern recognition molecules (PRMs) of the lectin pathway: MBL, the ficolins, and collectin-11. The complexes most often constitute of one MASP homodimer with one PRM, but a PRM may also include different homodimers or a heterodimer. Besides that, free circulating homo- or heterodimers of MASPs and MAps may occur. The PRMs bind to carbohydrates or acetylated residues on microorganism surfaces or to aberrant glycocalyx patterns on apoptotic, necrotic, or malignant cells. Upon binding, MASP-1 and MASP-2 convert from pro-enzymes to its active forms and activate the lectin pathway of the complement cascade. In contrast, MASP-3 activates the alternative pathway through pro-factor D cleavage and inhibits activation of the lectin pathway by competing with MASP-1 and MASP-2 for binding sites on PRMs, similarly to the truncated protein MAp44. Although found in complex with PRMs, the physiological function of MAp19 is still unclear. The *MASP1* gene, located on chromosome 3q27-q28, has a length of 5276 nucleotides and 18 exons. The *MASP2* gene, located on chromosome 1p36.22, comprises 12 exons. *MASP1* presents 4757 described variants. Of these, only 333 exhibit MAF $\geq$1%. At least 13 *MASP1* polymorphisms have been associated with modulation of MASP-1, MASP-3, and Map44 serum levels. *MASP2* is a polymorphic gene with 2882 described variants spread across the gene; however, only 182 present global MAF $\geq$1%. Some of the *MASP2* polymorphisms have been described modulating MASP-2 and MAp19 serum levels. MASPs serum levels may influence the immune host response against pathogens. In fact, SNPs modulating protein concentration have been associated with the etiology of viral, parasitic, and bacterial diseases. They seem to have a dual role in disease development, in the same manner as observed for other complement proteins.

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