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Low levels of the innate immune system proteins MASP-2 and MAp44 in patients with common variable immunodeficiency

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Abstract

Patients with common variable immunodeficiency (CVID) display low antibody levels and associated symptoms, including an increased risk of infections. The causes of CVID are uncertain and likely heterogeneous. The complement system protects against pathogens and plays essential roles in homeostasis and development. The influence of the complement system in CVID is not established. We investigated CVID patients and healthy individuals for plasma levels of the complement proteins: MASP-1, MASP-2, MASP-3, MAp19 and MAp44. We also tested other patients with symptoms similar to the CVID patients. CVID patients had lower average MASP-2 and MAp44 levels than healthy individuals ($P < 0.01$); the MASP-2 level was 0.73-fold lower, and the MAp44 level was 0.87-fold lower. This was not observed in the other patient cohorts studied. Our findings in this exploratory study provide new insights into CVID and introduce a complement perspective for future investigations into the underlying mechanisms of the disease.

1 | INTRODUCTION

Infections are frequent causes of contact with the healthcare system. Some patients experience an exceptionally high burden of infections that may result from an underlying primary immune defect (PID). Common variable immunodeficiency (CVID) is among the most frequent significant PID diagnoses, with a prevalence of approximately 1:25 000 in adults.[1] According to the American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma & Immunology, CVID is defined as a marked decrease in plasma IgG and at least one of the classes IgM or IgA, and fulfilment of the following three criteria: (i) onset of immunodeficiency after age 2 years, (ii) poor ability to produce antibody response to antigenic challenge and (iii) defined causes of hypogammaglobulinemia have been excluded.[2] Common features are B cell perturbations and antibody deficiency,

typically causing recurrent airway infections.[1] Patients with CVID also frequently suffer from autoimmunity, enteropathy, granulomas and/or lymphoproliferative disorders.[3] The pathogenesis of the disease for most patients is not established despite great efforts but is likely heterogeneous. In a minority of CVID patients, various monogenic variants may drive the disease,[4,5] but the underlying cause remains unknown for most patients. Thus, new perspectives on possible causative factors seem relevant.

The complement system is a highly preserved part of the innate immune system and consists of more than 40 plasma proteins and surface-bound regulators.[6] The biological importance is emphasized by the courses of humans with rare detrimental defects in complement proteins who are prone to devastating bacterial infections and autoimmune diseases.[7] Furthermore, there is increasing awareness that complement is pivotal in homeostasis and development in some cell types (eg B cells)[8] as well as more broadly in the organism.[9,10] In this light, we found it of interest to further examine the connection between complement and CVID.

Activation of the complement system may proceed through three distinct pathways: the alternative, the classical and the lectin pathway.[11] All three pathways converge in a shared terminal pathway and may result in direct killing of the pathogen or in opsonization for elimination by phagocytosis. Activation of the lectin pathway is based on the ability of soluble pattern recognition molecules (PRMs) to bind to specific molecular structures on the surface of microorganisms or apoptotic cells.[12] The lectin pathway has five different PRMs in plasma; mannan-binding lectin (MBL), collectin-LK (a heteromer of CL-L1 (collectin-10) and CL-K1 (collectin-11)), H-ficolin, L-ficolin and M-ficolin (also termed ficolin-3, ficolin-2 and ficolin-1, respectively). The PRMs circulate in plasma in complexes with three serine proteases or with two so-called associated proteins. For historical reasons, the three serine proteases are termed MBL-associated serine proteases MASP-1, MASP-2 and MASP-3, and the two associated proteins are termed MBL-associated proteins MAp19 (also termed sMAP) and MAp44 (also termed MAP-1).[13] MASP-1, MASP-3 and MAp44 are produced through alternative splicing of RNA from the same gene, *MASP1*, whereas MASP-2 and MAp19 are produced through alternative splicing of RNA from the gene *MASP2*.[14]

When the lectin pathway PRMs bind their ligands, the accompanying MASP-1 autoactivates and subsequently activates neighbouring MASP-2.[15] Activated MASP-2 cleaves circulating complement factors C4 and C2, generating the C3 convertase, C4bC2a.[16] The C3 convertase drives downstream complement activation leading to (1) release of anaphylactic factors (ie C3a, C4a and C5a) for stimulation of cells of the immune system, (2) deposition of fragments on surfaces that enhance phagocytosis and (3) assembling of the (cell-killing) membrane attack complex. MASP-3 has the ability to cleave pro-Factor D to generate Factor D, which is an essential protein of the alternative pathway of the complement system.[10,13,17] The MAps (MAp19 and MAp44) are able to complex with the PRMs but lack the serine protease domain and hence enzymatic activity. They are thought to attenuate complement activation by competing with MASPs for binding to the PRMs.[9,18]

Deficiencies in the MASPs (MASP-1, MASP-2 and MASP-3) involved in the lectin pathway have previously been reported, primarily as single patient cases. MASP-2 deficiency was proposed to promote invasive pneumococcal infections as well as other symptoms such as ulcerative colitis and erythema multiforme bullosum in an adult male patient.[19] However, MASP-2 deficiency has later also been found in asymptomatic individuals.[7] The clinical role of MASP-2 deficiency therefore remains controversial.

Apart from having a direct influence on the activation of enzymes in the alternative pathway of the complement system,[20] MASP-3 is also very important in embryonic development, as complete MASP-3 deficiency causes severe malformations causing so-called 3MC syndrome.[10,21,22] Thus, the serine proteases and associated proteins of the lectin pathway may serve additional roles in humans beyond the killing of pathogens and should therefore be of interest in the heterogeneous CVID patients.

We have previously investigated the PRMs of the lectin pathway in CVID patients in a cohort of suspect immunodeficiency patients.[23] Our aim of the present study was to expand this work by focusing on MASPs and MAps of the lectin pathway of complement. With this, we aim to provide the first comprehensive overview of the lectin pathway proteins in CVID patients in a cohort of suspect immunodeficiency patients.

2 | MATERIALS AND METHODS

2.1 | Study population

Twenty-nine patients suffering from CVID were identified among 332 patients with frequent and/or severe infections referred for evaluation of immunodeficiency at the Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark, between February 2011 and October 2013. One-hundred and fifty (75 females and 75 males) anonymized blood donors at the Blood Bank, Aarhus University Hospital, Aarhus, Denmark, served as healthy controls.

The characteristics of the entire patient cohort have been described previously.[23] Briefly, patients referred for

laboratory assessment of immunodeficiency were categorized into the following five groups: (1) CVID according to criteria set by the American Academy of Allergy, Asthma & Immunology and the American College of Allergy, Asthma & Immunology[2]; (2) lung transplantation candidates (LTX) (n = 25); (3) recurrent airway infections (RAI) without CVID or LTX (n = 122); and (4) recurrent abscesses (n = 25); and (5) 'other causes' (n = 131). The present study primarily focuses on the CVID group. The other patient groups, which were included and analysed in parallel, served as additional controls. Genetic analyses were not available on all patients, but genetic deficiencies determined for some of the patients have previously been described.[23]

Females comprised 51.7% of CVID patients compared with 50.0% of the healthy controls (non-significant).[23] CVID patients were slightly older than healthy controls; the median age in CVID patients was 42.8 years, interquartile range between 33 and 65 years, and the median age in controls was 29.0 years, interquartile range between 23 and 41 years ($p < 0.01$).[23]

2.2 | Methods

Heparin blood samples were centrifuged at $1849 \times g$ for 5 min, and plasma was collected and subsequently frozen at −80°C. To enable the tests, the samples were thawed, diluted 1/4 in Tris-buffered saline (10 mM Tris, 145 mM NaCl, pH 7.4) (TBS), and stored at −80°C. The stability of the lectin pathway proteins is high, and plasma concentration varies negligibly after repeated freeze/thaw cycles.[24]

All of the following specific measurements of proteins were performed blinded to patient data. The MASPs (MASP-1, MASP-2 and MASP-3) and MAps (MAp19 and MAp44) involved in the lectin pathway were measured by sandwich-type immunoassays based on the principle of time-resolved immunofluorometric assays (TRIFMAs). The specific antibodies used in the TRIFMAs were developed and produced in-house; the assays have been described in detail elsewhere.[25–28] Briefly, for all assays, microtiter wells were coated with capture antibody, followed by the addition of diluted samples and subsequently biotinylated detection antibody. Next, europium-labelled streptavidin was added, and the signals from the wells were read by time-resolved fluorometry. Sample dilution and loading on microtiter plates were automated using a pipetting robot (JANUS, Perkin Elmer, Hamburg, Germany). Each sample was analysed as technical duplicates. Measurements were repeated if the coefficient of variation (CV) of the duplicates was above 15%. Inter-assay CV based on three internal controls was also determined for each protein, and CVs were ≤11% for all proteins (MASP-1 < 10%, MASP-3 < 9.2%, MAp44 < 6.1%, MASP-2 < 11% and MAp19 < 8.5%).

2.3 | Statistics

Plasma protein concentrations were compared using a multiple linear regression model and t tests. Gaussian distributions were approximated by a logarithmical transformation of the measured concentrations of all proteins except MAp19, which followed a Gaussian distribution without transformation. Frequencies of low concentrations (defined as below the 10th percentile of healthy controls) and high concentrations (defined as above the 90th percentile of healthy controls) of MASPs and MAps in CVID patients were compared with healthy controls using Fisher's exact test. Correlations between concentrations of MASPs and MAps and C-reactive protein (CRP) were performed using Spearman correlation. All tests were two-sided. P values < 0.05 were defined as significant. Geometric mean and 95% CI and arithmetic mean and 95% CI (MAp19) are depicted as error bars in figures. Correction for mass significance was not performed in this exploratory study. Analyses were performed using Stata 16 software (StataCorp, TX, USA). Graphical representations were performed using GraphPad Prism software (GraphPad Software, CA, USA).

2.4 | Ethics

The study was conducted under the approval of the Central Denmark Region Committees on Health Research Ethics (1-10-72-127-12) and the Danish Data Protection Agency (1-16-02-40-12). The study was performed according to the Declaration of Helsinki.

3 | RESULTS

3.1 | Levels of MASPs and MAps in CVID patients and healthy individuals

MASP-1, MASP-2, MASP-3, MAp44 and MAp19 were quantified in heparin plasma samples from 29 patients with CVID and 150 healthy individuals by sandwich-type immunoassays (Figure 1). All five proteins were quantifiable in all samples.

The mean level of MASP-1 was 11 μg/mL (95% confidence intervals [CI]: 11; 12) in healthy individuals and 11 μg/mL (95% CI: 10; 12) in CVID patients. The mean
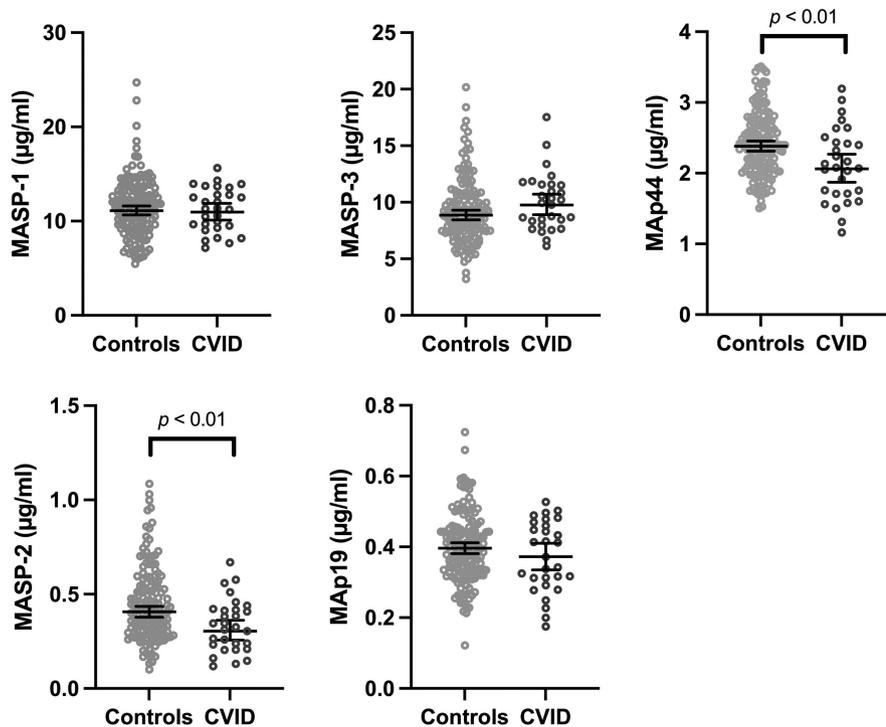
FIGURE 1 Plasma (heparin) concentrations of pattern recognition molecule (PRM)-associated proteins of the lectin pathway in CVID patients and healthy individuals (controls). Bars indicate geometric mean and 95% CI or mean and 95% CI (MAp19). The two groups' mean were compared by *t* tests. CVID, common variable immunodeficiency; MAp, MBL-associated protein; MASP, MBL-associated serine protease

TABLE 1 Prevalence of deficiencies and abundancies of single proteins in patients relative to healthy individuals

	10th %			90th %		
	Controls	CVID		Controls	CVID	
	10th % (µg/mL)	n (%)	*P* value[a]	90th % (µg/mL)	n (%)	*P* value[a]
MASP-1	7.567	1 (3.5)	0.48	14.719	1 (3.5)	0.48
MASP-3	6.125	0 (0)	0.14	12.859	3 (10.3)	1.00
MAp44	1.857	10 (34.5)	**<0.01**	3.050	1 (3.5)	0.48
MASP-2	0.247	10 (34.5)	**<0.01**	0.708	0 (0)	0.14
MAp19	0.278	5 (17.2)	0.33	0.516	1 (3.5)	0.48

Note: Deficiency: Protein levels below controls' 10th percentile.

Abundancy: Protein levels above controls' 90th percentile.

[a]Compared by Fisher's exact test.

P values <0.05 are marked in bold.

level of MASP-2 was 0.41 µg/mL (95% CI: 0.38; 0.44) in healthy individuals and 0.30 µg/mL (95% CI: 0.26; 0.36) in CVID patients. The mean level of MASP-3 was 8.9 µg/mL (95% CI: 8.5; 9.3) in healthy individuals and 9.8 µg/mL (95% CI: 8.9; 11) in CVID patients. The mean level of MAp44 was 2.4 µg/mL (95% CI: 2.3; 2.5) in healthy individuals and 2.1 µg/mL (95% CI: 1.9; 2.3) in CVID patients. The mean level of MAp19 was 0.40 µg/mL (95% CI: 0.38; 0.41) in healthy individuals, and MAp19 was 0.34 µg/mL (95% CI: 0.31; 0.39) in CVID patients.

Comparison between the CVID and the healthy individuals revealed significantly lower levels of MASP-2 and MAp44 in CVID patients. Mean MASP-2 concentration in CVID patients was 75% (95% CI: 63%; 89%) of healthy individuals. Mean MAp44 concentration in CVID patients

was 86% (95% CI: 79%; 95%) of healthy individuals. No significant differences in plasma levels were observed for MASP-1, MASP-3 or MAp19. We also determined the concentration of MASPs and MAps in 303 additional patients encompassing RAI, LTX, recurrent abscesses or other causes. Results for these patients are depicted in the supplementary data file (Figure S1). Low levels of MASP-2 and MAp44 were not observed in any of the other patient groups; however, MASP-1 levels were significantly higher in RAI and other causes, MASP-3 levels were significantly higher in RAI, abscesses and other causes, whereas MAp44 levels were significantly higher in abscesses and other causes.

Among all the included patient groups, only CVID patients displayed low levels of MASP-2 and MAp44.

3.2 | Prevalence of low and high concentrations

We subsequently examined whether the prevalence of single MASPs and MAPs in concentrations defined as 'low' or 'high' differed in CVID patients and healthy individuals (Table 1). Low concentrations were defined as below the 10th percentile of healthy individuals, and high concentrations were defined as above the 90th percentile of healthy individuals. Low levels of MASP-2 and MAp44 were observed in 34.5% (n = 10 (34.5%) CVID patients compared with 10.0% (n = 15 (10.0%) of the healthy individuals ($P < 0.01$), respectively. Low levels of both MASP-2 and MAp44 were co-existing in five CVID patients. No significant differences in the prevalence of low concentrations of MASP-1, MASP-3 and MAp19 were observed. No significant differences in the prevalence of high concentrations of any of the MASPs and MAps were observed. The prevalence of low and high levels of MASPs and MAps in other patient groups referred for an immunological evaluation are depicted in supplementary (Tables S1 and S2).

3.3 | Prevalence of combined deficiencies

and MAps are both able to complex with PRMs of the lectin pathway. The three MASPs possess enzymatic activities and are crucial for activating the lectin pathway of complement. We, therefore, investigated the combined prevalence of low concentrations of the MASPs (Figure 2). For each MASP, a low level was defined as stated above (below the 10th percentile of plasma levels in healthy individuals). We disclosed low levels of one MASP in 11 of the CVID patients (37.9%) and in 41 of the healthy individuals (27.3%). Low levels of two out of the three MASPs were observed in none of the CVID patients and two of the healthy individuals (1.3%) (Fisher's exact $P = 0.49$). The two MAps have no enzymatic activity and are suggested to serve regulatory functions by attenuating complement activation through competitive binding to the PRMs. Thus, we examined the prevalence of combined high concentrations of the two MAps. High levels were defined as above (higher than the 90th percentile of plasma levels in healthy individuals). High levels of one MAp were observed in 2 (6.9%) CVID patients and 22 (14.7%) of healthy controls. High levels of both MAps were not observed in any CVID patient but in 4 of the healthy controls (2.7%) ($P = 0.45$). The prevalence of combined deficiencies of MASPs and MAps in other patient groups referred for an immunological evaluation are depicted in supplementary (Figure S2).
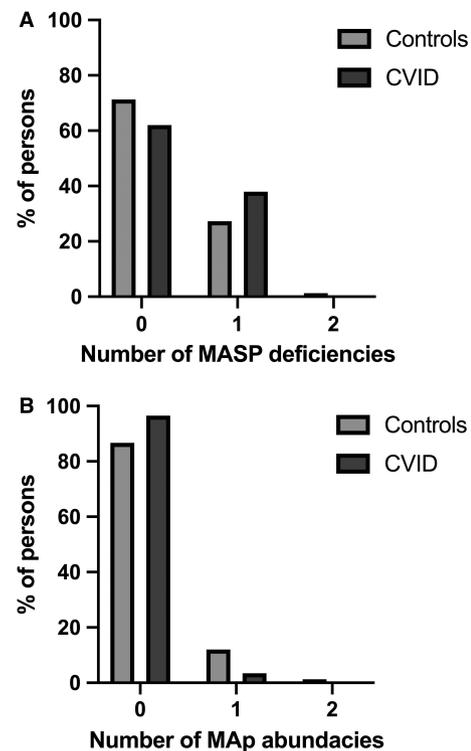

FIGURE 2 Constellations of deficiencies and abundancies among the lectin pathway activating proteins. (A) The number of deficient proteins among proteins MASP-1, MASP-2 and MASP-3. Each protein was defined as deficient when its level was below the 10th percentile in healthy persons. (B) The number of abundant proteins among the speculated/suspected (lectin pathway) inhibitory proteins MAp44 and MAp19. Each protein was defined as abundant when its level exceeded the 90th percentile in healthy persons (Fisher's exact test)

3.4 | Correlations between proteins

The MASPs and MAps are produced through alternative splicing of two genes, *MASP1* (giving rise to MASP-1, MASP-3 and MAp44) and *MASP2* (giving rise to MASP-2 and MAp19), this is why we examined a potential correlation between protein levels (Figure 3). We observed that MASP-2 correlated positively with MAp19 in CVID patients ($r = 0.40$, $P = 0.03$) but not in healthy individuals ($r = -0.06$, $P = 0.48$). We also observed a positive correlation among healthy individuals for MAp44 and MASP-1 ($r = 0.27$, $P < 0.001$), MASP-1 and MASP-2 ($r = 0.24$, $P < 0.001$), MAp44 and MAp19 ($r = 0.27$, $P < 0.001$), which was not observed for CVID patients.

The levels of the MASPs and MAps may be influenced by inflammation. We have previously found higher levels of CRP in CVID patients compared with healthy individuals.[23] Using the earlier published data set for CRP in the patient samples,[23] we found that CRP correlated positively with MAp19 in both CVID patients ($r = 0.51$, $P < 0.001$) and healthy individuals ($r = 0.26$,
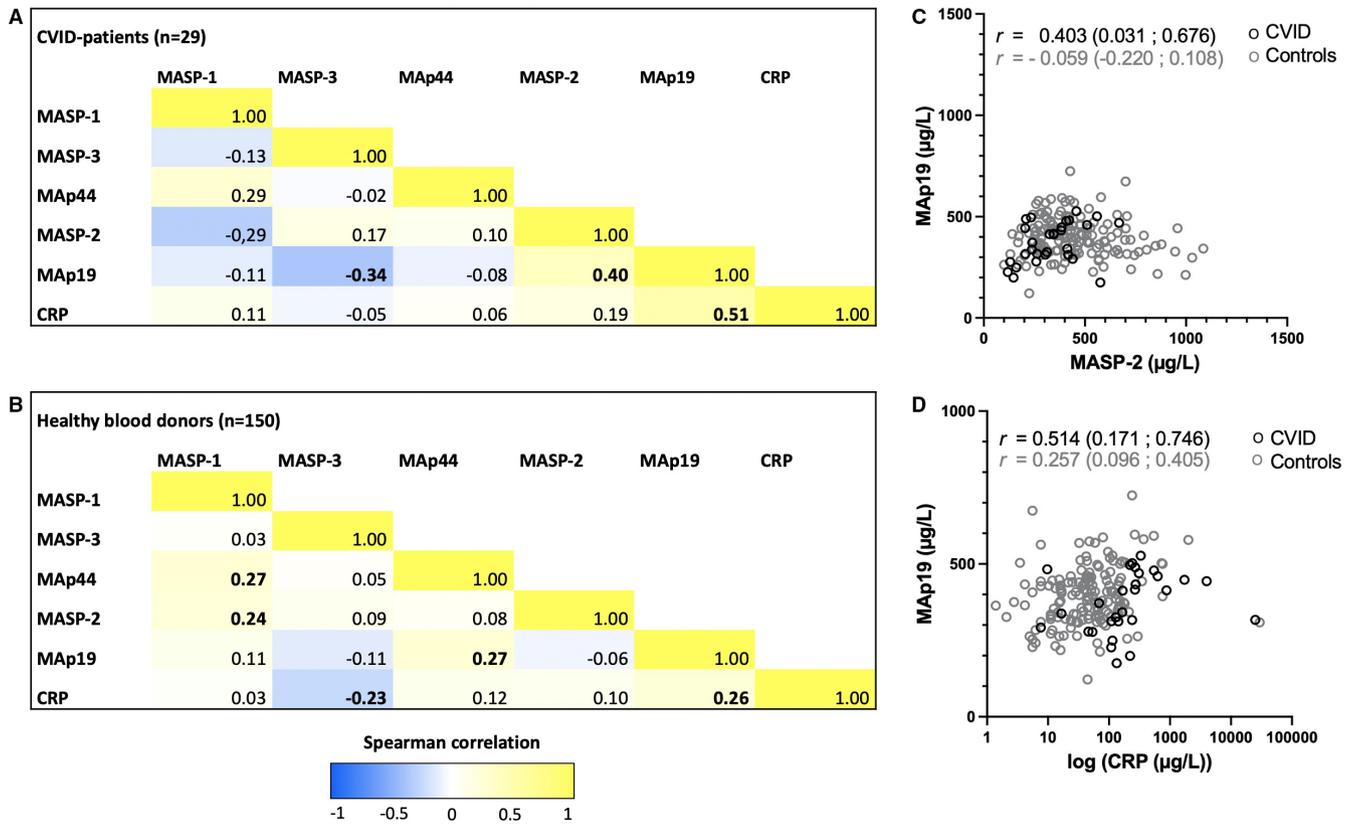
FIGURE 3 Correlations of protein levels in plasma. Data from CVID patients (n = 29) are shown in black and data from healthy persons (n = 150) in grey. (A) Spearman's rho with *P* value for all pairwise correlations in CVID patients. The colouring indicate the correlation coefficient, *P* values <0.05 er marked in bold. (B) Spearman's rho with *P* value for all pairwise correlations in healthy persons. The colouring indicate the correlation coefficient, *P* values <0.05 are marked in bold. (C) Data points for the correlation between MAp19 and MASP-2. (D) Data points for the correlation between MAp19 and CRP

$P < 0.001$). We also observed a positive correlation among healthy individuals between MASP-3 and CRP ($r = 0.26$, $P < 0.001$); this was not observed for patients with CVID. Correlations of lectin pathway proteins and proteases in the entire cohort of patients referred for evaluation of immunodeficiency (n = 332) are depicted in supplementary (Table S3).

4 | DISCUSSION

The growing understanding of the complex interactions of the immune system urged us to explore the levels of the innate immune system proteins, MASPs and MAps, in patients with CVID and other patients tested for primary immunodeficiency diseases. Together with a previous study,[23] the present study provides a complete description of the levels of all 10 lectin pathway proteins in patients referred for clinical investigation of immunodeficiency. To the best of our knowledge, this is the first complete report of the lectin pathway in patients with CVID.

Interestingly, we disclosed low levels of MASP-2 and MAp44 in patients with CVID compared with healthy

individuals. MASP-2 is known to be crucial for activation of the lectin pathway of the complement system[15] and the only protease of the lectin pathway able to cleave C4 under physiological conditions.[16] MASP-2 is thus essential for the function of the lectin pathway. Variants in *MASP2* are accepted as a potential cause of PID as stated in the Human Inborn Errors of Immunity: 2019 Update on the Classification from the International Union of Immunological Societies Expert Committee.[29] MASP-2 deficiency was first identified in an adult male patient suffering from severe infections and several autoimmune symptoms including recurring pneumonia, pulmonary fibrosis, ulcerative colitis and erythema multiforme bullosum.[19] The deficiency was caused by biallelic missense variants in *MASP2* causing p.D120G, which impairs the ability to form complexes with PRMs. However, it has turned out that 0.1%–0.3% of European descent carry the underlying genetic variants in homozygous form and many appear healthy.[30,31] A recently published study reviewed p.D120G homozygous individuals in the literature, and reported 11 patients with a wide range of disorders, whereas nine persons were healthy, supporting the heterogeneous clinical

presentations.[31] Other variants that cause MASP-2 deficiency are described in other ethnic groups,[32] and yet other polymorphisms influence the function of MASP-2.[33] The clinical significance of low levels of MASP-2 remains unsettled. Our finding of low MASP-2 levels in CVID patients could be due to a common polymorphism as stated above, but it also opens a new perspective on the clinical significance of the protein. The latter is nourished by the growing understanding of clinical differences in CVID phenotypes as either dominated primarily by infections or by autoimmune manifestations.[34] Moreover, autoimmune manifestations common in CVID may be caused by the inability to eradicate microbial antigens, resulting in an excessive or misguided immune response due to a chronic inflammation.[35] The MASP-2 deficiency may be a consequence of CVID or it may be a driver of CVID in some patients. We speculate that yet unknown effects of MASP-2 could play a role in the development of CVID, for example analogous to the recently discovered additional role of MASP-3 in the 3MC syndrome.[10,21] Further studies are required to conclude on the possible causality between MASP-2 deficiency and CVID.

The biological function of MAp44 has not been finally established. However, MAp44 possesses the ability to interact and possibly regulate the complex formation between PRMs and the associated proteases or proteins and thus consequently to inhibit the activity of the lectin pathway.[27] MAp44 has no enzymatic activity but is able to compete with MASP-1, MASP-2 and MASP-3 for binding to PRMs. Very high doses of MAp44 injected in mice did, however, not reduce MBL pathway activation.[36] On the contrary, in vivo expression of MAp44 in the liver of mice resulted in lower lectin pathway activity.[37] MAp44 has been found to be produced primarily in extrahepatic tissue such as the heart,[18] which suggests that unknown mechanisms account for the low plasma levels observed in CVID patients. In zebrafish, lack of MAp44 influenced heart function, suggesting an influence on the development of cardiac tissue.[38] It is thus possible that MAp44 plays an unknown role in the development of CVID in some individuals, but this requires further investigations.

A subset of patients with CVID has an abnormal liver function,[39] which may cause lower plasma levels of liver synthesized proteins. As MASP-2 is liver synthesized, a lower level of the protein might relate to decreased liver function in CVID patients. Unfortunately, we had no access to patient details on liver function. However, an argument against reduced liver function explaining the lower MASP-2 level is that the other proteins synthesized in the liver, such as MASP-1 (data presented in this report) as well as MBL and L-ficolin (both described in[23]), were not

lower in CVID patients than in the controls. Alternatively, low levels of MASP-2 and MAp44 could also be explained by increased consumption of these proteins, for example consumption of MASP-2 through complement activation followed by elimination of the activated MASP-2 by C1-inhibitor,[40,41] or possibly Inter-α-inhibitor heavy chain 4, a recently described inhibitor of the MASPs.[42] The design of our study does not permit us to settle this question here.

We further examined the prevalence of combined deficiencies of the MASPs as well as combined high levels of MAps but observed no significant difference between CVID patients and healthy individuals.

A strong correlation was observed between MAp44 and MASP-2 as well as MAp19 and CRP in CVID patients. The latter correlation was also observed in healthy individuals. In healthy individuals, we also observed correlations between MASP-1 and MASP-2, MASP-1 and MAp44 and MAp44 and MAp19. As described previously, MASP-1, MASP-3 and MAp44 are produced through alternative splicing of *MASP1* RNA,[43] whereas MASP-2 and MAp19 are produced through alternative splicing of *MASP2* RNA.[44] All of the five proteins are able to bind PRMs of the lectin pathway, but their possible interdependences in terms of plasma levels are not clarified. In a recent study of a cohort of healthy blood donors, similar correlations were observed in EDTA plasma: MASP-1 and MAp44 ($r = 0.31$ $P < 0.001$), MASP-1 and MASP-2 ($r = 0.23$ $P = 0.001$), and MAp44 and MAp19 ($r = 0.24$ $P < 0.001$).[24] The biological significance of these observations is yet unknown, and further investigations may provide important insights into the functions of the lectin pathway.

A limitation to our study is that the possible contributions of common polymorphism, for example p.D120G on plasma levels of MASP-2 were not examined. DNA was however not available for the present study. This will be important in to examine in future studies. Another limitation is the small sample sizes in some of the patient groups. However, the patients with suspected immunodeficiencies are indeed a heterogenous group and hereby provide clinically relevant controls to CVID patients.

The slightly higher age of the CVID patients relative to the healthy individuals encompasses another limitation. However, age seems to have a negligible effect on the concentrations of MASPs and MAps in healthy individuals.[24] We adjusted for age and gender and found only marginal changes in estimated difference in MASP-3 levels (mean MASP-3 concentration in CVID patients compared with healthy individuals increased from 110% [96%; 126%] to 116% [101%; 131%] ($P = 0.03$). The difference is unlikely of practical importance, and in the present study, we report unadjusted data.

5 | CONCLUSIONS

In conclusion, the results of our study suggest that CVID patients have lower levels of MASP-2 and MAp44 compared with healthy individuals, whereas the prevalence of combined deficiencies of MASP-1, MASP-2 and/or MASP-3 did not differ between CVID patients and healthy individuals. The findings need to be reinvestigated in larger patient cohorts but may pave the way for new insights into the pathogenesis of CVID.

AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST
None.

DATA AVAILABILITY STATEMENT
Data available on request due to privacy/ethical restrictions

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REFERENCES

1. Westh L, Mogensen TH, Dalgaard LS, et al. Identification and characterization of a Nationwide Danish adult common variable immunodeficiency cohort. *Scand J Immunol*. 2017;85(6):450-461.
2. Bonilla FA, Khan DA, Ballas ZK, et al. Practice parameter for the diagnosis and management of primary immunodeficiency. *J Allergy Clin Immunol*. 2014;136(5):1186-1205.
3. Chapel H, Lucas M, Lee M, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. *Blood*. 2008;112(2):277-286.
4. Bogaert DJA, Dullaers M, Lambrecht BN, Vermaelen KY, De Baere E, Haerynck F. Genes associated with common variable immunodeficiency: one diagnosis to rule them all? *J Med Genet*. 2016;53(9):575-590.
5. Christiansen M, Offersen R, Jensen JMB, Petersen MS, Larsen CS, Mogensen TH. Identification of novel genetic variants in CVID patients with autoimmunity, autoinflammation, or malignancy. *Front Immunol*. 2020;10(3022):1-11.
6. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement system part II: role in immunity. *Front Immunol*. 2015;6:1-26.
7. Skattum L, Van Deuren M, Van Der Poll T, Truedsson L. Complement deficiency states and associated infections. *Mol Immunol*. 2011;48(14):1643-1655.
8. Roozendaal R, Carroll MC. Complement receptors CD21 and CD35 in humoral immunity. *Immunol Rev*. 2007;219(1):157-166.
9. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010;11(9):785-797.
10. Pihl R, Jensen L, Hansen AG, et al. Analysis of factor D isoforms in Malpuech–Michels–Mingarelli–Carnevale patients highlights the role of MASP-3 as a maturase in the alternative pathway of complement. *J Immunol*. 2017;199(6):2158-2170.
11. Merle NS, Church SE, Fremeaux-Bacchi V, Roumenina LT. Complement system part I - molecular mechanisms of activation and regulation. *Front Immunol*. 2015;6:1-30.
12. Bajic G, Degn SE, Thiel S, Andersen GR. Complement activation, regulation, and molecular basis for complement-related diseases. *EMBO J*. 2015;34(22):2735-2757.
13. Dobó J, Pál G, Cervenak L, Gál P. The emerging roles of mannose-binding lectin-associated serine proteases (MASPs) in the lectin pathway of complement and beyond. *Immunol Rev*. 2016;274(1):98-111.
14. Yongqing T, Drentin N, Duncan RC, Wijeyewickrema LC, Pike RN. Mannose-binding lectin serine proteases and associated proteins of the lectin pathway of complement: two genes, five proteins and many functions? *Biochim Biophys Acta Proteins Proteomics*. 2012;1824(1):253-262.
15. Degn SE, Kjaer TR, Kidmose RT, et al. Complement activation by ligand-driven juxtaposition of discrete pattern recognition complexes. *Proc Natl Acad Sci USA*. 2014;111(37):13445-13450.
16. Thiel S, Vorup-Jensen T, Stover CM, et al. A second serine protease associated with mannan-binding lectin that activates complement. *Nature*. 1997;386:506-510.
17. Hayashi M, Machida T, Ishida Y, et al. Cutting edge: role of MASP-3 in the physiological activation of factor D of the alternative complement pathway. *J Immunol*. 2019;203(6):1411-1416.
18. Degn SE, Hansen AG, Steffensen R, Jacobsen C, Jensenius JC, Thiel S. MAp44, a human protein associated with pattern recognition molecules of the complement system and regulating the lectin pathway of complement activation. *J Immunol*. 2009;183:7371-7378.
19. Stengaard-Pedersen K, Thiel S, Gadjeva M, et al. Inherited deficiency of mannan-binding lectin-associated serine protease 2. *N Engl J Med*. 2003;349:554-560.
20. Dobó J, Szakács D, Oroszlán G, et al. MASP-3 is the exclusive pro-factor D activator in resting blood: the lectin and the alternative complement pathways are fundamentally linked. *Sci Rep*. 2016;6(31877):1-12.
21. Sirmaci A, Walsh T, Akay H, et al. MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of carnevale, malpuech, OSA, and michels syndromes. *Am J Hum Genet*. 2010;87(5):679-686.

22. Atik T, Koparir A, Bademci G, et al. Novel MASP1 mutations are associated with an expanded phenotype in 3MC1 syndrome. *Orphanet J Rare Dis*. 2015;10:1-10.

23. Jørgensen CM, Jensen L, Christiansen M, Bjerre M, Jensen JMB, Thiel S. Pattern recognition molecules of the lectin pathway—screening of patients with suspected immunodeficiency. *J Clin Immunol*. 2019;39:668-677.

24. Troldborg A, Hansen A, Hansen SWK, Jensenius JC, Stengaard-Pedersen K, Thiel S. Lectin complement pathway proteins in healthy individuals. *Clin Exp Immunol*. 2017;188(1):138-147.

25. Thiel S, Jensen L, Degn SE, et al. Mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1), a serine protease associated with humoral pattern-recognition molecules: Normal and acute-phase levels in serum and stoichiometry of lectin pathway components. *Clin Exp Immunol*. 2012;169(1):38-48.

26. Møller-Kristensen M, Jensenius JC, Jensen L, et al. Levels of mannan-binding lectin-associated serine protease-2 in healthy individuals. *J Immunol Methods*. 2003;282:159-167.

27. Degn SE, Jensen L, Gál P, et al. Biological variations of MASP-3 and MAp44, two splice products of the MASP1 gene involved in regulation of the complement system. *J Immunol Methods*. 2010;47:2253-2254.

28. Degn SE, Thiel S, Nielsen O, Hansen AG, Steffensen R, Jensenius JC. MAp19, the alternative splice product of the MASP2 gene. *J Immunol Methods*. 2011;48:1709.

29. Tangye SG, Al-Herz W, Bousfiha A, et al. Human inborn errors of immunity: 2019 update on the classification from the International Union of Immunological Societies Expert Committee. *J Clin Immunol*. 2020;40(1):24-64.

30. Degn SE, Jensenius JC, Thiel S. Disease-causing mutations in genes of the complement system. *Am J Hum Genet*. 2011;88(6):689-705. gnomad.broadinstitute.org

31. García-Laorden MI, Hernández-Brito E, Muñoz-Almagro C, et al. Should MASP-2 deficiency be considered a primary immunodeficiency? Relevance of the lectin pathway. *J Clin Immunol*. 2020;40(1):203-210.

32. Thiel S, Steffensen R, Christensen IJ, et al. Deficiency of mannan-binding lectin associated serine protease-2 due to missense polymorphisms. *Genes Immun*. 2007;8(2):154-163.

33. Thiel S, Kolev M, Degn S, et al. Polymorphisms in mannan-binding lectin (MBL)-associated serine protease 2 affect stability, binding to MBL, and enzymatic activity. *J Immunol*. 2009;182(5):2939-2947.

34. Lee EY, Betschel S, Grunebaum E. Monitoring patients with uncomplicated common variable immunodeficiency: a systematic review. *Allergy Asthma Clin Immunol*. 2022;18(1):1-10.

35. Costagliola G, Cappelli S, Consolini R. Autoimmunity in primary immunodeficiency disorders: an updated review on pathogenic and clinical implications. Journal of. *Clin Med*. 2021;10(20):1-20.

36. Pavlov VI, Skjoedt MO, Tan YS, Rosbjerg A, Garred P, Stahl GL. Endogenous and natural complement inhibitor attenuates myocardial injury and arterial thrombogenesis. *Circulation*. 2012;126(18):2227-2235.

37. Banda NK, Mehta G, Kjaer TR, et al. Essential role for the lectin pathway in collagen antibody–induced arthritis revealed through use of adenovirus programming complement inhibitor MAp44 expression. *J Immunol*. 2014;193(5):2455-2468.

38. Mortensen SA, Skov LL, Kjaer-Sorensen K, et al. Endogenous natural complement inhibitor regulates cardiac development. *J Immunol*. 2017;198(8):3118-3126.

39. Ward C, Lucas M, Piris J, Collier J, Chapel H. Abnormal liver function in common variable immunodeficiency disorders due to nodular regenerative hyperplasia. *Clin Exp Immunol*. 2008;153(3):331-337.

40. Kozarcanin H, Lood C, Munthe-Fog L, et al. The lectin complement pathway serine proteases (MASPs) represent a possible crossroad between the coagulation and complement systems in thromboinflammation. *J Thromb Haemost*. 2016;14(3):531-545.

41. Petersen SV, Thiel S, Jensen L, Vorup-Jensen T, Koch C, Jensenius JC. Control of the classical and the MBL pathway of complement activation. *Mol Immunol*. 2000;37(14):803-811.

42. Pihl R, Jensen RK, Poulsen EC, et al. ITIH4 acts as a protease inhibitor by a novel inhibitory mechanism. *Sci Adv*. 2021;7(2):1-17.

43. Degn SE, Jensen L, Gál P, et al. Biological variations of MASP-3 and MAp44, two splice products of the MASP1 gene involved in regulation of the complement system. *J Immunol Methods*. 2010;361(1–2):37-50.

44. Degn SE, Thiel S, Nielsen O, Hansen AG, Steffensen R, Jensenius JC. MAp19, the alternative splice product of the MASP2 gene. *J Immunol Methods*. 2011;373(1–2):89-101.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.