Mannose-binding lectin (MBL2) and ficolin-2 (FCN2) polymorphisms in patients on peritoneal dialysis with staphylococcal peritonitis

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Abstract

Background. Mannose-binding lectin (MBL) and ficolin-2 (FCN) are activators of the lectin pathway of complement and act as primary defences against infection. Single-nucleotide polymorphisms (SNPs) in the MBL2 and FCN2 genes influence the functionality of the proteins. Both proteins are capable of binding staphylococci, which are pathogens that frequently cause peritonitis in patients on continuous ambulatory peritoneal dialysis (CAPD). We studied the role of polymorphisms in the MBL2 and FCN2 genes as a risk factor for developing CAPD peritonitis caused by staphylococci.

Methods. We analysed SNPs in the MBL2 and FCN2 genes in 40 CAPD patients with staphylococcal peritonitis and in 65 CAPD patients without any history of peritonitis. Additionally, we analysed the prevalence of exit site infections and nasal Staphylococcus aureus carriage in both groups.

Results. The +6359C>T SNP leading to the Thr236Met amino acid alteration in the FCN2 gene, associated with decreased substrate binding, was significantly more prevalent in CAPD patients with a history of staphylococcal peritonitis compared with patients on CAPD without a history of peritonitis (P = 0.037). No difference was found in MBL2 genotypes between the two groups. In CAPD patients with a history of staphylococcal peritonitis, exit site infection with S. aureus was also more prevalent (P < 0.01), while S. aureus carriage was not (P = 0.073).

Conclusions. In addition to known risk factors such as exit site infection, the +6359C>T SNP in the FCN2 gene might be a risk factor for staphylococcal peritonitis in CAPD patients due to decreased binding of FCN to staphylococci.

Keywords: CAPD; ficolin-2; MBL; peritonitis; polymorphism

Introduction

Patients with end-stage renal disease have an increased susceptibility to infection, partly due to the impaired immunity caused by uraemia. In patients on continuous ambulatory peritoneal dialysis (CAPD), peritonitis is a common complication with significant morbidity and mortality. The most commonly found causative microorganism is Staphylococcus aureus. Staphylococcal peritonitis is caused by local spreading from the skin via the catheter. Furthermore, nasal carriage of S. aureus is a known risk factor for S. aureus peritonitis. The role of intrinsic factors (e.g. the patient’s immune system) is a subject of research.

Mannose-binding lectin (MBL) is a calcium-dependent C-type lectin that acts as a primary defence mechanism against infections. MBL is an activator of the complement system and enhances phagocytosis. MBL deficiency may confer a risk of infection, especially when other mechanisms of immunity are impaired. MBL is capable of binding to a broad range of microorganisms and has a strong binding capacity for S. aureus [1,2]. Ficolin-2 (FCN) is a serum protein that is similar to MBL in structure and function. After binding microorganisms, it can activate complement and enhance phagocytosis by opsonization. FCN can recognize S. aureus by binding lipoteichoic acid (LTA) moieties on the bacterial surface [3].

Functional MBL levels are influenced by single-nucleotide polymorphisms (SNPs) in exon 1 and the MBL2 gene promoter region. The combination of these SNPs results in sufficient or deficient MBL serum levels [4]. Polymorphisms in the FCN gene FCN2 have been described [5,6]. Genetic differences in the 5′ untranslated region (5′-UTR) influence FCN levels and two coding SNPs in the fibrinogen-like domain alter the substrate binding affinity [6–8].
In CAPD patients, MBL2 and FCN2 gene SNPs may influence defences against staphylococci, since the overall immunity is already impaired. This might lead to an increased risk to staphylococcal peritonitis. The aim of this study was to examine whether polymorphisms in the genes encoding MBL and FCN act as risk factors for staphylococcal peritonitis in CAPD patients.

Materials and methods

Patients and controls

In this retrospective study, we used information from databases of all CAPD patients in two centres from January 1997 through August 2009. Subjects were included in the study if they were at least 18 years old and were on CAPD for more than 6 months. Staphylococcal peritonitis was defined as a white blood cell count in PD fluid $>100 \text{mm}^3$, with PD fluid cultures that were positive for staphylococcal species. Relapsing peritonitis, defined as the development of peritonitis caused by the same microorganism within 2 weeks, was regarded as the same peritonitis episode. Using these criteria, 40 patients with at least one episode of staphylococcal CAPD peritonitis and 65 control patients without any history of peritonitis were included. In the peritonitis group, 32 patients were asked to donate whole-blood samples for DNA analysis. Of eight peritonitis patients who were deceased, serum samples were available for analysis. In the 65 control patients, 45 patients donated whole blood, and from 20 patients, serum samples were available. Exit site infection was defined as colonization of the PD catheter exit site with staphylococcal species and clinical signs of inflammation (e.g. redness of the skin). Nasal S. aureus carriage was defined by at least two positive nasal cultures with S. aureus. A previously described second control group consisted of 223 Caucasian blood donors, not on CAPD, who originated from the same geographical area as the patients [5,9]. Written informed consent was obtained from all living patients. The study protocol was approved by the institutional medical ethics committee.

DNA isolation

Genomic DNA was isolated from whole-blood samples obtained from 76 patients. To increase the number of patients available for genotypic analysis, genotyping from previously stored serum samples from 29 patients was used. Genomic DNA was isolated from 100 μL of whole blood or sera with the MagNAPure LC robot (Roche Diagnostics, Mannheim, Germany), using the MagNAPure DNA Isolation Kit according to the manufacturer’s protocol.

Genotyping of MBL2

The X/Y promoter (rs7096206) and exon 1 SNPs (wild-type ‘A’ and variants ‘O’ rs5030737, rs1800450 and rs1800451) of MBL2 were determined using a previously described denaturing gradient gel electrophoresis (DGGE) assay with modifications in a nested PCR protocol [9,10]. Two PCR assays specific for the promoter X or Y SNP were run in a nested PCR assay. For whole-blood samples, the PCR was run for 25 cycles as previously described [9]. For serum samples, it was run for 40 cycles. After a 1:100 dilution in PCR-grade water (Sigma-Aldrich, Zwijndrecht, The Netherlands), MBL2 exon 1 was amplified from these PCR products with an additional GC clamp attached to one primer to meet DGGE requirements. Amplified DNA fragments from the second PCR assay were analysed on a polyacrylamide gel with a linear denaturing gradient of formamide and urea. All MBL2 exon 1 haplotypes could be distinguished by their different patterns of migration. Genotypes YA/YA,XA/YA,XA/XA and YA/O were considered ‘MBL-sufficient’ and genotypes XA/O and O/O were considered ‘MBL-deficient’ [4,9,11].

Genotyping of FCN2

Three FCN2 genotypes were determined using a previously described DGGE assay [5]: one SNP in the 5′-UTR (−4A>G, rs17514136, associated with elevated FCN serum levels) and two coding SNPs in exon 8 (+6359C>T, rs7851696, associated with increased substrate binding). For DNA isolated from serum samples, this protocol was modified to incorporate a nested PCR assay. An initial PCR for exon 1 (forward primer TCG GAA GAT GAG AAA TTG G, reverse primer CAG GGA CGA GAA GTT TCC) and exon 8 (forward primer CCT GCC TAA CCA TAC ATG G, reverse primer AAC AGA GCT GGA TTT GAA CC) was performed (annealing for 60 s at 57°C, 40 cycles). A 1:100 dilution of this PCR product served as the template for further amplification in the whole-blood genotyping assay as described above.

Statistical analysis

All statistical analyses were performed using statistical software (SPSS version 15.0 for Windows, Chicago). The contributions of polymorphisms in the MBL2 and FCN2 genes and clinical characteristics (e.g. age, sex, nasal exit site infection, nasal S. aureus carrier status) to the development of CAPD staphylococcal peritonitis were analysed using univariate analysis (Pearson’s χ² test or the Fisher’s exact test, as appropriate). Statistical significance was reached at the P = 0.05 level. All polymorphisms adhered to the Hardy–Weinberg expectations (P > 0.05).

Results

Baseline characteristics

There were no significant differences in age, sex or mean time on CAPD between patients and controls (Table 1). Nasal S. aureus carriage was not measured in one of the centres. Therefore, carriage was scored in 56 patients only. Nasal S. aureus carriage was equally prevalent between both groups (P = 0.073). Significantly more patients with a history of peritonitis had one or more exit site infections with S. aureus, compared with the CAPD patients without a history of peritonitis (P < 0.01).

Genotype distribution

No differences were seen in MBL2 genotype distribution (sufficient vs insufficient) between CAPD patients who...
had a history of staphylococcal peritonitis and patients without a history of peritonitis (Table 2; Pearson’s χ², P = 0.240). Eight percent of the patients with CAPD peritonitis had a genotype coding for deficient MBL production (O/O or XA/O), compared with 17% of the patients without peritonitis. This distribution did not differ from that of healthy individuals (data not shown).

The +6359C>T SNP in the FCN2 gene, associated with decreased substrate binding, was significantly more prevalent in patients with a history of staphylococcal peritonitis compared with patients on CAPD without a history of peritonitis (Table 3; 18 vs 5% respectively, Pearson’s χ², P = 0.037). Moreover, there is a tendency towards a gene dose effect. Patients with genotype +6359TT had more frequent peritonitis than patients with genotype +6359CC [odds ratio (OR) = 5.57, 95% CI = 1.29–24.05, P = 0.013]. There is a trend that, in patients with genotype +6359TT, peritonitis was more frequent than in the patients with the +6359CT genotype (OR = 3.05, 95% CI = 0.66–14.14, P = 0.144). The OR for genotype +6359CT compared with genotype +6359CC is 1.83 (95% CI = 0.74–4.53, P = 0.191). No differences were seen for the other two SNPs in the FCN2 gene.

### Discussion

In this cohort of 105 patients on CAPD, the +6359C>T SNP leading to the Thr236Met amino acid alteration in the FCN2 gene was associated with an increased risk of developing staphylococcal peritonitis in patients on CAPD. SNPs in the MBL2 gene were not associated with an increased risk of staphylococcal peritonitis. Significantly more patients with a history of peritonitis had one or more exit site infections with *S. aureus*, compared with the control patients without peritonitis.

FCN levels and polymorphisms in the FCN2 gene have been associated with a variety of infectious diseases and autoimmune diseases [12–15]. Several SNPs have been demonstrated to influence FCN serum levels as well as ligand binding affinity [6,8]. Furthermore, low levels of ficolin may contribute to susceptibility of respiratory infections [13].

The +6359C>T SNP in the FCN2 gene is associated with decreased ability of carbohydrate binding, but it has no effect on FCN levels [6,8].

Several SNPs in the promoter region of FCN2 have been associated with susceptibility for rheumatic fever and Behcet’s disease [14,15].

CAPD has been associated with lower serum MBL levels, compared with healthy controls [16]. These lowered MBL levels in patients on CAPD were not associated with exon 1 SNPs but were proposed to be due to MBL loss via peritoneal clearance and reduced MBL synthesis in the liver due to uraemia [16]. A decreased MBL level may be an underlying risk factor for infections in patients on peritoneal dialysis. On the other hand, in haemodialysis patients, MBL levels were significantly higher [17]. In a subsequent study it was suggested that lower MBL levels predict unfavourable outcome in haemodialysis patients [18].

Besides exon 1 SNPs, the promoter X/Y SNP greatly influences MBL levels. The genotype YA/O is considered to be MBL-sufficient, while XA/O is considered to be MBL-deficient [11]. Furthermore, genotype XA/XA is not able to upregulate production when MBL is consumed (e.g. during the acute phase of infection) [4]. Our data suggest that the differences in distribution of X/Y promoter SNPs is not responsible for staphylococcal infections in CAPD patients.

In a previous study, peritonitis in CAPD patients was not associated with peritoneal MBL levels or exon 1 SNPs of MBL2 [16]. However, in that study, all causes of peritonitis were considered, including Gram-positive and

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**Table 2. MBL genotypes**

<table>
<thead>
<tr>
<th>MBL2 genotype</th>
<th>Patients with a history of staphylococcal peritonitis, n = 40 (%)</th>
<th>Patients without a history of peritonitis, n = 65 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL-sufficient</td>
<td>37/40 (92)</td>
<td>54/65 (83)</td>
<td>NS*</td>
</tr>
<tr>
<td>A/A</td>
<td>27/40 (68)</td>
<td>30/65 (46)</td>
<td></td>
</tr>
<tr>
<td>XA/XA</td>
<td>0/40 (0)</td>
<td>3/65 (5)</td>
<td></td>
</tr>
<tr>
<td>YA/YA</td>
<td>10/40 (25)</td>
<td>21/65 (32)</td>
<td></td>
</tr>
<tr>
<td>MBL-deficient</td>
<td>3/40 (8)</td>
<td>11/65 (17)</td>
<td></td>
</tr>
<tr>
<td>XA/O</td>
<td>2/40 (5)</td>
<td>6/65 (9)</td>
<td></td>
</tr>
<tr>
<td>O/O</td>
<td>1/40 (3)</td>
<td>5/65 (8)</td>
<td></td>
</tr>
</tbody>
</table>

*P = 0.240, MBL-sufficient vs MBL-deficient.

**Table 3. FCN genotypes**

<table>
<thead>
<tr>
<th>FCN2 genotype</th>
<th>Patients with a history of staphylococcal peritonitis, n = 40 (%)</th>
<th>Patients without a history of peritonitis, n = 65 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>−4A&gt;G9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>20/37 (54)</td>
<td>42/63 (67)</td>
<td></td>
</tr>
<tr>
<td>A/G</td>
<td>13/37 (35)</td>
<td>18/63 (29)</td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>4/37 (11)</td>
<td>3/63 (5)</td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A allele %</td>
<td>72</td>
<td>81</td>
<td>NS</td>
</tr>
<tr>
<td>G allele %</td>
<td>28</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>+6359C&gt;T</td>
<td>0.037#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>18/38 (47)</td>
<td>43/63 (68)</td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>13/38 (34)</td>
<td>17/63 (27)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>7/38 (18)</td>
<td>3/63 (5)</td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C allele %</td>
<td>64</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>T allele %</td>
<td>36</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>+6424G&gt;T</td>
<td>NS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>28/38 (74)</td>
<td>45/63 (71)</td>
<td></td>
</tr>
<tr>
<td>G/T</td>
<td>10/38 (26)</td>
<td>17/63 (27)</td>
<td></td>
</tr>
<tr>
<td>T/T</td>
<td>0/38 (0)</td>
<td>1/63 (2)</td>
<td></td>
</tr>
<tr>
<td>Allele frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G allele %</td>
<td>87</td>
<td>85</td>
<td>NS</td>
</tr>
<tr>
<td>T allele %</td>
<td>13</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

A/A, C/C, G/G, wild-type homozygous; A/G, C/T, G/T, wild-type/variant-type heterozygous; G/G, T/T, variant-type homozygous.

#Due to missing values, the denominator has been changed.

Statistically significant.
Gram-negative pathogens, mixed flora and culture-negative peritonitis. Furthermore, the study was limited to MBL only as the activating molecule of the lectin pathway of complement. Our study specifically looked at the association of staphylococcal peritonitis with genetic variations in two initiators of the lectin pathway, MBL and FCN; both have a strong affinity for staphylococci. In our cohort, MBL genetic variations did not explain why some CAPD patients had staphylococcal infections and why some CAPD patients did not have peritonitis at all.

Other predisposing risk factors for peritonitis are also of importance (i.e. exit site infections, connecting technique of the catheter, nasal S. aureus carrier status, personal hygiene) [19,20]. Also, in our study, we observed an increased prevalence of exit site infection in those who developed staphylococcal peritonitis.

The limited sample size is a potential weakness of the study. In this relatively small study population, we did not observe any effect of MBL on staphylococcal peritonitis. We, therefore, consider major effects of MBL genotype on the occurrence of staphylococcal peritonitis unlikely. However, we did find a significant difference in the +6359C>T SNP of the FCN2 gene. Larger study populations are needed to investigate potential weaker effects in the other SNPs.

**Conclusion**

In conclusion, the +6359C>T SNP in the FCN2 gene might be a risk factor for staphylococcal peritonitis in CAPD patients due to decreased binding of variant FCN to staphylococci. This study shows that, besides classical risk factors for CAPD peritonitis (e.g. exit site infection), genetic variations in the immune system can lead to an increased risk of peritonitis in patients on CAPD.

**Conflict of interest statement.** None declared.

**References**


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