Genetic and clinical factors influence the baseline permeability of the peritoneal membrane

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Genetic and clinical factors influence the baseline permeability of the peritoneal membrane.

Background. Patients starting peritoneal dialysis (PD) show a significant variability in small solute transport across the peritoneal membrane (PM). The latter parameter determines dialysis prescription and survival. Clinical factors probably influence solute transport across the PM, but the putative role of genetic variants is unknown.

Methods. We have investigated the influence of functional polymorphisms of VEGF, ENOS, and IL-6, together with clinical and biological factors, on baseline peritoneal equilibration test (PET) parameters in a homogeneous population of 152 unrelated Caucasian PD patients from Belgium and the North of France.

Results. The distribution of the 21 alleles (7 polymorphisms) and linkage disequilibrium parameters were similar in PD patients and healthy subjects. Univariate and multivariate analyses identified comorbidity, serum albumin, and the −174G/C polymorphism of IL-6 as independent predictors of small solute transport. The −174G/C polymorphism of IL-6 was associated with significantly higher IL-6 mRNA levels in the PM and higher plasma and dialysate IL-6 concentrations, suggesting a dominant effect of the C allele. Patients harboring the CC and GC genotypes (N = 92) were characterized by significantly higher permeability parameters and inflammatory markers than patients harboring the GG genotype (N = 60). In contrast with IL-6, VEGF and ENOS polymorphisms had no influence on baseline peritoneal permeability.

Conclusion. These data show that, together with clinical parameters, the functionally relevant −174G/C polymorphism of IL-6 contributes to the interpatient variability in small solute transport rate at the start of PD; and (2) substantiate the critical role played by IL-6 in the PM.

Key words: peritoneal dialysis, interleukin-6, polymorphisms, inflammation, PET.

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Peritoneal dialysis (PD) is used in approximately 15% of dialysis patients worldwide. The small solute transport rate, which is clinically assessed by the peritoneal equilibration test (PET), determines the dialysis prescription, and is also a major predictor of patient and technique survival [1–3]. Twardowski et al first showed that approximately two thirds of patients have average transport rate, the remaining one third being almost equally distributed between high and low transporters [1]. Subsequent series confirmed the existence of a significant interpatient variability in the baseline solute transport characteristics of the peritoneal membrane (PM) [2–4]. Clinical factors such as diabetes, cardiovascular comorbidity, and malnutrition likely influence the peritoneal permeability [2, 5]. Despite recent insights into the molecular mechanisms regulating PM permeability during PD [6, 7], it remains unknown whether genetic variants influencing such mechanisms contribute to the baseline interpatient variability in peritoneal transport.

The small solute transport rate depends mainly on the effective surface area (EPSA), which corresponds to the amount of perfused capillaries within the PM [8]. Clinical and experimental studies have shown that increased EPSA is associated with increased transport of small solutes and, eventually, ultrafiltration (UF) failure [6, 7]. Accumulating evidence suggests that growth factors such as vascular endothelial growth factor (VEGF) and cytokines such as interleukin-6 (IL-6), together with the release of nitric oxide (NO) by endothelial cells, play a central role in the regulation of vascular density and permeability within the peritoneum [6–9]. The regulation of VEGF expression depends on a variety of hormones, growth factors, and cytokines, including IL-6 [10]. VEGF is expressed within the human PM, and its abundance in the dialysate directly correlates with the permeability of the PM and the loss of UF [11, 12]. The pleiotropic
IL-6 is an important mediator of inflammation that increases vascular permeability and stimulates the production of acute-phase proteins, such as the C-reactive protein (CRP) [13]. Several lines of evidence suggest that the signaling mediated by IL-6 and its soluble receptor (sIL-6R) plays a key role in both acute and chronic inflammation [14, 15]. Various cells within the PM secrete IL-6 [16], and its plasma and dialysate concentrations have been associated with high peritoneal solute transport rate [12]. Among its many biological functions, NO is involved in the regulation of vascular tone and permeability [17], and modulation of angiogenesis [18]. A significant endothelial NO synthase (eNOS) activity is detected in the peritoneum [19], and eNOS up-regulation is clearly involved in permeability changes associated with peritonitis [20].

Several polymorphisms within the regulatory region of the genes coding for VEGF, IL-6, and eNOS have recently been identified. These polymorphisms modify the amount of gene expression in vitro [21–26], which suggests that they could modulate the expression of important mediators and influence the permeability of the PM. The aim of the present study was to evaluate whether genetic factors contribute, in parallel with clinical factors, to the small solute transport rate at baseline. Our data, obtained in a large series of homogeneous PD patients, suggest that a promoter polymorphism of \textit{IL-6} influencing the expression of IL-6 at the mRNA and protein levels could influence the baseline solute transport across the PM.

**METHODS**

**Patients and controls**

Patients from a restricted geographic area (150 km radius) covering central Belgium and the North of France were consecutively recruited from June 2002 to June 2003 in Saint-Luc Academic Hospital (Brussels, Belgium), Bichat Academic Hospital (Paris, France), University Hospital Gasthuisberg (Leuven, Belgium), Clinique Ste-Elisabeth (Namur, Belgium), University Hospital Ghent (Ghent, Belgium). All Caucasian patients treated by PD and having an initial PET performed within one to six months after PD initiation were included. Patients with active peritonitis at the time of the PET were excluded. The patients were unrelated. The Ethics Committee of each center involved approved the study protocol (including biopsy analysis when appropriate), and informed consent was obtained from all patients. A population of 103 healthy Caucasian men and women recruited from University Hospital Gasthuisberg and Saint-Luc Academic Hospital was used to assess genotype distributions and allele frequencies.

**Clinical and biological parameters**

Detailed physical, clinical, and PD parameters were obtained for all patients. The intake of drugs, including angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor antagonists (ARA), statins, nitrates, corticosteroids, and immunosuppressive treatment was also recorded. The Wright comorbidity score, which includes age, diabetes mellitus, active malignancy, coronary heart disease, peripheral vascular disease, and inflammatory disease, was determined for each patient [27]. Cardiovascular comorbidity was defined by at least one cardiovascular event, including angina pectoris, myocardial infarct, and peripheral arterial disease. Body surface area (BSA) was calculated as \[\sqrt{\text{weight (kg)} / 3600}\]. Serum albumin at the time of PET was determined using bromocresol green (serum) or immunochemistry (dialysate) (Dade Behring, Marburg, Germany). CRP level at the time of PET was determined by enzyme-linked immunosorbent assay (ELISA) (Dade Behring) in a subset of 56 patients.

**Study of small solute transport**

The dialysate over plasma concentration ratio for creatinine at 4 hours (D/P\text{creat}), dialysate over initial dialysate concentration ratio for glucose at 4 hours (D/D\text{0}), and glucose absorption were determined during the PET performed within one to six months after PD initiation [1, 2]. Peritoneal transport category was based on the D/P\text{creat}, which has been shown to be minimally influenced by dialysate tonicity [28]. The mass transfer area coefficient for creatinine (MTA\text{creat}), normalized for BSA, was calculated according to the Waniewski model, which corrects for convective transport [29].

**DNA extraction and genotyping**

Genomic DNA was purified from peripheral blood leukocytes or frozen dialysate samples using the Purgene kit® (Gentra, Minneapolis, MN, USA). All polymerase chain reaction (PCR) amplifications were carried out in 20 μL, using 100 ng of genomic DNA. Genotyping for the intron 4 VNTR and exon 7 Glu298Asp polymorphisms of \textit{ENOS} were performed using primers, PCR conditions, and digestion by \textit{MboI} and \textit{BanII} (Life Technologies, Carlsbad, CA, USA), as described previously [21, 22]. Genotyping for the VEGF polymorphism −2578C/A was performed using primers and PCR conditions described earlier [24], whereas the −1154G/A and +405G/C \textit{VEGF} polymorphisms were analyzed by PCR followed by \textit{MnlI} and \textit{BsmFI} digestion, respectively [23, 24]. Genotyping for the −174G/C and −597G/A polymorphism of \textit{IL-6} were performed by PCR followed by \textit{NlaIII} and \textit{FokI} digestion, respectively [25, 26]. The −174G/C genotype...
was subsequently verified by pyrosequencing analysis, as described previously [30].

Determination of IL-6, VEGF, and NO metabolites in plasma and dialysate

The plasma and dialysate IL-6 concentrations at time of the PET were determined using ELISA (Quantikine®, R&D Systems, Inc., Minneapolis, MN, USA) in all 56 patients for which samples were available. These 56 patients were similar in terms of peritoneal permeability (D/Pcreat, MTACcreat), age, gender, plasma albumin, and Wright score than the rest (N = 96) of the cohort. The human IL-6 assay has been validated in the plasma, with an intra-assay \((N = 20)\) and interassay \((N = 36)\) precision averaging 7% and 8%, respectively. The average recovery of IL-6 spiked to levels throughout the range of the assay in the EDTA plasma was 97% \((N = 8)\). The immunoassay is calibrated against a highly purified Escherichia coli-expressed recombinant human IL-6 (R&D Systems). The mean minimum detectable dose of IL-6 was 0.039 pg/mL. The linearity of the assay was systematically verified >99% using standards that have appropriately been diluted to produce samples within the dynamic range of the assay. There was no cross-reactivity for other human cytokines, including IFN-\(\gamma\) and IL-4. The concentrations of VEGF (Quantikine®) and nitrate/nitrite (NOx; Cayman Chemical, Ann Arbor, MI, USA) were determined in 32 randomly selected dialysate samples. All analyses were performed in duplicate.

Quantitative real-time RT-PCR analysis of IL-6 mRNA expression in human peritoneum

The transcriptional effect of the −174G/C polymorphism of IL-6 was investigated by quantitative real-time reverse transcription (RT)-PCR on eight human peritoneum biopsies (parietal peritoneum) from patients included in the study. These PD patients were similar to the rest of the cohort in terms of peritoneal transport and clinical parameters. Four patients harbored the −174GG, and four the −174CC genotype. Two biopsies were obtained at time of catheter insertion, and two at renal transplantation in each group. The samples were homogenized in Trizol (Invitrogen, Merelbeke, Belgium). RNA samples were treated with DNase I (MBI Fermentas GMBH, St. Leon-Rot, Germany), and reverse transcribed into cDNA by using Superscript II (Invitrogen). The primers for GAPDH and IL-6 were as follows: 5′ AGAGGCACCTGGCAGAAACA 3′ antisense 5′ CACAGCTCTGGCTTGTTCCT 3′ (IL-6); 5′ GGGGCTCCTCCAGAACATCAT 3′ antisense 5′ TCTAGACGGCGAGTCAGGT 3′ (GAPDH). The length of amplicons were 184 bp (IL-6) and 149 bp (GAPDH). The PCR products were size fractionated on 1.5% agarose, stained with ethidium bromide, purified by Qiaquick gel extraction kit (Qiagen, Leusden, The Netherlands), and sequenced by Genome Express (Grenoble, France).

Real-time RT-PCR (iCycler, Bio-Rad, Hercules, CA, USA) analyses were performed in duplicate with 200 nmol/L of both sense and antisense primers in a final volume of 25 μL using iSupermix (Bio-Rad). PCR conditions were settled as incubation at 94°C for three minutes, followed by 40 cycles of 30 seconds at 95°C, 30 seconds at 61°C, and one minute at 72°C. The melting temperature of PCR product was checked at the end of each PCR by recording SYBR Green fluorescence increase upon slowly renaturing DNA. A control PCR without template DNA was performed in each experiment. To determine the absolute copy number of the target transcript, a cloned plasmid DNA for IL-6 and GAPDH was used to generate a standard curve. The PCR products were cloned into a pTZ57R vector (MBI Fermentas) and transformed in JM 107 competent cells (MBI Fermentas). A standard curve was generated from serial 10-fold dilutions of the purified plasmid containing the appropriate cDNA. Regression analyses of the purified plasmid containing the appropriate cDNA. Linear associations were assessed by Pearson correlation. The plasma and dialysate concentrations of IL-6 according to the genotype were compared by extrapolating the Ct values from the standard curves. The standardized ratio (IL-6 mRNA/GAPDH mRNA) will be referred to as the relative amount of IL-6 mRNA [31].

Statistical analysis

Continuous data are given as mean ± SD (Tables 1 to 5) and mean ± SEM (Figures 1 to 5). Categorical data are given as absolute counts and percentage. The chi-square test \(\chi^2\) was used to compare genotype and allele frequencies in PD patients and controls. Comparison between transport groups was assessed by two-tailed unpaired \(t\) test for continuous data, and Fisher exact test for categorical variables. Linear associations were assessed by Pearson correlation. The plasma and dialysate concentrations of IL-6 according to the genotype were compared using analysis of variance (ANOVA). The two-tailed unpaired \(t\) test, with Welch’s correction when appropriate, was used to compare the effect of IL-6 genotype on small solute transport. No adjustments have been made for multiple comparisons in the univariate analysis [32].

Multivariate regressions were conducted in order to verify whether small solute transport (assessed by the D/Pcreat) still correlated with genetic polymorphisms after adjustment to potential confounders [32]. In the studied population, M represents the more frequent (major) allele, and m represents the less frequent (minor) allele. Polymorphisms were coded into two classes to test dominant \((0 = M/M, 1 = M/m or m/m)\) and recessive models \((0 = M/M or M/m, 1 = m/m)\), or in three classes to test the
and the change in log-likelihood were introduced as a sixth variable in linear regression, VEGF, ENOS, and albumin as dummy variables. Next, four polymorphisms with the D/Pcreat. The polymorphism with the highest \( \chi^2 \) was kept as the sixth variable in linear regression, and the three remaining polymorphisms were introduced in turn as a seventh variable to check for an additional correlation value with the D/Pcreat. Statistical analysis was performed using Instat 3 software (GraphPad Software, Inc.). Significance is defined as \( P < 0.05 \).

**RESULTS**

**Clinical and peritoneal permeability characteristics**

A total of 206 PD patients were recruited in Belgium and France, 54 of which were excluded because the initial PET was performed during active peritonitis (\( N = 5 \)) or outside the one- to six-month period (\( N = 36 \), or clinical data were lacking (\( N = 13 \)). Thus, the study population consisted of 152 PD patients with a mean age of 57 ± 1.4 years, and a male to female ratio of 87:65. Causes of renal failure included: chronic glomerulonephritis (\( N = 46 \)), hypertensive nephropathy (\( N = 30 \)), interstitial nephritis (\( N = 22 \)), diabetic nephropathy (\( N = 16 \)), polycystic kidney disease (\( N = 12 \)), Alport’s syndrome (\( N = 5 \)), and other/unknown (\( N = 21 \)).

The mean duration of PD before the initial PET was 11 ± 1 weeks (range 4–24 weeks). Thirteen (9%) patients had one episode of treated peritonitis at least one month before the PET. The mean characteristics of the initial PET were the following: D/Pcreat, 0.72 ± 0.12, MTACcreat, 12.3 ± 3.6 mL/min/1.73m², glucose absorption, 70 ± 9%. Although only five patients (4%) fell into the previously described low category [1], the D/Pcreat among the study population showed a Gaussian distribution (23 patients (15%) low (L), 41 patients (27%) low-average (LA), 56 patients (37%) high-average (HA), and 32 patients (21%) high (H) when using the criteria defined by Rodby et al [4].

### Table 1. Comparison of peritoneal equilibration test (PET) and clinical parameters between the different transport groups in 152 PD patients

<table>
<thead>
<tr>
<th></th>
<th>Low (L)</th>
<th>Low average (LA)</th>
<th>L/LA</th>
<th>High average (HA)</th>
<th>High (H)</th>
<th>HA/H</th>
</tr>
</thead>
<tbody>
<tr>
<td>D/Pcreat</td>
<td>0.54 ± 0.05</td>
<td>0.66 ± 0.02</td>
<td>0.62 ± 0.07</td>
<td>0.75 ± 0.03</td>
<td>0.86 ± 0.05</td>
<td>0.79 ± 0.06</td>
</tr>
<tr>
<td>MTACcreat</td>
<td>7.5 ± 1.5</td>
<td>9.6 ± 1.5</td>
<td>8.9 ± 1.6</td>
<td>12.7 ± 2.2</td>
<td>17 ± 4</td>
<td>14.3 ± 3.7</td>
</tr>
<tr>
<td>mL/min/1.73m²</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu Abs%</td>
<td>56 ± 6</td>
<td>66 ± 5</td>
<td>63 ± 7</td>
<td>72 ± 6</td>
<td>80 ± 5</td>
<td>75 ± 3</td>
</tr>
<tr>
<td>Time on PD before PET weeks</td>
<td>13 ± 8</td>
<td>10 ± 8</td>
<td>11 ± 8</td>
<td>11 ± 8</td>
<td>12 ± 9</td>
<td>11 ± 8</td>
</tr>
<tr>
<td>Age years</td>
<td>52 ± 18</td>
<td>52 ± 18</td>
<td>52 ± 18</td>
<td>59 ± 16</td>
<td>62 ± 12</td>
<td>60 ± 15</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>15/7</td>
<td>17/25</td>
<td>32/32</td>
<td>20/36</td>
<td>13/19</td>
<td>33/55</td>
</tr>
<tr>
<td>BSA m²</td>
<td>1.68 ± 0.25</td>
<td>1.76 ± 0.25</td>
<td>1.73 ± 0.24</td>
<td>1.77 ± 0.14</td>
<td>1.73 ± 0.18</td>
<td>1.76 ± 0.16</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (14%)</td>
<td>2 (5%)</td>
<td>5 (8%)</td>
<td>10 (18%)</td>
<td>11 (34%)</td>
<td>21 (24%)</td>
</tr>
<tr>
<td>(type 1 and 2) N (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comorbid grade N (%)</td>
<td>2 (9%)</td>
<td>1 (2%)</td>
<td>3 (5%)</td>
<td>5 (9%)</td>
<td>6 (19%)</td>
<td>11 (12%)</td>
</tr>
<tr>
<td>Peritonitis before PET N (%)</td>
<td>4 (14%)</td>
<td>4 (14%)</td>
<td>6 (10%)</td>
<td>10 (18%)</td>
<td>11 (34%)</td>
<td>21 (24%)</td>
</tr>
<tr>
<td>MTACcreat,m</td>
<td>7.5 ± 1.5</td>
<td>9.6 ± 1.5</td>
<td>8.9 ± 1.6</td>
<td>12.7 ± 2.2</td>
<td>17 ± 4</td>
<td>14.3 ± 3.7</td>
</tr>
</tbody>
</table>

**Notes:**
- D/Pcreat, dialysate over plasma ratio for creatinine at 4 hours; MTACcreat, mass transfer area coefficient for creatinine; Glu Abs, glucose absorption; PET, peritoneal equilibration test; PD, peritoneal dialysis; BSA, body surface area; ACEi, angiotensin-converting enzyme inhibitor; ARA, angiotensin II receptor antagonist. Values are given as mean ± SD.
- Comparison between L/LA and HA/H groups was assessed by unpaired \( t \) test for continuous data and Fisher exact test for categorical variables.
- \( \chi^2 \) Wright index [27].

**Distribution of VEGF, ENOS, and IL-6 polymorphisms**

The distribution of the 21 alleles (7 polymorphisms in 3 genes) was similar in the different centers and, for the whole study population, did not differ from the healthy Caucasian population. For each polymorphism, the observed genotype frequencies did not deviate from the Hardy-Weinberg equilibrium (data not shown). Similar linkage disequilibrium parameters were observed in our study population and previously studied Caucasian populations regarding the association between the a allele of intron 4 VNTR and the Glu allele of the Glu298Asp polymorphism of ENOS [33], the −2578G/A and −1154G/A VEGF polymorphisms [24], and the IL-6 −174G/C and −597G/A polymorphisms [26].
Influence of clinical parameters on the initial PET characteristics

The univariate analysis of clinical parameters according to PET characteristics in the 152 PD patients is shown in Table 1. The patients were defined according to the PET, and subsequently included into L/LA (N = 64) and HA/H (N = 88) groups for statistical analyses. The mean D/P creat was 0.62 ± 0.07 versus 0.79 ± 0.06, the mean MTAC creat 8.9 ± 1.6 versus 14.3 ± 3.7, and the mean glucose absorption 63 ± 7% versus 75 ± 3% in the L/LA versus HA/H groups, respectively. The groups were similar for duration of PD before the PET, gender ratio, BSA, type of nephropathy, and peritonitis episodes. The HA/H group showed a significantly higher age (60 ± 15 years vs. 52 ± 18 years in L/LA), a higher proportion of diabetic patients (24% vs. 8% in L/LA), a higher prevalence of high-grade comorbidity (30% vs. 10% in L/LA), and a higher proportion of patients with cardiovascular disease and treatment with ACEi or ARA (60% vs. 37% in L/LA). Ultrasensitive CRP levels tended to be higher in the HA/H group. The proportions of prior renal transplanted patients and patients under steroid and/or immunosuppressive treatment were significantly higher in the L/LA group.

The relationship between the comorbidity grade and serum albumin and permeability parameters is illustrated in Figure 1. The MTAC creat was significantly higher in PD patients with a high or medium grade of comorbidity (high: 13.4 ± 6.3 mL/min/1.73m²; medium: 12.9 ± 5.3 mL/min/1.73m²; low: 10.7 ± 3.2 mL/min/1.73m²) (Fig. 1A). Serum albumin was significantly lower in the HA/H versus the L/LA group (3.4 ± 0.4 vs. 3.8 ± 0.5 g/dL, P < 0.0001), and a negative correlation between serum albumin and the MTAC creat (Pearson r = −0.22, P = 0.005) was observed (Fig. 1B).

Distribution of genotypes according to the initial PET characteristics

The distribution of the ENOS, VEGF, and IL-6 genotypes among PD patients according to PET characteristics is shown in Table 2. The distribution of ENOS and VEGF polymorphisms was similar in each transport group. However, the distribution of the −174G/C and −597G/A polymorphisms of IL-6, which were in complete linkage disequilibrium, was significantly different among the two transport groups. In comparison with the HA/H group, where the genotype distribution was similar to that observed in the general population, the L/LA group was characterized by a lower prevalence of the CC/GC alleles (69% vs. 49%, respectively) and a higher prevalence of the GG allele (31% vs. 51%, respectively) of the IL-6 −174G/C polymorphism.
IL-6−dependent variables were (step 1) the Wright index, R−VEGF [MTACcreat:G G(0.19, P(N = 0.10, F-change = P)], Polymorphisms (dominant model)

Baseline variables

Male gender 0.03 ± 0.02 0.07
Age 0.0001 ± 0.001 0.86
Diabetes 0.008 ± 0.023 0.73
Wright score 0.031 ± 0.014 0.02
Albumin −0.04 ± 0.02 0.01

Polymorphisms (dominant model)

ENOS Glu298Asp 0.01 0.94
VEGF −1154G/A 4.26 0.04
VEGF +405G/C 4.10 0.05
IL-6 −174G/C 4.69 0.03

Influence of IL-6 genotype on biochemical, clinical, and transport parameters

To document the putative biological effect of the −174G/C polymorphism of IL-6, we performed detailed expression studies of IL-6 mRNA in eight peritoneal biopsies from patients included in the study. Quantitative real-time RT-PCR showed that the expression of IL-6 mRNA was significantly higher in the parietal peritoneum of patients harboring the −174CC genotype (Fig. 2A to C).

Plasma and dialysate concentrations of IL-6 at time of the initial PET were determined to substantiate the biological link between the −174G/C polymorphism of IL-6 and peritoneal permeability (Fig. 3). The plasma and dialysate concentrations of IL-6 were significantly higher in patients harboring the CC and GC genotypes compared with the GG patients [plasma IL-6 (geometric means 95% CI): 6.75 (4.07–10.96) and 5.5 (3.72–7.94) vs. 2.58 (1.82–3.55) respectively, ANOVA, P = 0.002] [dialysate IL-6 (geometric means 95% CI): 86.81 (40.74–181.97) and 97.95 (51.29–181.97) vs. 29.83 (20.89–40.74) respectively, P = 0.003]. Furthermore, the 34 patients harboring the GC/CC genotype had a significantly higher CRP level than the 22 GG patients (1.55 ± 0.44 vs. 0.60 ± 0.21, P = 0.04). In contrast, the dialysate concentrations of VEGF and NOx metabolites were similar in all patients. These data suggest that the C allele of the IL-6 −174G/C polymorphism acts in a dominant way to increase the production of IL-6 in plasma and dialysate, which could be reflected by a selective increase in the
production of CRP. Of note, the albumin concentration in the dialysate of the 34 patients with the GC/CC genotype was significantly higher than in the 22 patients harboring the GG genotype (1328 ± 746 mg/L vs. 848 ± 324 mg/L, \( P = 0.006 \)), further evidencing higher peritoneal permeability in the former.

These biochemical findings were reflected by the influence of the \(-174G/C\) polymorphism on solute transport parameters (Fig. 4). The MTAC\(_{\text{creat}}\) was indeed significantly lower among patients harboring the GG genotype than CC or GC patients (10.7 ± 0.4 vs. 12.9 ± 0.8 or 12.8 ± 0.5, ANOVA, \( P = 0.006 \)), confirming the dominant effect of the C allele. Analysis of the PD transport and clinical parameters according to the dominant model (Table 4) confirmed that MTAC\(_{\text{creat}}\), D/P\(_{\text{crea}}\), and glucose absorption were all significantly lower in patients harboring the GG genotype. The GG patients were also characterized by significantly higher serum albumin levels, whereas other clinical parameters, including age, gender, BSA, comorbidity grade, number of peritonitis episodes, and duration of PD were similar in both groups. It must be noted that the results were similar when the 13 patients with peritonitis more than one month before the PET were excluded from the analysis. For the remaining 139 patients, the values for the MTAC creatinine were the following: CC + GC (\( N = 85 \)): 12.7 ± 4.2 vs. GG (\( N = 54 \)): 10.6 ± 3.2; \( P = 0.0023 \).

**DISCUSSION**

We have investigated the effects of polymorphisms of \(\text{ENOS, VEGF, and IL-6}\) together with clinical parameters on the small solute transport rate during the initial PET in a large series of PD patients. No effect was found for \(\text{ENOS}\) and \(\text{VEGF}\) polymorphisms. In contrast, the \(-174G/C\) polymorphism of \(\text{IL-6}\) was identified as an independent predictor of peritoneal permeability, together with comorbidity and serum albumin level. Most importantly, this promoter polymorphism of \(\text{IL-6}\) influenced the expression of \(\text{IL-6}\) both at the mRNA and protein levels, with a dominant effect of the C allele on the plasma and dialysate concentrations of \(\text{IL-6}\), mirroring the effect on solute transport.

Our study provides the first detailed investigation of the potential role of genetic factors in regulating the baseline solute transport across the PM. Based on the emerging framework of molecular mechanisms that regulate the microvascular density and permeability during PD [6–9], we selected genes coding for the key mediators \(\text{IL-6, VEGF, and eNOS}\). Considering the inclusion criteria...
and the necessity to investigate patients similar in terms of PD modalities and care in general, we could analyze a large series of unrelated PD patients with a similar ethnic background, and originating from a relatively restricted geographic area. For each of the three genes considered, we selected frequent polymorphisms that have previously been shown to regulate the transcription in vitro [21–26]. Population stratification and spurious allelic associations should be a concern in association studies in general. However, the inclusion criteria and general characteristics of our cohort are clearly distinct from the conditions associated with stratification bias [34]. First, our population is homogenous in terms of race, ethnic background, and geographic area. Second, the patients were systematically included in the study, and the origin of renal failure in this group is similar to our dialysis population at large. Third, the population showed a Gaussian distribution of the permeability parameter, and the influence of classic clinical factors was verified by univariate analysis. Fourth, two types of genomic controls have been performed. The distributions of the 21 alleles (7 polymorphisms on different chromosomes) were similar in our cohort of patients and healthy controls from the same area. Similar linkage disequilibrium parameters were also found for the polymorphisms of ENOS, IL-6, and VEGF in our cohort and the healthy population. We feel that these clinical and

### Table 4. Influence of the −174G/C polymorphism of IL-6 on peritoneal transport and clinical and biochemical parameters in the 152 PD patients

<table>
<thead>
<tr>
<th>IL-6-174G/C polymorphism</th>
<th>GG (N = 60)</th>
<th>GC+CC (N = 92)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTAC&lt;sub&gt;creat&lt;/sub&gt; mL/min/1.73m²</td>
<td>10.70 ± 3.38</td>
<td>12.85 ± 4.21</td>
<td>0.002</td>
</tr>
<tr>
<td>D/P&lt;sub&gt;creat&lt;/sub&gt;</td>
<td>0.69 ± 0.11</td>
<td>0.74 ± 0.10</td>
<td>0.004</td>
</tr>
<tr>
<td>Glu Abs%</td>
<td>67 ± 9</td>
<td>72 ± 9</td>
<td>0.002</td>
</tr>
<tr>
<td>Age years</td>
<td>58 ± 17</td>
<td>55 ± 16</td>
<td>0.3</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>28/32</td>
<td>37/55</td>
<td>0.5</td>
</tr>
<tr>
<td>BSA m²</td>
<td>1.78 ± 0.2</td>
<td>1.72 ± 0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>Peritonitis episodes before PET</td>
<td>5 (8%)</td>
<td>6 (6%)</td>
<td>0.4</td>
</tr>
<tr>
<td>Comorbidity grade&lt;sup&gt;a&lt;/sup&gt; N (%)</td>
<td></td>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>Low</td>
<td>30 (50%)</td>
<td>39 (42%)</td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>21 (35%)</td>
<td>30 (33%)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>9 (15%)</td>
<td>23 (25%)</td>
<td></td>
</tr>
<tr>
<td>Albumin g/dL</td>
<td>3.7 ± 0.5</td>
<td>3.5 ± 0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Time on PD before PET weeks</td>
<td>12 (4–24)</td>
<td>11 (4–24)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>Wright index [27].
genetic data strongly argue against population admixture in our study population.

Among a wide range of biological activities, IL-6 promotes the release of CRP and simultaneously suppresses albumin production by hepatocytes, and it influences vascular permeability in vitro [13]. Moreover, the IL-6/sIL-6R signaling emerges as a complex regulator of cytokines and growth factors involved in acute and chronic inflammation [14, 15]. Increased levels of plasma IL-6 and CRP predict cardiovascular morbidity and overall mortality [35]. Relevant to this study, inflammation has been associated with the peritoneal transport status in prevalent PD patients [36], and plasma and dialysate IL-6 concentrations are increased among H/HA PD patients [12]. There is, thus, a rationale to investigate whether peritoneal permeability could be influenced by functional polymorphisms of IL-6, including the $-174G/C$ polymorphism known to influence both the endothelial function [37] and the inflammatory response [38].

Our univariate and multivariate analyses show that the $-174G/C$ polymorphism of IL-6 influences the baseline permeability for small solutes in this series of PD patients. The conventional analysis confirmed that, together with the Wright index and serum albumin, the IL-6 polymorphism is a significant predictor of peritoneal transport at baseline. In this model, the independent variables account for $\sim 20\%$ of the variability in solute transport, whereas the IL-6 polymorphism alone accounts for only $2\%$ of the variability. It is interesting to note that similar conclusions have recently been evidenced in a large cohort of 574 new PD patients, in which the clinical variables accounted for $\sim 20\%$ of the variability in solute transport, whereas individual factors had little influence (for instance, age accounted only for $2\%$ of the variability) [39]. Subsequent analysis in our population showed a strong effect of the C allele of this polymorphism on the MTACcr (Fig. 4), and the association of the $-174G/C$ polymorphism with small solute transport was also found in subgroups stratified by country (Belgium vs. France). The molecular basis of the effect of this $-174G/C$ polymorphism has been previously attributed to the association of the C allele with higher plasma levels of IL-6 and CRP [40, 41], although these results have been challenged by others [25].

Based on quantitative real-time RT-PCR analysis, we have shown that the $-174G/C$ promoter polymorphism exerts an influence on the transcription of IL-6 in the PM (Fig. 2). Patients harboring the GC and CC genotypes have higher IL-6 levels in plasma and dialysate (Fig. 3) and higher transport parameters (Fig. 4) than patients with the GG genotype, suggesting that the C allele exerts a dominant effect to increase local IL-6 production and influence solute transport. It is tempting to speculate that inflammation may be the link between the genetic variant and peritoneal transport. This hypothesis is supported by the fact that increased IL-6 production in our PD patients is associated with a higher CRP level, as well as by the demonstration of low serum albumin as an independent predictor of PM transport (see below). Beyond the potential genetic influence, the production of IL-6 may also be regulated by other, clinical factors. Elevated levels of IL-6 are associated with aging, type 2 diabetes mellitus, cardiovascular morbidity, and chronic stress [13, 42]. Yet the H/HA patients in our cohort were older and characterized by a higher comorbidity (as assessed by the Wright score that includes age and diabetes), a higher percentage of diabetics, and a higher proportion of treatment with ACEI/ARA (Table 1). After multivariate analysis, the Wright score, as well as serum albumin and the IL-6 polymorphism, remained independent predictors of small solute transport across the membrane. It is, thus, possible that high comorbidity, including older age and diabetes, is associated with increased inflammatory response and IL-6 production, which, in turn, could modify the peritoneal microcirculation and small solute transport.

In addition to pleiotropic cytokines such as IL-6, growth factors and vasoactive mediators have a potential influence on the structure and permeability of the peritoneum [6, 7]. In contrast with IL-6, VEGF and ENOS polymorphisms were not identified as independent predictors of baseline solute transport (there was a minor effect of VEGF polymorphisms that was not confirmed in the multivariate analysis). This negative result may reflect the lack of biological effect of these variants, since no significant differences in VEGF and NOx levels were detected. The effect of probiotic growth factors such as TGF-$\beta$ or FGF2 was not investigated here. It is tempting to hypothesize that genetic variants in growth factors may be particularly important for the longitudinal changes in the membrane exposed to the dialysate [6–9]. Investigation of this hypothesis will have to take into account the potential influence of many factors, including drugs (such as ACEi or ARA) that may interfere with the release of such factors.

Baseline peritoneal transport is associated with comorbidity and albumin in this series of PD patients. The Wright score of comorbidity includes clinical factors (age, diabetes mellitus, cardiovascular disease) associated with peritoneal transport in univariate analysis. Although potentially interesting, interpretation of the effect of prior renal transplantation, and immunosuppressive drugs, ACEi, or ARA, is hampered by low numbers or correlation with cardiovascular comorbidity. The association between low albumin and increased transport rate has been reported by others [2, 12, 39]. Recently, Davies et al have demonstrated that, in addition to plasma albumin, comorbidity at the start of treatment was associated with increased solute transport in PD patients [5, 39]. However, one should remain cautious in interpreting albumin levels in relation to solute transport: rather
than being a marker of inflammation, lower systemic albumin concentrations may reflect the fluid overload encountered in PD patients with high peritoneal permeability. High transporters may also lose albumin in the dialysate, as suggested by the data in the GC/CC patients studied here.

**CONCLUSION**

Our studies, based on genetic and biochemical investigations, support the hypothesis that underlying mechanisms (regulation of IL-6 gene expression) could regulate systemic and local inflammation and, in association with comorbidity and uremia, affect the transport of small solutes across the PM (Fig. 5). These data also suggest that genetic variants, together with clinical factors, could contribute to the variability in peritoneal transport observed at baseline. Confirming the strength of the present association, and deciphering the influence of genetic determinants on peritoneal transport will require well-designed, adequately powered studies, in different populations and different settings [43], as well as a detailed assessment of the function of the polymorphisms [44].

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