Phenotype and genotype: perspectives for peritoneal dialysis patients

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Introduction

Peritoneal dialysis (PD) is currently used on ~15% of the worldwide dialysis population. Recent improvements in connectology and antibiotics management have led to a dramatic reduction in the rate and consequences of peritonitis in PD patients. Deterioration of membrane permeability, with modifications in the transport of small solutes across the peritoneal membrane (PM) and loss of ultrafiltration (UF), is the most frequent abnormality in long-term PD patients, and the main reason for technical failure. A better understanding of the mechanisms and determinants of the small solute transport and UF is now essential to achieve long-term preservation of the PM together with improvements in patient morbidity and mortality.

The transport of solutes and water across the PM occurs through three types of pores located in the capillary endothelium [1]. The ‘small pores’ (radius 40–50 Å), which correspond to the clefts located between endothelial cells, account for ~95% of the hydraulic conductance (UF coefficient, \(L_pS\)). A second population of pores, the ‘large pores’ (radius 250 Å), thought to correspond to the venular interendothelial gaps, accounts for 5% of the UF coefficient. These pores are involved in the transport of macromolecules and mediate an important part of UF via fluid convection from blood to the peritoneal cavity. The third population of pores consists of water-specific, ‘ultrasmall pores’ located in the endothelial cells; these correspond to aquaporin-1 (AQP1). Although they account for only 1–2% of the hydraulic conductance, ultrasmall pores reject solutes and facilitate the transport of water during crystalloid osmosis. Data obtained on the AQP1 mouse model have confirmed that ultrasmall pores do mediate ~50% of the UF, as well as the ‘sodium sieving’, i.e. the rapid fall in dialysate sodium concentration during a dwell with hypertonic glucose [2].

In this editorial comment, we will review the clinical tools available to assess the transport properties of the PM at the start of PD, corresponding to a given phenotype. We will next summarize what is known about the clinical determinants of individual variability in baseline peritoneal transport. Based on the fact that clinical factors only account for a small part of the variability, we will then discuss how genetic factors may contribute to the baseline transport—opening a perspective for genotype—phenotype correlations in PD.

Clinical importance of assessing transport properties of the peritoneum

The transport properties of the PM (i.e. the three populations of pores) are usually assessed by the peritoneal equilibration test (PET) [3]. The PET, performed during a 4-h dwell with a 2.27% glucose solution, evaluates the small solute transport rate, using the dialysate/plasma (D/P) ratio of creatinine at the end of the procedure, and the ratio of dialysate glucose concentration at 240 min/initiation of the test (D/D0), as well as the net UF. According to the D/P ratio of creatinine, patients are categorized as low (L), low-average (LA), high-average (HA) and high (H) transporters. Recently, the substitution of the 2.27% by a 3.86% glucose solution has provided better information on free water transport across the ultrasmall pores [4]. In parallel, evaluation of the clearance of macromolecules (albumin, α2-macroglobulin) may reflect large pore patency [5]. Alternative methods to assess the transport properties of the PM are the standard peritoneal permeability analysis (SPA) or the peritoneal dialysis capacity (PDC) tests [6].

Assessing individual variability in peritoneal transport has major clinical importance. The CANUSA study first documented the association between higher transport for small solutes and lower combined patient and technical survival [7], an association that has been confirmed in various populations [8]. Indeed, a high D/P ratio for creatinine is paralleled by a low D/D0 ratio for glucose with, in consequence, a reduction in the osmotic gradient, loss of UF and fluid retention.
A high transport status may also be associated with increased peritoneal albumin losses, and thus potential denutrition. Furthermore, individual variability in peritoneal transport status also influences PD prescription: high transporters benefit from short dwells and icodextrin, while low transporters are prescribed longer dwells.

Twardowski et al. [3] first showed that approximately two-thirds of the patients have an average transport rate, the remaining one-third being almost equally distributed between high and low transporters. Subsequent series confirmed the existence of a significant interpatient variability in the baseline solute transport characteristics of the PM [7,9–12], raising the critical issue of the determinants involved in such variability.

Clinical determinants of the baseline peritoneal transport

In 1988, the CANUSA study (606 patients) demonstrated that older age, male gender, diabetes and low serum albumin concentration were all independent factors associated with a high D/P creatinine ratio at the onset of PD [7]. More recently, Davies [9] (574 patients) also documented significant correlations between small solute transport rate and albumin, gender and residual urine volume. Similarly, the Australian and New Zealand Dialysis and Transplant Registry (3188 patients) found independent associations between high peritoneal transport status and race (Maori and Pacific Islander origin), older age and lower body mass index (BMI) [10]. In a recent European multicentre study, Gillerot et al. [12] (152 patients) documented that high/high-average transporters were more likely to be older, diabetics, hypoalbuminaemic, treated by angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor antagonists, and to have a higher comorbidity grade than low-average/low transporters. In a study including 367 Spanish patients tested between the 2nd and 6th week on PD, Selgas et al. [11] detected a significant inverse linear correlation between UF and age and creatinine mass transfer area coefficient—confirming the association between older age and higher transporters. Finally, Clerbaux et al. [13] (72 patients) found that small solute transport rate was higher in patients given an ACE inhibitor, with a low serum albumin concentration, and in those with the highest body surface area (BSA). It must be pointed out that differences in the timing of initial testing, or the possible effect of peritonitis prior to the transport study, could explain some discrepancies in the above studies. We must also acknowledge that some of the clinical determinants interact with each other, which may explain why age or gender, for instance, predominate in one study and BMI in another.

Taken together, these studies tell us that a high comorbidity score, including hypoalbuminaemia, older age, diabetes and ACE inhibitor prescription is likely to influence the transport properties of the PM at baseline. In particular, systemic inflammation, associated with comorbid states and hypoalbuminaemia as surrogate marker, may result in structural changes such as neoangiogenesis, leading to modifications in transport. Of note, one should remain cautious in interpreting albumin levels in relation to solute transport: rather than being a marker of inflammation, lower systemic albumin concentration may reflect the fluid overload encountered in high transporters. The latter may also lose albumin in the dialysate [12]. At any rate, the complexity of the relationships between UF and small solute transport [11], as well as the fact that independent clinical variables account for only ~20% of the individual variability of solute transport [9,12], strongly suggest that factors that are not routinely measured do play a role in the baseline functional characteristics of the peritoneum.

The determinants of other transport parameters have been addressed in some studies. Clerbaux et al. [13] identified BSA as a determinant of sodium sieving. As BSA correlates with the effective peritoneal surface area (EPSA), this finding is likely to reflect a secondary association: the larger the BSA, the higher the number of small pores available for glucose absorption, with reduction in AQP1-mediated water transport. Using the PDC test, Van Biesen et al. [5] (135 patients) showed that a large-pore flux is an indicator of systemic inflammation, related to increased mortality [5]. In our population, the clearance of α2-macroglobulin was affected by age, diabetes and renal residual function [13]. Individual characteristics, probably linked to comorbidity and peritoneal inflammation, may thus influence the density and/or patency of large pore population, hence the PM leakiness.

Genetic determinants of the transport properties of the peritoneal membrane

The small solute transport rate depends mainly on the amount of perfused capillaries within the PM, the blood flow and the physical area of membrane contact with the dialysate. Accordingly, an increased EPSA is associated with higher transport of small solutes and eventually, UF failure [14,15]. 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 of the PM [14–16]. Because angiogenesis and fibrosis are two essential—and possibly related—events in the PM exposed to PD, other mediators such as transforming growth factor-β (TGF-β) and the plasminogen activator inhibitor (PAI-1) have also been involved [14,17]. Several polymorphisms within the regulatory region of the genes coding for VEGF, IL-6, the endothelial NO synthase (eNOS) and PAI-1 modify the amount of
gene expression in vitro, which suggests that they could modulate the expression of these mediators. Furthermore, variants in these genes have been associated with diseases—such as diabetic retinopathy and/or nephropathy—that are potentially relevant for the structural changes that occur in the PM exposed to dialysis. Based on the finding that most of the variability in peritoneal transport remains unexplained by clinical factors, recent studies (summarized in Table 1) have investigated whether genetic variants (polymorphisms) could influence transport properties of the PM at baseline [12,17–19].

Nitrous oxide regulates vascular tone and permeability and modulates angiogenesis. A significant NOS activity is detected in the peritoneum, and modifications in the expression of eNOS, for instance, during acute peritonitis, are associated with major changes in transport across the PM [20]. Wong et al. [18] were the first to observe a positive association between a variable number of tandem repeats (VNTR) in the intron 4 of ENOS (ENOS4a/b) and transport properties in incident PD patients. Of note, the ENOS genotype remained an independent predictor for peritoneal transport after adjustment for clinical parameters (gender, age, diabetes) by multivariate analysis. However, there was no biological counterpart to the effect observed, and the study was limited by the low prevalence (12%) of the a allele in this peculiar variant.

Different cell types synthesize VEGF in the PM and its abundance in the dialysate directly correlates with the small solute transport rate and the loss of UF [21]. Szeto et al. [19] investigated whether two promoter polymorphisms of VEGF could influence peritoneal transport, in a series of 135 incident PD patients. There was no association between VEGF polymorphisms and transport at baseline. However, in a sub-analysis restricted to 83 patients for which a 12-month follow-up was available, there was a positive association between the two variants and the longitudinal changes in D/P creatinine. Of note, the protective effect of the CC genotype was reflected by significantly lower VEGF mRNA in a subset of dialysate effluent samples [19]. Although the study used a non-classical method for the longitudinal monitoring of transport, with a limited population and a low prevalence (6%) of the deleterious AA genotype, it showed that a genetic variant may influence longitudinal changes in membrane transport, with a biological counterpart. Furthermore, these data supported previous evidence suggesting the deleterious effect of VEGF on the human PM exposed to PD [14,15,22].

Interleukin-6 is a pleiotropic cytokine that mediates inflammation by increasing vascular permeability and stimulating the production of acute-phase proteins such as the C-reactive protein. Several lines of evidence suggest that the signalling mediated by IL-6 and its soluble receptor (sIL-6R) plays a key role in both acute and chronic inflammation [23]. Various cells within the PM secrete IL-6, and its plasma and dialysate concentrations have been associated with high

| Table 1. Association studies addressing peritoneal transport parameters in patients treated by peritoneal dialysis (PD) |
|-------------------------------------------------|---------------------------------|-------------------|-------------------|------------------|-------------------|-------------------|
| Variant, polymorphism(s)                       | Type of study, country          | No. of patients, PD population | Transport parameter | Biological parameter | Association | Authors (REF) |
| 4G/5G promoter                                  | Cross-section, L, Hong Kong     | 103 incident (48 prevalent)    | DATT baseline      | NOx mRNA in dialysate (NS) | Positive at baseline | Szeto et al. [17] |
| ENOS 1154G/A, Intron 4 VNTR                    | Single center, MV, Belgium, France | 86 incident (Chinese)         | DATT baseline      | VEGF mRNA in dialysate (NS) | Positive at baseline | Szeto et al. [18] |
| VEGF −2578C/A, −1154G/A                        | Single center, MV, Belgium, France | 133 incident (Chinese)       | PET baseline       | VEGF in dialysate (NS) | Negative at baseline | Gillerot et al. [12] |
| IL-6 597G/A, −4058G/C                           | Cross-section, L, Hong Kong     | 132 incident (Caucasians)     | DATT baseline      | VEGF in dialysate (NS) | Positive at baseline | Gillerot et al. [17] |
| ENOS 1154G/A, Intron 4 VNTR                    | Single center, MV, Belgium, France | 83 incident (Chinese)       | DATT baseline      | VEGF mRNA in dialysate (NS) | Positive at baseline | Gillerot et al. [18] |

NS, not significant; (*), significant association between the genotype and the biological parameter; **, significant association after adjustment for clinical variables; PET, peritoneal equilibration test; DATT, dialysis adequacy and transport test; NOx, nitrate and nitrite; VNTR, variable number of tandem repeats.
peritoneal solute transport rate [21]. Recently, Gillerot et al. [12] investigated the respective contributions of seven common polymorphisms (21 alleles) in ENOS, VEGF and IL-6, in parallel with clinical factors, to the small solute transport rate in a multicentric series of 152 incident PD patients. The distribution of the 21 alleles 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 harbouring the CC and GC genotypes ($n=92$) were characterized by significantly higher permeability parameters and inflammatory markers than patients harbouring the GG genotype ($n=60$). These data identified a common polymorphism of IL-6 as an independent predictor of peritoneal transport, together with comorbidity and serum albumin level. The effect was reflected by significant changes in IL-6 at the mRNA and protein levels, pointing to the role of local and systemic inflammation in regulating the small solute transport rate [12]. In contrast with Wong et al. [18], ENOS polymorphisms were not identified as independent predictors of baseline solute transport in the above study [12].

Conflicting results in association studies are common, and may have different causes. The size of the population is essential: many negative studies are simply explained by a lack of power. Differences in ethnicity, environment and inclusion strategy (e.g. related/unrelated patients) must be considered. The number of polymorphisms within a gene of interest and the allelic frequencies in the population are critical. As mentioned earlier, particular caution should be taken when interpreting results based on rare alleles. A negative result may also reflect the lack of biological effect of a given variant, or the insufficient pathophysiological relevance of the gene tested in this clinical setting. Equally important is a careful phenotyping. Factors such as the method used for testing peritoneal transport, the timing of testing after initiation of PD, the incidence of peritonitis, treatment strategies, or the underlying nephropathy may have a significant influence on the functional properties of the peritoneum.

**Conclusion**

There are more than 1 million common polymorphisms in the human genome. Recent studies support the hypothesis that inherited genetic variants could regulate specific mediators and, in association with clinical factors, affect the transport properties of the PM. Analysis of the variability in baseline peritoneal transport in unrelated vs related PD patients will be useful to evaluate the contribution of environment vs genetic factors on this parameter [24]. In addition to the genes described above (Table 1), future studies will probably include genes involved in longitudinal changes in the PM (host defence, matrix synthesis, . . . ); incidence and severity of acute peritonitis, or sclerosing peritonitis; regulation of AQP1 and UF: cardiovascular or metabolic complications and drug response—just to mention a few candidates. Useful insights will also probably arise from large association studies currently performed in diabetic nephropathy or retinopathy.

In parallel, genome-wide association studies will be important to confirm the role of candidate genes, and to reveal unexpected pathways influencing baseline and/or longitudinal changes in peritoneal transport. The limitations of association studies with candidate genes are now clearly delineated, leading to specific guidelines [25,26]. Confirming the strength of the positive associations and deciphering the influence of genetic determinants on peritoneal transport will require well-designed, adequately powered studies, in different populations and different settings, as well as a detailed assessment of the biological role of the polymorphisms. Undoubtedly, the task will be difficult. But the promise of individualized medicine in PD patients, with improved detection, follow-up and treatment, is worth the effort.

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**References**

4. ISPD Ad hoc Committee on Ultrafiltration Management in Peritoneal Dialysis. *Perit Dial Int* 2000; 20 [Suppl 4]: S3–S4


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