Permeability factors in idiopathic nephrotic syndrome: historical perspectives and lessons for the future

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ABSTRACT

The term idiopathic nephrotic syndrome (iNS) traditionally covers minimal change disease and primary focal segmental glomerulosclerosis (FSGS), now thought to be separate disease entities. Clinical and experimental evidence suggest that circulating permeability factors are involved in their pathogenesis. In the past four decades, many investigators have searched for the responsible factors, thus far with little success. The recent report of the soluble urokinase plasminogen activator receptor as a causative factor in FSGS has received much attention, but again the initially promising findings were not confirmed. We describe the history of the search for permeability factors, discuss the pitfalls that are likely responsible for the lack of success and propose criteria that should be used in future studies when evaluating candidate permeability factors.

Keywords: focal segmental glomerulosclerosis, idiopathic nephrotic syndrome, minimal change disease, permeability factor, suPAR

INTRODUCTION

The term idiopathic nephrotic syndrome (iNS) was used to describe a condition consisting of proteinuria, hypo-albuminaemia and oedema, caused by a glomerular disease characterized by the absence of distinctive glomerular abnormalities in conventional light microscopy. Nowadays, minimal change disease (MCNS) and primary focal segmental glomerulosclerosis (FSGS) are considered main causes of iNS. Most experts consider these as separate disease entities, although this is still debated [1]. Already in 1954 Gentili et al. [2] hypothesized that iNS was caused by a circulating factor, based on rather daring experiments in which they administered plasma from infants with iNS to non-nephrotic children and observed a minimal increase in proteinuria. In 1974, Shalhoub [3] suggested that iNS was mediated by (a) T-cell-dependent circulating factor(s) that would affect glomerular permeability. Since then many investigators have tried to identify putative permeability factors. Still, despite the development of many new research techniques, thus far the responsible factors have escaped identification, with soluble urokinase plasminogen activator receptor (suPAR) being the latest example of an unfulfilled prophecy [4]. In this review, we describe the history of the search for permeability factors in iNS, discuss potential pitfalls and provide guidance for future studies.

PERMEABILITY FACTORS IN iNS: A HYPOTHESIS

The best evidence for the existence of circulating permeability factors and their role in glomerular disease came from clinical observations in recurrent FSGS after kidney transplantation [5]. In 1972, Hoyer et al. [6] presented the cases of two children and one young adult with iNS and no or minimal sclerotic lesions on initial kidney biopsy. Subsequent biopsies showed progressive sclerotic lesions. End-stage kidney disease (ESKD) developed within 2–6 years, despite treatment with high-dose corticosteroids combined with other immunosuppressive drugs. After kidney transplantation, proteinuria recurred and kidney biopsy showed a similar histological pattern and no evidence of rejection. Additional evidence for a permeability factor was derived from studies reporting remission of proteinuria by plasma exchange or immunoadsorption, especially if instituted early in the course of recurrent disease [7–9]. Moreover, serum or plasma from patients with recurrent FSGS induced proteinuria in rats and increased albumin permeability in isolated glomeruli [10, 11]. Case reports demonstrated transmission of FSGS from a mother to her child, and remission of proteinuria following implantation of a kidney with FSGS in recipients with other kidney diseases [12–14]. In contrast, there is less clinical evidence for the existence of a circulating permeability factor in MCNS. The idea that a permeability factor produced by T-cells is responsible for MCNS was based on combining
several observations: (i) the association with non-Hodgkin lymphoma and atopy, the onset of remission of MCNS after measles infection and the induction of remission by immunosuppressive agents that inhibit T-cell function (corticosteroids or cyclophosphamide) all suggested involvement of T cells; (ii) the absence of T-cells or immunoglobulins in kidney biopsies and (iii) the discovery of lymphokines, biologically active products of lymphocytes present in plasma [3, 15, 16]. Evidence for a circulating factor was also supported by a single case report of remission of proteinuria after transplantation of two kidneys from a donor with biopsy-proven active MCNS [17].

### PERMEABILITY FACTORS IN MCNS

**Vascular permeability factor**

Lagrué et al. [24] were the first to systematically study the effect of potential plasma factors on *vascular* permeability using a defined model. Their investigations were stimulated by clinical observations and the experimental observation that a human lymphocyte product termed ‘skin reactive factor’ increased permeability in guinea pig skin capillaries [6, 25]. Therefore, Lagrué et al. used isolated lymphocytes from patients with different histological types of nephrotic syndrome and healthy controls (Table 2). Cell culture supernatants were injected into guinea pig skin, followed by immediate intravenous injection of a blue dye. Vascular permeability was measured as the surface of blue dye at the skin injection site. Culture medium derived from lymphocytes of nephrotic patients resulted in significantly higher permeability compared with controls. Culture of lymphocytes in serum, as well as lymphocyte stimulation with concanavalin A (Con A) or phytohaemagglutinin (PHA) increased the response. It was concluded that lymphocytes from nephrotic patients produced a vascular permeability factor (VPF) that may be related to increased glomerular vascular permeability and the mechanism of proteinuria. Although Lagrué et al. are credited for being the first to document the presence of a permeability factor in MCNS, their pivotal study actually showed that VPF was present in most but not all patients with nephrotic syndrome and certainly was not unique to MCNS. Still, their work formed the basis for following studies, mainly conducted in MCNS [27]. These studies reported that VPF bioactivity corresponded with disease activity and remission and was inhibited

### THE SEARCH FOR PERMEABILITY FACTORS

Many investigators have evaluated the role of circulating permeability factors in iNS. When comparing literature data, it is obvious that different models have been used to identify the factor, with most authors using only one model [18]. Moreover, few studies have validated their results. Also patient populations have been divergent. It is evident that strict criteria are needed to evaluate the pathogenic and causative role of a putative disease-causing permeability factor. In the nineteenth century, Koch [19] proposed a set of criteria to judge the pathogenic role of micro-organisms as cause of disease. Johnson et al. [20] adapted these criteria to be applicable to the studies of the pathobiology of growth factors in glomerular disease. Such criteria are also needed in the search for glomerular permeability factors (GPFs) [21]. Table 1 summarizes the conditions which should be met before a permeability factor can be considered the pathogenic culprit.

### Table 1. Conditions which should be met before a permeability factor can be considered pathogenic

<table>
<thead>
<tr>
<th>Criteria to establish the causality of a putative permeability factor</th>
<th>Requirements</th>
<th>Comments</th>
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</thead>
</table>
| I. The permeability factor must have biologic effects *in vitro* and *in vivo*, and be confirmed in validation studies | - Development of a suitable model, e.g. an animal model, cell culture or permeability assay  
- Validation of the model by different research groups or by using more than one model | Response to a permeability factor depends on expression of the target molecule in an experimental model. Thus response may differ between animal and human models |
| II. Identification of the permeability factor in well-phenotyped patients but not in appropriate controls and validation in independent patient cohorts | - Use of biomaterial from well-phenotyped patients and appropriate healthy and disease controls  
- Validation of the specificity of the permeability factor in an independent patient cohort, preferably in a multicenter study | Good phenotyping requires exclusion of patients with secondary FSGS. This also includes use of serum albumin and amount of proteinuria to diagnose FSGS secondary to glomerular hyperfiltration [22, 23]  
Controls should be matched for confounders such as eGFR, amount of proteinuria, treatment |
| III. Temporal relation of the permeability factor with disease activity and remission | - Collection of multiple samples per patient during follow-up  
- Adequate follow-up data | Follow-up data should include information on treatment response and proteinuria outcome, independent of blood pressure changes |
| IV. Specific removal or inhibition of the permeability factor *in vivo* blocks the biologic effect | - Specificity of a method used to remove or inhibit the permeability factor should be demonstrated | Plasmapheresis is a rather crude method and removes many proteins. Thus, plasmapheresis can be used to obtain information relevant for item III, but cannot be used as condition to fulfil the requirement of this item (IV) |

Adapted from references [19–21].
### Table 2. Studies into VPF using skin blueing area (see the text) in patients with nephrotic syndrome and controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Controls</th>
<th>Model</th>
<th>Patient material</th>
<th>Outcome</th>
<th>Comment</th>
</tr>
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<tbody>
<tr>
<td>Lagrue et al. [24]</td>
<td>MCNS/FSGS (n = 18)</td>
<td>MPGN (n = 14)</td>
<td>Guinea pig VPF assay</td>
<td>Supernatant of cultured lymphocytes stimulated with serum, ConA or PHA</td>
<td>MCNS/FSGS: 210 ± 112 mm²</td>
<td>VPF disappeared after successful steroid therapy (n = 3)</td>
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<td></td>
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<td>MN (n = 14)</td>
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<td>MPGN: 174 ± 103 mm²</td>
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<td></td>
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<td>Healthy (n = 23)</td>
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<td>MN: 161 ± 80 mm²</td>
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<td>Healthy: 50 ± 71 mm²</td>
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<td>(P &lt; 0.0005 NS versus normal)</td>
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<td>Trompeter et al. [26]</td>
<td>SSNS (n = 6)</td>
<td>Healthy (n = 6)</td>
<td>Guinea pig VPF assay</td>
<td>Supernatant from unstimulated T-cells</td>
<td>SSNS: 40 ± 10 mm²</td>
<td>– No significant differences</td>
</tr>
<tr>
<td>Jones and Simpson [27]</td>
<td>MCNS (n = 5)</td>
<td>Healthy (n = 10)</td>
<td>Guinea pig VPF assay</td>
<td>Supernatant from unstimulated T-cells</td>
<td>MN: 161 ± 80 mm²</td>
<td>– Only negative study of VPF in NS versus controls</td>
</tr>
<tr>
<td>Bakker et al. [28]</td>
<td>IgAN (n = 11)</td>
<td>MCNS (n = 5)</td>
<td>Wistar rat VPF assay</td>
<td>Supernatant from ConA stimulated PBMC</td>
<td>P &lt; 0.01 for IgAN and MCNS versus MN and healthy controls</td>
<td>No correlation between VPF and proteinuria</td>
</tr>
<tr>
<td>Tomizawa et al. [29]</td>
<td>MCNS active disease (n = 9)</td>
<td>MCNS (n = 5)</td>
<td>Guinea pig VPF assay</td>
<td>Supernatant from unstimulated T-cells</td>
<td>MCNS active: 108 mm²</td>
<td>– VPF secretion was inhibited by plasma from active MCNS</td>
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<td>MCNS remission (n = 7)</td>
<td>MN (n = 5)</td>
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<td>MCNS inactive: 33 mm²</td>
<td>– VPF activity was not neutralized by plasma from active MCNS</td>
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<td>Healthy (n = 14)</td>
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<td></td>
<td>Healthy 20 mm²</td>
<td>– VPF was present in fraction &lt;10 kDa</td>
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<td>(P &lt; 0.001, MCNS active versus other groups)</td>
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<tr>
<td>Matsumoto et al. [30]</td>
<td>MCNS active disease (n = 8)</td>
<td>IgAN with NS (n = 8)</td>
<td>Guinea pig VPF assay</td>
<td>Supernatant from T-cells + 10% FCS or MCNS plasma active/inactive</td>
<td>Plasma from active MCNS significantly decreased VPF production (P &lt; 0.001 active MCNS versus other groups)</td>
<td>– Six MCNS and two IgAN patients were treated with prednisolone</td>
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<td></td>
<td>MCNS remission (n = 8)</td>
<td>IgAN without NS (n = 8)</td>
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<td>– This study is part of several papers on the effect of interleukins/cytokines on VPF production (see the text), including almost the same patients [31–34]. Two papers found a significant difference for active MCNS/IgAN with NS versus healthy controls using supernatant from unstimulated PBMC [31, 34]</td>
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<tr>
<td></td>
<td></td>
<td>Healthy (n = 16)</td>
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</table>

MCNS, minimal change disease; FSGS, focal segmental glomerulosclerosis; MPGN, membranoproliferative glomerulonephritis; MN, membranous nephropathy; NS, nephrotic syndrome; VPF, vascular permeability factor; Con A, concanavalin A; PHA, phytohaemagglutinin; SSNS, steroid-sensitive nephrotic syndrome; NS, nephrotic syndrome; IgAN, IgA nephropathy; PBMC, peripheral blood mononuclear cell; FCS, foetal calf serum.
by cyclosporine [29, 35]. Remarkably, plasma from patients with active MCNS inhibited VPF production, but had no direct blocking effect on its activity [29]. Matsumoto et al. [30–34, 36] studied the effect of several cytokines on VPF and found that IL-4, IL-10, IL-13 and TGF-β had blocking effects, whereas IL-12 and IL-15 stimulated its release.

Physicochemical characteristics of the VPF were investigated using culture medium of cells obtained from an unspecified group of patients with nephrotic syndrome [37]. Using Sephadex column chromatography and isoelectric focussing, VPF activity was identified in a fraction with a molecular weight (MW) of 12 kDa and an isoelectric point of 6.4, respectively. Furthermore, it was established that VPF activity mainly resided in T lymphocytes, and specifically in the CD4+ fraction [29, 38, 39]. IL-2 is produced by T lymphocytes and can induce a vascular leakage syndrome, but was shown to be distinct from VPF [40]. The exact composition of VPF remains unknown to date. Measurement of VPF is a complex technique and has never been standardized. Of note, VPF is not specific for MCNS/FSGS, but also common in other glomerular diseases and not all studies confirmed elevated VPF activity in MCNS versus healthy controls [24, 26, 28, 30]. More importantly, there is no direct evidence that the VPF causes proteinuria or even has an effect on the glomerular capillary wall permeability [21, 41, 42]. In fact, lymphocytes of patients with IgA nephropathy and mild proteinuria released more VPF than lymphocytes of patients with MCNS and heavy proteinuria [28].

**Glomerular permeability factor**

The limitations of the guinea pig skin model and the possible discrepancies between vascular and glomerular permeability prompted experiments in which Con A-stimulated lymphocyte culture supernatants were administered to rats, and glomerular histology and/or proteinuria were used as markers of glomerular permeability [43]. A transient increase in proteinuria along with rat podocyte foot process effacement was elicited by cell culture supernatant of patients with MCNS, but not of healthy or nephrotic controls. Another study also suggested the presence of a GPF in MCNS using a reduction in anionic sites in the glomerular basement membrane of rat kidney as read-out [42]. Several authors [42, 44, 45] demonstrated that GPF activity was present in some patients with FSGS and investigated the relationship between VPF and GPF in MCNS. Clearly, VPF and GPF were not similar, as VPF activity was observed in the absence of GPF (Table 3) [42, 45].

Koyama et al. [47] were able to construct stable T-cell hybridomas, derived from T cells from patients with MCNS. GPF was identified by the ability of hybridoma supernatants to induce proteinuria and podocyte foot process effacement when injected intravenously in rats (Table 3). The putative GPF was present in fractions with a MW in the range of 60–160 kDa. Unfortunately, in spite of the continuous production of supernatant, the T-cell hybridomas have not resulted in the characterization of the GPF [21].

Bakker et al. [48, 49] found a vasoactive plasma fraction with a MW of 80–100 kDa that affected glomerular sialoglycoproteins in patients with MCNS. The responsible protein was identified as haemopexin, a 80–85 kDa abundant haem-scavenging plasma protein [50]. When purified human or recombinant haemopexin was infused into rats, it induced reversible proteinuria accompanied by podocyte foot process effacement [51, 52]. Haemopexin induced nephrin-dependent cytoskeletal rearrangement in cultured podocytes and increased albumin passage across monolayers of glomerular endothelial cells, possibly by affecting its glycocalyx [53]. In MCNS in relapse, lower titres of haemopexin but with enhanced protease activity were observed compared with MCNS in remission and disease controls, including FSGS [52]. It was suggested that various isoforms of haemopexin exist and that in normal conditions circulating haemopexin is inactive but that in certain conditions it can become activated and act as serine protease [54]. These observations thus far have not been validated. The studies also point to some important methodological issues: (i) it may not be sufficient to evaluate merely protein levels and (ii) the factor may not only affect podocytes but may also alter permeability by changing the endothelial cell layer.

In view of the varying sizes reported for the permeability factor, research has also focussed on cytokines. Especially IL8 and IL13 have been suggested. IL13 has been shown to stimulate podocyte protein trafficking and proteolysis in vitro [55]. Overexpression of IL13 in rats induced a MCNS-like nephropathy [56]. However, it is unknown if such high IL13 levels can be present in human MCNS [57]. A pathogenic role for IL8 in glomerular permeability was suggested because serum levels were increased in MCNS in relapse compared with MCNS in remission and disease controls and because IL8 affected the metabolism of glomerular basement membrane compounds in vitro [58]. However, rat podocytes incubated in vitro with high concentrations of human IL8 did not show any alteration in permeability, heparin sulphate proteoglycan gene expression or heparan sulphate synthesis [59].

**FSGS permeability factor**

Experimental models to study permeability factors in recurrent FSGS have used serum rather than cell culture supernatants. Sera from a single patient were shown to induce proteinuria in rats, but histological effects were not investigated [11]. Savin et al. [10] presented an in vitro model to measure the effect of the FSGS permeability factor. This model used isolated rat glomeruli, incubated in isotonic bovine serum albumin. The diameter of the glomeruli and glomerular volume changes were measured after changing the medium to one containing a lower bovine serum albumin concentration. The resulting oncotic pressure gradient caused swelling of glomeruli if glomerular permeability was maintained. Precipitation of isolated glomeruli in serum of patients with recurrent FSGS compared with healthy controls resulted in less glomerular swelling, explained by loss of glomerular permeability and thus dissipation of the oncotic gradient. Permeability to albumin (PAlb) was expressed as 1 minus the ratio of glomerular volume difference established by patient serum
### Table 3. Studies into GPF in patients with nephrotic syndrome and controls

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Controls</th>
<th>Model</th>
<th>Patient material</th>
<th>Outcome</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boulton Jones et al. [46]</td>
<td>MCNS (n = 5) FSGS (n = 1) MPGN (n = 1) FPGN (n = 1)</td>
<td>Healthy (n = 7)</td>
<td>Sprague-Davley rats (degree of FPE)</td>
<td>Supernatant from SKSD stimulated PBMC in 20% serum</td>
<td>Patients: 16.6 ± 2.4 slit pores/10 µm GBM Healthy: 21.6 ± 3.1 slit pores/10 µm GBM (P &lt; 0.005)</td>
<td>Reduced colloidal GBM iron staining (suggests reduction of GBM charge)</td>
</tr>
<tr>
<td>Yoshizawa et al. [43]</td>
<td>MCNS (n = 4 active disease, n = 1 remission)</td>
<td>MPGN (n = 2) IgAN (n = 1) MN (n = 1) FSGS (n = 1)</td>
<td>Wistar rats (proteinuria &gt;2 SD of normal)</td>
<td>Supernatant from Con A stimulated PBMC in 10% heat-inactivated FCS</td>
<td>MCNS: 4/5 positive Controls: 0/10 positive (P &lt; 0.05)</td>
<td>MCNS patient in remission had GPF activity – GPF positive supernatants induced FPE in rat glomeruli</td>
</tr>
<tr>
<td>Maruyama et al. [42]</td>
<td>MCNS (n = 9)</td>
<td>Healthy (n = 5)</td>
<td>Wistar rats (proteinuria)</td>
<td>Supernatant from unstimulated T-lymphocytes in 10% FCS</td>
<td>Patients: 71 ± 5 mg/12 h Controls: 44 ± 4 mg/12 h (P &lt; 0.01) No significant correlation between proteinuria and VPF</td>
<td>Reduced PEI binding to GBM (suggests loss of GBM anionic sites) – No FPE was observed</td>
</tr>
<tr>
<td>Tanaka et al. [44]</td>
<td>SSNS (n = 12)</td>
<td>FSGS (n = 8) Healthy (n = 9)</td>
<td>Sprague-Dawley rats (proteinuria)</td>
<td>Supernatant from Con A stimulated PBMC in 10% heat-inactivated FCS</td>
<td>Patients: 3/10 GPF positive Controls: 0/15 GPF positive, 6/15 VPF positive (P &lt; 0.01)</td>
<td>Supernatant from FSGS also increased proteinuria – Significant reduction of GBM anionic sites after injection of supernatants from MCNS and FSGS versus controls (P &lt; 0.01)</td>
</tr>
<tr>
<td>Kondo et al. [45]</td>
<td>FSGS (n = 10)</td>
<td>MN (n = 1) IgAN (n = 2) MPGN (n = 2)</td>
<td>Sprague-Dawley rats (proteinuria &gt;2 SD of normal)</td>
<td>Supernatant from Con A stimulated PBMC in 10% heat-inactivated FCS</td>
<td>Patients: 7/10 GPF positive Controls: 1/5 (with MN) GPF positive</td>
<td>GPF activity in a minority of patients with FSGS</td>
</tr>
<tr>
<td>Koyama et al. [47]</td>
<td>MCNS (n = 10)</td>
<td>Healthy (n = 10) Culture medium only (n = 5)</td>
<td>Sprague-Dawley rats (proteinuria &gt;2 SD of normal)</td>
<td>Supernatant from unstimulated T-cell hybridoma</td>
<td>Patients: 61.5 mg/24 h Healthy: 0.96 mg/24 h</td>
<td>Minor histological lesions with partial FPE – GPF was present in fraction 60–160 kDa – Reduced PEI binding to GBM (suggests loss of GBM anionic sites)</td>
</tr>
</tbody>
</table>

*Con A, concanavalin A; FCS, foetal calf serum; FPE, podocyte foot process effacement; FSGS, focal segmental glomerulosclerosis; IgAN, IgA nephropathy; MCNS, minimal change disease; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; FPGN, focal proliferative glomerulonephritis; NS, nephrotic syndrome; VPF, vascular permeability factor; GPF, glomerular permeability factor; PBMC, peripheral blood mononuclear cell; PEI, poly-ethyleneimine; PHA, phytohaemagglutinin; SKSD, streptokinase-streptodornase; SSNS, steroid-sensitive nephrotic syndrome.*

*P < 0.01 versus before infusion.

**P < 0.05 versus before infusion.
to control serum. In patients with FSGS, increasing Palb was associated with increasing risk of post-transplant proteinuria. A Palb cut-off of 0.50 predicted recurrence with a sensitivity of 60% and specificity of 95%. Compared with patients with post-transplant FSGS recurrence, Palb was also lower in patients with steroid-sensitive nephrotic syndrome and post-transplant membranous nephropathy. A clinical response to plasmapheresis coincided with a decrease in Palb. The fraction with Palb activity precipitated in 70–80% ammonium sulphate solution, inconsistent with immunoglobulin. It appeared to have a molecular mass of 50 kDa and was bound by protein A. The latter is consistent with a report of proteinuria remission after protein A column ex vivo adsorption [8]. In rats, infusion of the purified 30–50 kDa fraction with high Palb activity caused increased proteinuria [60].

The predictive value of albumin permeability for post-transplant FSGS recurrence has been investigated by only two other groups with equivocal results. In a cohort of 32 children with FSGS, a pre-transplant Palb value >0.6 predicted post-transplant proteinuria recurrence with a sensitivity of 73% and a specificity of 80% [61]. Godfrin et al. [62] used a very similar model, but measured permeability to albumin using electric impedance. Compared with a control group of 63 patients with ESKD due to various causes, 80 patients with FSGS had significantly higher albumin permeability. However, some patients with membranous nephropathy had similar high values, and pre-transplant albumin permeability did not predict proteinuria recurrence in patients with FSGS.

Several investigators have tried to apply the findings to patients with native kidney FSGS. Palb >0.5 was present in 42% of children with iNS [63]. However, Palb did not discriminate between steroid-responsive and steroid-resistant patients. In adult patients with FSGS, Palb was <0.50 in the majority of patients, and there was no good correlation between change in Palb and treatment-induced changes in proteinuria [64].

Savin et al. [65] hypothesized that the FSGS permeability factor interacts with sugars of the podocyte glycocalyx, which may be prevented by the monosaccharide galactose. Indeed, galactose had strong affinity for the plasma fraction <30 kDa with high Palb in FSGS patient plasma. Oral galactose caused a decrease in Palb in a patient with plasmapheresis-resistant post-transplant FSGS. There was no effect on proteinuria, which was attributed to irreversible glomerular damage. Proteomic analysis of the galactose-affinity fraction with high Palb suggested that cardiotrophin-like cytokine 1 was the culprit [66]. Unfortunately, these preliminary findings, reported more than 5 years ago, still await further confirmation. Of note, after 1999 there are no reports of investigators who have validated the model used by Savin et al. We were unable to reproduce the findings and observed large variations when using isolated glomeruli as model (unpublished observations).

Other investigators have used podocyte cell cultures as model to study the presence of putative permeability factors in the serum or plasma of patients with FSGS. Read-out parameters have varied and included a change in podocyte cell shape or altered expression of podocyte-specific proteins such as nephrin and podocin. Most studies have only been published in abstract form and have not been validated. In a recent study, Harris et al. [67] demonstrated increased protease-activated receptor 1-mediated phosphorylation of the vasodilator stimulated protein (VASP) in response to FSGS plasma in human conditionally immortalized podocytes, suggesting that circulating proteases could be involved. However, which protease(s) would be responsible is yet to be determined.

THE suPAR AS A PERMEABILITY FACTOR IN FSGS

In the above-mentioned studies, the search for a causative circulating permeability factor in FSGS was guided by models that were used as biomarkers for the activity of the factor. Total serum, plasma or fractions thereof were used in an ‘untargeted’ approach toward the identification of the putative factor. In recent studies, investigators have used a more targeted approach, based on more detailed knowledge of podocyte pathobiology. Using such an approach, suPAR was recently proposed as a candidate protein and received much attention. The membrane-bound urokinase plasminogen activator receptor (uPAR) is a three-domain glycoprotein with a MW of 35–60 kDa, depending on its degree of glycosylation [68]. Proteolytic cleavage at the membrane anchor and the linker region between domains 1 and 2 results in different circulating (soluble) forms of uPAR. Originally identified as the receptor for urokinase, uPAR also interacts with transcellular receptors such as integrins [69]. The uPAR–integrin interaction causes a motile cell phenotype seen in circumstances such as inflammation and neoplasia. A similar observation of increased podocyte motility in proteinuric diseases stimulated research into the role of uPAR in FSGS. Wei et al. [70] studied uPAR in the lipopolysaccharide (LPS) mouse model of proteinuria and in podocyte cultures. In the respective models, increased podocyte uPAR expression resulted in foot process effacement and actin cytoskeleton rearrangement, likely by activation of αvβ3-integrin. Subsequent studies addressed the inferred hypothesis that circulating suPAR is a circulating factor causing FSGS. We discuss these studies and show how too early optimism could have been prevented by applying the criteria as proposed in Table 1.

The permeability factor must have biologic effects in vitro and in vivo and be confirmed in validation studies

Wei et al. [71] showed that sera from patients with post-transplant FSGS recurrence activated podocyte αvβ3-integrin, which was demonstrated with the β3-integrin-specific antibody AP5. Antibodies against uPAR blocked the effect on AP5. From these studies, it cannot be determined if uPAR or suPAR interacted with αvβ3-integrin. In fact, other pathways such as TNF-α may also be involved [72].

To ascertain the relevance of suPAR, studies were conducted in vivo in uPAR-null mice. These mice developed dose-dependent proteinuria after injection of a chimeric full-domain murine suPAR linked to a human IgG1-Fc. The investigators evaluated the effects of a fragment of suPAR, produced by a plasmid and containing domains 1 and 2 in wild-type mice.
These mice developed proteinuria and early FSGS lesions. A recent study by Cathelin et al. [73] failed to validate the role of intact suPAR. These investigators showed that the same suPAR chimera as used by Wei et al. failed to cause proteinuria in wild-type mice despite glomerular suPAR deposition. Thus, studies with intact suPAR have failed criterion I. Fragments of suPAR may play a role; however, these findings need further confirmation and validation.

**Identification of the permeability factor in well-phenotyped patients but not in appropriate controls and validation in independent patient cohorts**

Wei et al. reported increased serum suPAR concentrations in patients with FSGS versus controls with or without glomerular disease. The highest values were found in patients who developed post-transplant FSGS recurrence. Based on their initial data, serum suPAR concentrations of 3000 pg/mL measured by a commercial assay were proposed as a diagnostic cut-off value. In a subsequent study, suPAR levels >3000 pg/mL were confirmed in 84% of FSGS patients from the NIH-FSGS Clinical Trial, and in 55% from the PodoNet consortium, respectively [74]. In contrast, only 6% of controls had elevated suPAR levels. Although these findings were used as strong supportive evidence, the studies do not fulfill criterion II. Many patients that were included in the seminal studies were not well phenotyped. In fact, a closer look at the individual patient data showed that the studies included patients with secondary FSGS as well as patients with hereditary FSGS, conditions that are not associated with recurrent disease after transplantation. Moreover, the control patients were not matched for critical parameters such as estimated glomerular filtration rate (eGFR). We found an inverse correlation between suPAR and eGFR in a small group of patients with primary and secondary FSGS, and MCNS, and serum suPAR concentration did not distinguish between respective patient groups [75]. When comparing suPAR concentrations in patients with primary FSGS with eGFR-matched controls from the Leuven mild-to-moderate chronic kidney disease study, suPAR was not higher in patients with FSGS [76]. Cohorts from Japan and India also failed to demonstrate elevated suPAR in patients with FSGS compared with other glomerular diseases and confirmed the negative correlation between eGFR and suPAR [77, 78]. Even in FSGS patients with ESKD who had developed post-transplant disease recurrence, we and others did not find elevated suPAR compared with non-FSGS controls [79, 80]. In conclusion, criterion II is not fulfilled, and the role of intact suPAR in recurrent FSGS remains unproven. Importantly, the investigators who first introduced the commercial R&D assay in FSGS research have recently suggested that a specific form of suPAR unrecognized by this enzyme-linked immunosorbent assay is involved in FSGS [81]. Clearly, new assays will need to be developed, which should be validated in independent cohorts.

**Temporal relation of the permeability factor with disease activity and remission**

Few studies have performed serial measurement of suPAR in different disease states. In a subgroup analysis from the NIH-FSGS Clinical Trial, there was a correlation between decrease in proteinuria and suPAR [74]. On the other hand, Sinha et al. [78] found no relationship between suPAR and disease state in patients with FSGS and MCNS. In the setting of post-transplant FSGS recurrence, Wei et al. [71] found a pronounced decrease in serum suPAR in two patients who responded to plasmapheresis, compared with stable levels or slightly decreasing suPAR in two patients who did not respond. Of note, corresponding eGFR values during follow-up were not reported. Morath et al. [82] demonstrated that suPAR was reduced by both plasmapheresis and immunoadsorption. In addition, there was a clear relationship between amount of proteinuria and AP5 activation by serum on podocytes. However, plasmapheresis and immunoadsorption are not specific for removal of suPAR, and the authors admitted that the effect may have been related to other substances. Remarkably, a recent study showed that protein A column immunoadsorption also induced proteinuria remission, but did not remove suPAR [83].

**Specific removal or inhibition of the permeability factor in vivo blocks the biologic effect**

No studies with suPAR have been done. Researchers are developing specific methods for the removal or inhibition of suPAR and have alluded to a trial of specific suPAR removing in patients with FSGS. This would indeed be a strong proof of principle and provide relief for patients who long for improved and less toxic treatment.

**The search for permeability factors: future perspectives**

The history of the search for a GPF is a learning experience. It is evident that circulating factors must be present. The search for the responsible factor has been difficult, and thus far the causative factor has escaped identification. We expect that causative factors will be identified in the very near future, due to the major advances in technology. However, there are certain caveats that should be taken into account.

(i) We propose that collaborative efforts are needed to ascertain the validity of findings and define disease causality. We suggest that the criteria as illustrated in Table 1 could give guidance, and emphasize the need for appropriate patient phenotyping, the use of appropriate controls and validation studies.

(ii) When searching for the permeability factor, one must bear in mind that there may be more than one factor. It is evident that multiple candidate proteins have been proposed (Table 4). Interestingly, the activity of the permeability factor has resided in plasma fractions with divergent MW (Table 4). One simple explanation could be the existence of multiple factors.

(iii) Increased permeability may not be caused by the presence of a causative permeability factor, but related to the absence of an inhibitor. Already in 1976 Moorthy
et al. [84] described a factor in the sera of patients with MCNS in relapse that could inhibit the mitogenic response of PHA-stimulated lymphocytes. However, the relevance remained uncertain because other groups have reported inhibition of lymphokines (macrophage migration inhibitor factor) not associated with iNS and an inhibitory effect on lymphocytes by sera from patients with FSGS and membranous nephropathy [46, 85]. The group of Savin [86] demonstrated that normal serum from a variety of species contains (a) factor(s) that block the increase in P Alb by FSGS serum in vitro. These blocking factors may exert their protective effects by competing with the binding of the permeability factor to glomerular cells. Alternatively, normal components of serum may bind or enzymatically degrade the permeability factor, with several apolipoproteins (Apo J, Apo L, Apo E2 and E4 and a fragment of Apo-IV) being proposed as relevant substances [87–89].

During the editorial process of this manuscript an additional study into the role of suPAR in FSGS appeared (Spinale et al. Kidney Int 2014). Both the mouse model (criterion I) and clinical studies with repeated suPAR measurement (criteria II and III) failed to confirm suPAR as a cause or biomarker of FSGS.

**Table 4. Putative permeability factors in MCNS and FSGS**

<table>
<thead>
<tr>
<th>Putative permeability factors</th>
<th>Molecular weight</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MCNS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GPF-NOS [47]</td>
<td>60–160 kDa</td>
<td>Obtained from T-cell hybridoma made from patients with MCNS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induced proteinuria when injected into rats</td>
</tr>
<tr>
<td>Haemopexin [54]</td>
<td>80–85 kDa</td>
<td>Both recombinant and human haemopexin induced reversible proteinuria accompanied by FPE in rats</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased serum haemopexin with increased protease activity in MCNS in relapse</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Induced nephrin-dependent cytoskeletal rearrangements and increased albumin permeability across monolayers of glomerular endothelial cells</td>
</tr>
<tr>
<td>Interleukin 13 [56]</td>
<td>16 kDa</td>
<td>Increased expression of mRNA and cytoplasmic IL13 in CD4+/CD8+ T cells from children with steroid-sensitive iNS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulates podocyte protein trafficking and proteolysis in vitro</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Overexpression of IL13 in rats induces MCNS-like disease</td>
</tr>
<tr>
<td><strong>FSGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLC-1 [66]</td>
<td>22–25 kDa</td>
<td>Increased glomerular permeability</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased nephrin expression in cultured podocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Antibody to CLC-1 reverse the permeability effect of FSGS sera</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Concentrations up to 100 times higher in recurrent FSGS</td>
</tr>
<tr>
<td>suPAR [71]</td>
<td>20–50 kDa</td>
<td>Activated podocyte β3 integrin, resulting in reorganization of the actin cytoskeleton of podocytes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>High-dose recombinant mouse suPAR induced proteinuria, increased podocyte β3 integrin activity and foot process effacement in mice lacking the gene for uPAR but not in wild-type mice</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Experimental data were not supported by clinical data</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– When adjusted for renal function suPAR levels did not differentiate between FSGS and other kidney diseases</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– No correlation between serum suPAR and degree of proteinuria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>– Higher serum or plasma levels of suPAR were also reported in patients with cancer, sepsis, atherosclerosis and PNH without FSGS</td>
</tr>
</tbody>
</table>

FSGS, focal segmental glomerulosclerosis; GPF-NOS, glomerular permeability factor not otherwise specified; MCNS, minimal change disease; PNH, paroxysmal nocturnal haematuria; CLC-1, cardiotrophin-like cytokine-1; suPAR, soluble urokinase plasminogen activator receptor.

**ACKNOWLEDGEMENTS**

R.J.M. is supported by a grant of the Dutch Kidney Foundation (OW08).

**CONFLICT OF INTEREST STATEMENT**

None. This review has not been published previously in whole or part.

(See related article by Meyrier and Ronco. Allotransplantation using a diseased kidney: when a swallow makes a summer. Nephrol Dial Transplant 2014; 29: 2164–2166.)

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Received for publication: 14.10.2014; Accepted in revised form: 15.10.2014