Recurrent focal glomerulosclerosis in the era of genetics of podocyte proteins: theory and therapy

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Introduction

Focal segmental glomerulosclerosis (FSGS) is the most frequent cause of intractable proteinuria in children and adults and is emerging as a major glomerular cause of chronic kidney disease [1]. Most of the aspects related to its pathogenesis remain unknown, one major issue being post-transplant recurrence. Recent advances in...
molecular genetics of FSGS led to the identification of several genes responsible for familial forms. In general, they code for proteins of the podocyte and are specifically localized in the glomerular slit-diaphragm where they play a critical role in the control of glomerular permeability. The clinical implications of one of them, that is NPHS2 coding for podocin, are obvious as its involvement has been extended to sporadic FSGS [2–4]. Experience with inherited sporadic FSGS due to NPHS2 mutations is accumulating and shows strong clinical homologies with idiopathic FSGS, in spite of clear pathogenic differences. This offers an invaluable opportunity for a critical re-evaluation of the mechanisms, which are presently thought to be involved in the pathogenesis of idiopathic FSGS and in its recurrence after renal transplantation.

Recurrent FSGS: the classical point of view

Recurrent FSGS has remained an enigma ever since the first report of three patients with rapid recurrence of FSGS after transplantation was published by Hoyer et al. [5]. Since then, the clinical course and outcome of recurrent FSGS have been extensively described [6–9]. Approximately 30% of patients with idiopathic FSGS recur after renal transplantation, although in very young paediatric recipients and in patients with a rapid course of the disease in their native kidneys and with Caucasian ethnicity there is a higher risk of recurrence [8,9]. Patients who have recurrence of FSGS in the first year after transplantation with rapid loss of their graft are at a very high risk (>80%) of having recurrences in subsequent grafts [9]. In fact, at the University of California, San Francisco we currently avoid re-transplanting patients who lose their graft rapidly due to recurrent FSGS.

Several observations support the hypothesis of a causative factor present in the circulation of patients with FSGS. Recurrence of FSGS with proteinuria can occur within hours of transplantation and is associated with diffuse effacement of the foot processes [5,8]. In addition, both plasmapheresis and immunoadsorption with protein A [7,9] have been used successfully in inducing a remission of proteinuria as well as restoring the normal architecture of the foot processes [8–10]. In 1984, Zimmerman induced proteinuria in rats injected with serum from a patient with recurrent FSGS [11]. More recently, Sharma et al. [12] demonstrated the presence of a circulating permeability factor in a fractionated supernatant of plasma (obtained from plasmapheresis of patients with recurrent FSGS). Plasmapheresis supernatant, treated with 50–70% ammonium sulfate to precipitate plasma proteins such as albumin and immunoglobulins, contains the circulating permeability factor [13,14]. This supernatant contained a 100-fold purification of the factor as compared with untreated plasma and caused an increase in proteinuria when injected into rats. Dialysis and centrifugation-based membrane ultrafiltration indicated a molecular size between 30 and 50kDa. Dantal et al. [10] confirmed the findings above and reproduced experimental proteinuria in rats. They treated patients with recurrent FSGS with immunoadsorption and demonstrated that the material eluted from the protein A column induced proteinuria when injected in rats. The active fraction of the eluate had a molecular weight below 100,000 units. Thus, these studies indicate that a circulating factor is found in some patients with recurrent FSGS and may be responsible for initiating the foot processes renal injury.

An important advance in the relationship of the putative circulating factor to the recurrence of FSGS was the application of a technique described by Savin et al. for the measurement of albumin reflection coefficient in isolated rat glomeruli [13,14]. The use of this in vitro assay has allowed an indirect determination of the circulating factor activity in sera or plasma of patients with recurrent FSGS that causes an immediate and marked increase in the albumin permeability (Pant) of isolated glomeruli [14]. The capacity of this FSGS factor to increase Pant was found to be highest in the plasma or sera from patients with recurrent FGS and showed marked diminution in activity following plasmapheresis and remission of the nephrotic syndrome [14,15]. While this bioassay has many limitations, it can be useful both in estimating the risk of recurrence of FSGS and in evaluating the efficacy of various drug therapies in vitro.

Podocyte proteins as a clue for explaining proteinuria in inherited FSGS

The slit-diaphragm is the glomerular site for permeselectivity towards proteins. It is a continuous zipper-like structure that bridges two bordering foot processes of a podocyte. Most evidence indicates that abnormalities of the podocyte are major determinants of proteinuria. Structural components of the podocyte localized in the slit-diaphragm (nephrin and NEP1), in membrane (podocin and CD2AP) and in cytoskeleton (actinin 4) have been unequivocally linked to proteinuria [2–4,16–18]. Podocin is probably the most relevant among these proteins in human pathology. It is a stomatin homologue endowed in the lipid raft where it interacts with nephrin and CD2AP and probably functions as a stabilizing structure with signalling potential [19,20]. Stabilization of the slit-diaphragm structural assembly and functional integrity is essential for normal glomerular permeselectivity. Podocin knock-out mice develop proteinuria and die of renal failure within a few days of birth [21]; in human beings, molecular alterations of the podocin gene (NPHS2) are invariably linked to steroid-resistant nephrotic syndrome (see below).

Podocin mutations were first recognized as causes of familial steroid-resistant nephrotic syndrome with autosomal recessive inheritance, but several disease-associated mutations have also been reported in
sporadic cases. In their original report on NPHS2 identification by positional cloning, Boute et al. [2] described 14 families with 10 different mutations, comprising nonsense, frameshift and missense mutations. Screenings for NPHS2 mutations in sporadic nephrotic syndrome have been completed in Italy [3,4,22], Germany [23] and Turkey (personal observation) for a total of 328 cases (264 children, 64 adults) studied. Overall, 48 homozygous or compound heterozygous mutations were found; in 14 patients only one mutation was detected. Major clinical features in sporadic cases with homozygous or compound heterozygous mutations were: variable age at presentation of proteinuria in general within the first decade of life, strict steroid resistance and progression to end-stage renal failure. FSGS was the prevalent histological background. A molecular variant characterized by homozygosity of a complex haplotype with two mutations in cis was associated with an infantile and severe variant [24]. Heterozygous patients presented a more benign phenotype, which was characterized, in some cases, by responsiveness to steroids or cyclosporin [4]. The role of heterozygous podocin mutations associated with nephrotic syndrome is not clear, as FSGS associated with NPHS2 mutations is a recessive disease requiring a molecular defect on both alleles to produce a pathological effect. One possibility is that with heterozygous NPHS2 mutations, patients have another, albeit undiscovered mutation in the podocin gene, probably involving regulatory sequences or splicing sites in non-coding regions. Moreover, we cannot exclude a digenic inheritance with mutations involving one or more still undiscovered podocyte genes, producing an additive effect. A final possibility is that mutations occurring in heterozygosity confer a particular susceptibility or exert permissive effect to the disease requiring another factor for its clinical manifestation.

Homologies between genetic and ‘non-genetic’ conditions

Podocin and other structural components of the slit-diaphragm may be involved in acquired FSGS. Reduced expression of nephrin in glomeruli has been invariably observed in both experimental and human glomerular diseases [25,26]. Podocin also has been evaluated in several proteinuric conditions including FSGS and diabetic nephropathy. In FSGS, a marked reduction in glomerular podocin level was paralleled by an increase of mRNA expression and was related to the effacement of foot processes. Therefore, loss of podocin protein probably results from damage associated with a pathogenic event or with proteinuria, while increased mRNA may correspond with a compensatory reaction of the podocyte. In some way, loss of podocin is reminiscent of what happens in patients with NPHS2 mutations, confirming that proteinuria occurs in parallel with defects in glomerular slit-diaphragm assembly.

Post-transplant recurrence of FSGS in carriers of NPHS2 mutations

As already discussed, post-transplant recurrence of FSGS occurs in a relevant proportion of FSGS patients and represents an important clinical problem. It is taken as proof for the existence of circulating permeability plasma factor(s) that are also putative effectors of the original proteinuria in these patients. In the light of a possible implication of plasma factor(s), studies in patients with inherited forms of FSGS undergoing renal transplantation are essential to confirm or deny this possibility. It seems reasonable to hypothesize that proteinuria should not recur in patients with NPHS2 mutations as circulating plasma factors should not be implicated in the pathogenesis of the original disease. We reviewed recently the problem in 13 children carrying homozygous (\(n=9\)) or a heterozygous (\(n=4\)) mutation of NPHS2 and found recurrence in five, including three carriers of heterozygous mutations [27]. In our experience [27], the rate of FSGS recurrence in patients with NPHS2 mutations was comparable with that observed in acquired FSGS (15 out of 40) with the limitation of what represent heterogeneous cases. With the exception of our study above, literature data on recurrence of proteinuria in patients with NPHS2 mutations is scant [28].

As discussed already in the Introduction, the identification of Plasma Factor, which has long been a central issue in studies on the pathogenesis of FSGS, remains elusive [10,12,14,29]. The presence of circulating PF in FSGS is clearly inferred from the rapid post-transplant recurrence and trans-placental transmission. More direct data on PF has been derived from the \textit{in vitro} bioassay with isolated glomeruli. Carraro et al. [30] measured pre-transplant \(P_{\text{lab}}\) in five patients carrying a homozygous mutation of NPHS2 and found levels comparable with those observed in idiopathic FSGS. In a recent study Doublier et al. showed that shedding of nephrin occurred within minutes of incubating glomeruli with plasma from FSGS patients (personal unpublished observation). This may represent the cell counterpart of the same mechanism that eventually leads to loss of intra-capillary albumin and determines high \(P_{\text{lab}}\) within isolated glomeruli. It is worth reporting that same shedding was observed with pre-transplant serum of patients with NPHS2 mutations with high \(P_{\text{lab}}\), confirming a high homology between hereditary FSGS of the NPHS2 type and idiopathic FSGS.

Putative mechanisms for recurrence

The elucidation of the mechanisms underlying proteinuria and recurrence in FSGS could be facilitated by future studies in an intriguing experimental model, the Buffalo/Mna rat. This animal strain develops proteinuria and FSGS lesions as an effect of two autosomal recessive genes, one identified locus (Pur 1) being
mapped on chromosome 13, in a region that is syntenic with the long arm of human chromosome 1, which contains NPHS2. Recent observations by Le Berre et al. [31] in Buffalo/Mna rats may provide the key for overcoming the apparent paradox of post-transplant recurrence of FSGS in NPHS2. When kidneys from Buffalo/Mna rats were grafted into normal LEW.W1 rats, proteinuria and renal lesions disappeared. Vice versa, when normal LEW.W1 kidneys were transplanted into Buffalo/Mna rats proteinuria recurred, which is strongly reminiscent of what happens in humans. Overall, these experiments as well as the occurrence of post-transplant recurrence in NPHS2 patients and the observations by Carraro et al. of elevated values of P_{alb} in patients with homozygous mutations of NPHS2 [30] support an interaction of genetic and circulating PF in the pathogenesis of recurrent FSGS. One possibility is that the molecular defects of podocin, in patients who develop proteinuria, are part of a multi-factorial disease in which plasma factors also play a role. It could be postulated that heterozygous mutations of podocin as well as a few homozygous mutations require the participation of an extra-renal mechanism (i.e. PF) for developing proteinuria and that this mechanism may induce recurrence of proteinuria after renal transplantation. This would thus mimic the usual sequence observed in idiopathic FSGS. The specific role of PF in both the proteinuria and the recurrence in patients with NPHS2 mutations remains to be determined.

Current and future therapies of recurrent FSGS

Following several reports of anecdotal successes with the treatment of recurrent FSGS with plasmapheresis, we initiated a series of prospective studies whereby we treated patients with recurrent FSGS with a uniform approach consisting of nine plasmapheresis treatments performed daily or every other day in which 1.5 l plasma volume is phoresed and replaced with 5% albumin [7,15]. In a recent review of 35 patients with recurrent FSGS treated with plasmapheresis, 70% of patients had a reduction in proteinuria although a third of these patients relapsed and required additional courses of plasmapheresis. Thirty percent of patients showed no response to plasmapheresis and did not respond to additional courses of plasmapheresis. In addition, inducing high blood levels of the calcineurin inhibitors cyclosporine or tacrolimus has been shown to promote remission of the nephrotic syndrome in recurrent FSGS [32].

In an attempt to prevent recurrence of FSGS, we treated five patients at high risk for recurrence of FSGS (three had a recurrence of FSGS in a previous renal allograft, two had rapid course of FSGS in their native kidneys and all had markedly elevated P_{alb} with a variety of pre-transplant interventions including pre-transplant plasmapheresis and/or cyclosporine). Unfortunately, these pre-transplant interventions failed to prevent the rapid recurrence of FSGS after transplantation and all five patients subsequently lost the graft due to recurrent disease.

On the basis of the finding that the circulating factor has protease activity, Carraro et al. (submitted for publication) showed that protease inhibitors, especially serine protease inhibitors, can block in vitro the increased P_{alb} induced by sera from patients with recurrent FSGS. This intriguing observation has led us to initiate a study with a protease inhibitor in patients who have had graft loss from a previous recurrence of FSGS and wish to get retransplanted. P_{alb} is measured before and after 8 weeks of protease inhibitor therapy. Patients who respond favourably to the protease inhibitor (decrease in P_{alb}) will then undergo re-transplantation.

A more drastic approach for patients who have had a previous recurrence of FSGS and who are deemed untransplantable because of the very high risk of recurrence of FSGS, is to consider bone marrow transplantation from their prospective kidney donor. Nishimura et al. demonstrated in a mouse model for FSGS, that bone marrow transplantation from normal to FSGS mice ameliorated FSGS while transplantation of bone marrow cells from the FSGS mice induced FSGS in normal mice [33]. These findings suggest that FSGS may be a stem cell disorder and therefore could be ameliorated or prevented by stem cell transplantation. Patients who have a HLA identical living kidney donor and are at high risk of recurrent FSGS with re-transplantation could be considered for this experimental approach. Such a strategy is intriguing. However, it should only be utilized if all other efforts or therapies fail.

The recent unexpected finding by Peter Mundel’s lab of that the co-stimulatory molecule CD80 (i.e. B7.1) is up-regulated in podocytes under nephrotic conditions and contributes to the pathogenesis of proteinuria, may lead to new therapeutic options, especially for recurrent FSGS [34]. CD80 can be effectively targeted with monoclonal antibodies, anti CD80, or the fusion receptor protein CTLA4Ig [34]. Both of these biologic agents are being considered for chronic induction therapy in renal transplantation and may block CD80 signalling that leads to proteinuria [35].

Conclusions

While recurrent FSGS is enigmatic and frustrating, it remains a worthy challenge for serious investigation. A thorough analysis of the mutations of the various genes encoding for podocin, nephrin, CD2AP and α-actinin 4 and their association with the circulating permeability factor should represent a particularly useful area of investigation. While idiopathic non-familial FSGS is probably a heterogeneous disease, the recurrent variety may have a more homogeneous aetiology and is more likely a successful target for therapeutic intervention.
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