Glomerular expression of nephrin and synaptopodin, but not podocin, is decreased in kidney sections from women with preeclampsia

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Abstract

Background. Preeclampsia is a pregnancy-specific disorder characterized by hypertension and proteinuria. In other disease states, proteinuria has been linked to altered expressions of podocyte foot-process proteins, but this has not been studied in women with preeclampsia. We sought to test the hypothesis that proteinuria in preeclampsia is associated with dysregulated expression of the podocyte cytoskeleton and/or tight junction proteins.

Methods. Renal tissue was obtained from autopsy material from seven women who had severe preeclampsia during the second half of their pregnancies up to 48 h after delivery, and who subsequently died. As controls, we used autopsy material from two women who died accidentally during the second half of their otherwise normal pregnancies. Immunohistochemical stains for nephrin, synaptopodin and podocin were performed on representative sections prepared from paraffin-embedded material.

Results. Expression of both nephrin and synaptopodin was markedly decreased in preeclamptic compared with control kidney sections. By contrast, both cases and controls demonstrated strong staining for podocin.

Conclusions. We conclude that down-regulation of nephrin and synaptopodin is associated with proteinuria in women with preeclampsia. Recent studies have provided evidence that preeclampsia is associated with elevated levels of the soluble receptor for vascular endothelial growth factor (VEGF) [2,3], commonly referred to as sFlt-1 (from soluble, fms-like tyrosine kinase receptor-1) that may bind and neutralize VEGF. Elevations in sFlt-1 levels have been shown to correlate with both the severity of the disease [4,5] and the degree of proteinuria [6]. While studies in mice treated with intravenous infusion of sFlt-1 have shown that a possible mechanism of proteinuria relates to down-regulation of nephrin, a structural component of the epithelial slit diaphragm [7], no data are available regarding the expression of nephrin in kidney tissues of preeclamptic women.

The aim of this study was to test the hypothesis that proteinuria is associated with down-regulation of nephrin in the kidneys from women with preeclampsia. Mutations in the nephrin (NPHS1) gene have been...
found in patients with congenital nephrotic syndrome of the Finnish type [8]. In addition to nephrin, we studied two other proteins that localize either to the slit diaphragm (podocin) or to the foot-process cytoskeleton (synaptopodin), as studies of the nephrotic syndrome in humans have indicated that these proteins play important roles in maintaining the structural and functional integrity of the slit diaphragm. Mutations in the gene coding for podocin (NPHS2) result in recessive familial forms of early onset proteinuria, resistant to steroid treatment [9]. Podocin may regulate the structural organization and filtration function of the slit diaphragm by interacting directly with nephrin [10]. Synaptopodin is an actin-associated protein, which is linked to the formation of foot processes, a hallmark of the differentiated podocyte phenotype [11]. Comparative studies of synaptopodin expression in human glomerulopathies have suggested that the disappearance of synaptopodin may serve as a prognostic indicator [12]; expression is normal in minimal change disease, which is usually reversible and associated with a benign course, and absent in collapsing nephropathies (idiopathic and HIV-associated) which are known for their progressive character. As kidney biopsies are rarely performed during preeclampsia, we studied the expressions of these proteins in the kidney sections obtained from the autopsy material of women who died from preeclampsia. In addition, we studied expression of synaptopodin in kidney sections from mice treated with either anti-VEGF antibody or sFlt-1. This animal model has been shown to develop proteinuria presumably through the down-regulation of nephrin [7], while the expression of synaptopodin has not yet been reported.

**Materials and methods**

**Human renal tissue experiments**

Renal tissue was obtained from the autopsy materials from seven women who developed severe preeclampsia during the second half of their pregnancies up to 48 h after delivery, and who subsequently died (Table 1). As controls, we used autopsy material from two women who died accidentally during the second half of their otherwise normal pregnancies. Kidney sections for six cases (cases 1–6) were obtained through collaboration with the University of Cape Town, South Africa; one case (case 7) and both controls were obtained through the Department of Pathology at Mayo Clinic. Light microscopy and immunohistochemical stains for nephrin, synaptopodin and podocin were performed on representative sections prepared from paraffin-embedded material.

Preeclampsia was defined as a pregnancy-specific disorder characterized by hypertension (blood pressure

<table>
<thead>
<tr>
<th>Case number (year of death)</th>
<th>Clinical features and pertinent findings</th>
<th>Protein expressiona</th>
<th>Nephrin</th>
<th>Synaptopodin</th>
<th>Podocin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 (1995)</td>
<td>32-year-old, full-term pregnancy, complained of headache at home, died during the third eclamptic seizure in hospital from intracerebral haemorrhage.</td>
<td>±</td>
<td>0–1±</td>
<td>3±</td>
<td></td>
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<tr>
<td>Case 2 (2000)</td>
<td>20-year-old, presented at 39 weeks of gestation with epigastric pain, blurred vision and preeclampsia. Underwent emergent C-section, post-operatively progressed to HELLP syndrome and died of haemorrhagic stroke</td>
<td>0</td>
<td>0</td>
<td>3±</td>
<td></td>
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<tr>
<td>Case 3 (1954)</td>
<td>30-year-old who developed hypertension, proteinuria and oedema towards the end of pregnancy. Had three seizures at home. Upon arrival to hospital, suffered from a ruptured hepatic haematoma, became haemodynamically unstable and died.</td>
<td>1–2±</td>
<td>0–1±</td>
<td>3±</td>
<td></td>
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<tr>
<td>Case 4 1964</td>
<td>40-year-old, who delivered a stillborn at full-term pregnancy. Had post-partum eclamptic seizure and was found to be hypertensive with 4+ proteinuria. Two days after delivery developed profuse uterine bleeding and liver failure became haemodynamically unstable and died.</td>
<td>1–2±</td>
<td>0–1±</td>
<td>3±</td>
<td></td>
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<td>Case 5 (2001)</td>
<td>36-year-old who developed epigastric pain 1 day after normal vaginal delivery. Developed haemolysis, elevated liver enzymes and low platelet count (HELLP syndrome) and oliguria, died with multiorgan failure.</td>
<td>1–2±</td>
<td>1±</td>
<td>3±</td>
<td></td>
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<tr>
<td>Case 6 (2001)</td>
<td>31-year-old presented at 34 weeks of gestation of her fourth pregnancy with hypertension and proteinuria and subsequently had a seizure. Died 6 h after delivery from intracerebral haemorrhage.</td>
<td>1–2±</td>
<td>1±</td>
<td>3±</td>
<td></td>
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<tr>
<td>Case 7 (1990)</td>
<td>30-year-old, developed hypertension and proteinuria at 31 weeks of gestation, progressed to HELLP syndrome, had C-section, but died on the 5th post-operative day.</td>
<td>0</td>
<td>0</td>
<td>3±</td>
<td></td>
</tr>
<tr>
<td>Control 1 (1988)</td>
<td>21-year-old, first pregnancy, 28 weeks pregnant, no obstetric complications, killed in a motor vehicle accident (MVA).</td>
<td>3±</td>
<td>3±</td>
<td>3±</td>
<td></td>
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<tr>
<td>Control 2 (1988)</td>
<td>18-year-old, first pregnancy, 36 weeks pregnant, no obstetric complications, killed in a MVA.</td>
<td>3±</td>
<td>3±</td>
<td>3±</td>
<td></td>
</tr>
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</table>

a0, absent; 1, mild; 2, moderate; 3, strong expression.
≥ 140/90 mmHg) and proteinuria ≥ 300 mg/24 h urine, which roughly correlates with a qualitative measurement of 1+(30 mg/dl) on dipstick urinalysis. The convulsive form of preeclampsia, i.e. eclampsia, was diagnosed in four patients who presented with seizures up to 48 h after delivery. HELLP syndrome, a severe form of preeclampsia clinically characterized by haemolysis, elevated liver enzymes, and low platelet count, was confirmed in three cases based on the following criteria [13]: (i) Evidence of intravascular haemolysis [decreasing haemoglobin, abnormal peripheral blood smear (schistocytes), elevated lactate dehydrogenase (LDH), elevated total bilirubin], (ii) Elevated liver enzymes [alanine transaminase, aspartate transaminase and LDH], (iii) Low platelet count (<100 x 10^9/l).

**Results**

**Human renal tissue experiments**

We studied the expression of proteins that localize either to the slit diaphragm (nephrin and podocin) or the cytoskeleton (synaptopodin) in the podocyte foot processes of the renal tissue from seven women who died during the course of preeclampsia. As controls, we examined renal autopsy material of women who died accidentally during otherwise normal pregnancies. We obtained kidney tissue samples that were embedded in paraffin and stored anywhere between 5 and 50 years. The tissue preservation was variable, but in all cases and controls renal sections strongly stained for podocin, indicating that the immunoreactivity of the tissue samples was preserved. All kidney sections were stained initially with haematoxylin and eosin and examined under light microscopy, looking for the presence of the glomerular lesion of endotheliosis, a classic pathological renal lesion of preeclampsia. Once the presence of this lesion (capillary loops occlusion by swelling and hypertrophy of endocapillary cells) was confirmed, the remaining sections were stained for nephrin, podocin and synaptopodin.

Light microscopy showed normal histology in both controls (controls 1 and 2, Table 1). In all cases (cases 1–7, Table 1), florid endotheliosis was present (Figure 1A); this was frequently associated with thrombotic microangiopathy (Figure 1B). In the control kidneys, strong, diffuse capillary wall staining for both nephrin and synaptopodin was present as expected (Figures 2A and 3A, respectively). Expressions of both nephrin (Figure 2B and C) and synaptopodin (Figure 3B and C) were decreased in cases compared with controls; the degree of down-regulation varied from a marked decrease to almost complete absence of protein expression. Podocin did not appear to be affected, as both cases and controls demonstrated strong staining for podocin (Figure 4A and B, respectively). Electron microscopy (Figure 5) showed marked glomerular endotheliosis coupled with segmental foot-process effacement.

**Animal experiments**

The expressions of nephrin, podocin and synaptopodin were examined in the kidney sections from mice infused with either sFlt-1 (Figure 6A) or anti-VEGF antibodies (Figure 6B) at concentrations corresponding to 10 times the molar concentration of normal plasma VEGF. The expression of nephrin was significantly reduced both with anti-VEGF antibodies and sFlt-1 treatments, and that of podocin was unchanged. Both anti-VEGF antibodies and sFlt-1 significantly reduced the expression of synaptopodin.
that, similar to nephrin, was restored in rescue experiments when 32.5 pM of VEGF was delivered 5 min after infusion of either anti-VEGF antibodies or sFlt-1.

**Discussion**

To the best of our knowledge, this is the first study to report that proteinuria in patients with preeclampsia is associated with under-expression of podocyte foot-process proteins, namely nephrin and synaptopodin. Down-regulation of both nephrin and synaptopodin was observed in diagnostically unequivocal cases: all patients had severe forms of preeclampsia, including eclampsia and HELLP syndrome, as indicated by their clinical presentations. In addition, light microscopy of representative kidney sections showed glomerular endotheliosis, a renal lesion, which is characteristic for preeclampsia. Down-regulation of podocyte proteins may not be due merely to pregnancy, as expressions of nephrin and synaptopodin were normal in kidney sections from controls, i.e. women who died accidentally during the second part of otherwise normal pregnancies.
Recent studies have supported the role of low free VEGF levels in preeclampsia that may contribute to the pathogenesis of this condition in a dual fashion by causing (i) endothelial dysfunction and (ii) glomerular epithelial cell dysregulation, leading to the two main clinical findings of preeclampsia, hypertension and proteinuria, respectively (Figure 7). Experiments with normal healthy mice showed that intravenous infusion of sFlt-1 causes glomerular endotheliosis and proteinuria, likely by down-regulation of nephrin [7]. However, a potential role of nephrin dysregulation in the development of proteinuria in human disease remains unknown.

Kidney biopsies are performed rarely in patients with preeclampsia. Therefore, we used renal tissues from women who died from preeclampsia to study the expressions of podocyte proteins that play critical roles in maintaining the normal filtration barrier, namely nephrin, podocin and synaptopodin. We demonstrated that expressions of both nephrin and synaptopodin were decreased markedly in preeclampsia compared with controls. In contrast, expression of podocin was not affected: strong, diffuse capillary staining was present both in cases and controls. Similar results were obtained in experiments in mice injected with either sFlt-1 or anti-VEGF antibodies [7]: expression
of nephrin was reduced significantly while that of podocin was unchanged. The expression of synaptopodin was not studied in previous mice experiments. In this study, by using the same animal model, we have demonstrated that, similar to nephrin, synaptopodin expression is significantly decreased, but that it can be rescued by neutralizing anti-VEGF antibodies or sFlt-1 with equimolar VEGF concentrations. Collectively, these results suggest that elevation in sFlt-1 levels, well documented in preeclampsia, indeed may disrupt the slit diaphragm proteins, thus causing proteinuria. A pattern of podocyte protein dysregulation in human kidney sections (i.e. down-regulation of nephrin and synaptopodin with unchanged expression of podocin) was identical to that observed in the animal model of VEGF neutralization by either anti-VEGF antibodies or sFlt-1. The reasons for the different expressions of nephrin and podocin in both the animal sFlt-1 model and the kidney sections from women with preeclampsia remain unclear. These proteins are known to form a signalling complex that is essential for podocyte function and structural integrity [10]. However, a recent study provided evidence indicating that these two proteins may undergo differential regulation [14]. Nephrin Y1193 phosphorylation, mediated by the Src-family member Yes, enhances podocin–nephrin interactions, thereby augmenting nephrin-dependent signalling; reduced nephrin Y1193 phosphorylation promotes β-Arrestin2-nephrin interactions, which lead to endocytosis of nephrin, but not podocin, and reduced nephrin signalling. Previous studies have suggested that VEGF stimulates Src activity and promotes the Src-mediated phosphorylation [15]. In preeclampsia, it would be particularly intriguing to postulate that low free VEGF levels may down-regulate Src-mediated nephrin phosphorylation. This would favour the formation of β-Arrestin2-nephrin complexes, leading to nephrin endocytosis, while the expression of podocin may remain unaffected. The effects of VEGF dysregulation on this specific pathway will be the subject of our future research.

After pregnancies complicated by preeclampsia, proteinuria typically resolves by 12 weeks postpartum. Does this imply that down-regulation of the podocyte foot-process proteins is reversible? Indirect evidence suggesting that the process may indeed be reversible comes from studies of HIV-associated nephropathy: treatment strategies that successfully suppressed HIV transcription in podocytes resulted in re-expression of podocyte differentiation markers,

![Fig. 5. Electron microscopy of kidney sections from a patient who died from severe preeclampsia/HELLP (case 7 from Table 1), showing marked endothelial cell swelling and vacuolization with compromise of capillary loop lumens and extensive epithelial foot-process effacement. Magnification 7400×.](https://academic.oup.com/ndt/article-abstract/22/4/1136/1909365/1141)

![Fig. 6. Blocking of circulating VEGF reduces the expression of nephrin and synaptopodin, but does not affect podocin. Immunofluorescence staining of kidney sections for nephrin, synaptopodin and podocin, comparing control mice (injected with IgG1) to those receiving 32.5 pM of either s-Flt-1 (Figure 6A) or anti-VEGF antibody (Figure 6B). Rescue experiments with equimolar VEGF treatment restored nephrin and synaptopodin expression in both sFlt-1 and anti-VEGF pre-treated mice.](https://academic.oup.com/ndt/article-abstract/22/4/1136/1909365/1141)
including synaptopodin [16]. Conceivably, a dramatic decrease in sFlt-1 levels within 48 h after delivery (due to removal of the placenta, which likely is a source of elevated sFlt-1 levels in preeclampsia) may allow for the gradual re-expression of the podocyte proteins, leading to resolution of proteinuria.

Our study has several limitations. For most of the cases, we did not have a quantitative measurement of proteinuria from a 24 h urine collection that would have allowed studying the correlation and establishing a possible dose-effect relationship between the down-regulation of podocyte proteins and severity of proteinuria. In addition, we had a relatively small sample size and the tissue used was collected as many as 40 years prior to analysis. The concern may be raised that down-regulation of the podocyte proteins may not be reflective of the disease process, but rather caused by non-specific changes related to tissue decomposition. Reasonably well-preserved glomerular structures on light microscopy, as well as normal expressions of synaptopodin and nephrin in kidney sections from controls that, similar to the cases, were analysed years after actual collection, argue against it. Most importantly, the immunoreactivity of the tissues studied was preserved, as evidenced by strong podocin stainings in all cases and controls. Therefore, despite its limitations, our study provides important preliminary evidence that down-regulation of nephrin and synaptopodin is associated with proteinuria in preeclampsia.

Our results raise several important questions that set the stage for future studies. Most importantly, it remains unclear whether down-regulation of nephrin and synaptopodin is a cause or a consequence of proteinuria in preeclampsia. Future experiments with both cultured podocytes and sFlt-1 animal models of preeclampsia may provide a better understanding of the sequence of events and establish cause-effect relationships among elevated sFlt-1 levels, decreased free VEGF levels, down-regulation of podocyte proteins and proteinuria.

In summary, our findings suggest that down-regulation of nephrin and synaptopodin may cause podocyte dysfunction and proteinuria in preeclampsia. Future studies are required to elucidate the intermediate steps and molecular mechanisms by which abnormal VEGF signalling may disrupt the slit diaphragm and cause proteinuria.

Acknowledgements. We thank Mrs Morea Petersen from the Division of Anatomical Pathology, University of Cape Town for her help in identifying and processing the kidney sections that were used in this study.

Conflict of interest statement. None declared.

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Received for publication: 8.3.06
Accepted in revised form: 2.11.06