Podocyte Pathobiology in Preeclampsia
research update and clinical implications

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Conflict of interest and Funding

- I am the inventor of podocyturia technology for prediction and diagnosis of preeclampsia
- The technology has been licensed to a commercial entity; Dr. Garovic and Mayo Clinic have contractual rights to receive royalties from the licensing of this technology
- Funding P-50 AG44170 NIA

Paradigm Shift

Disease of theories

↓

Preeclamptic syndrome

Disease of the endothelium

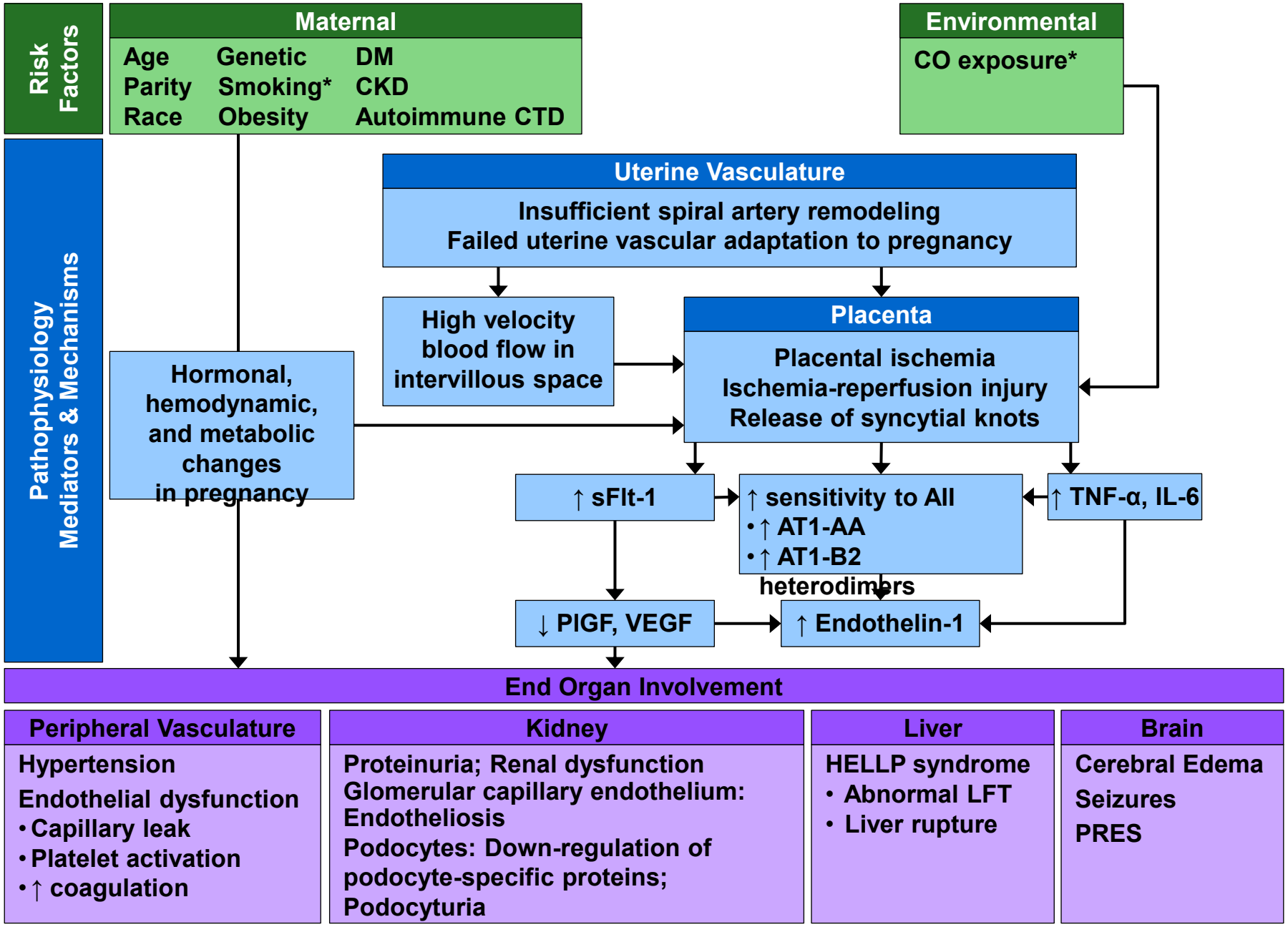
↓

Disease of the podocyte

Disease limited to pregnancy

↓

Disease with long-term effects


Risk Factors
Maternal
Age
Parity
Race
Genetic
Smoking*
Obesity
DM
CKD
Autoimmune CTD
Environmental
CO exposure*
Pathophysiology Mediators & Mechanisms
Uterine Vasculature
Insufficient spiral artery remodeling
Failed uterine vascular adaptation to pregnancy
High velocity blood flow in intervillous space
Placenta
Placental ischemia
Ischemia-reperfusion injury
Release of syncytial knots
Hormonal, hemodynamic, and metabolic changes in pregnancy
↑ sFlt-1
↑ sensitivity to AII
• ↑ AT1-AA
• ↑ AT1-B2 heterodimers
↑ TNF-α, IL-6
↓ PlGF, VEGF
↑ Endothelin-1
End Organ Involvement
Peripheral Vasculature
Hypertension
Endothelial dysfunction
• Capillary leak
• Platelet activation
• ↑ coagulation
Kidney
Proteinuria; Renal dysfunction
Glomerular capillary endothelium: Endotheliosis
Podocytes: Down-regulation of podocyte-specific proteins; Podocyturia
Liver
HELLP syndrome
• Abnormal LFT
• Liver rupture
Brain
Cerebral Edema
Seizures
PRES

Kidney injury in preeclampsia

- Podocyte pathobiology and markers in PE
- Interaction between endothelial dysfunction and podocyte injury
- Link between PE and CKD
- Future directives

Preeclampsia

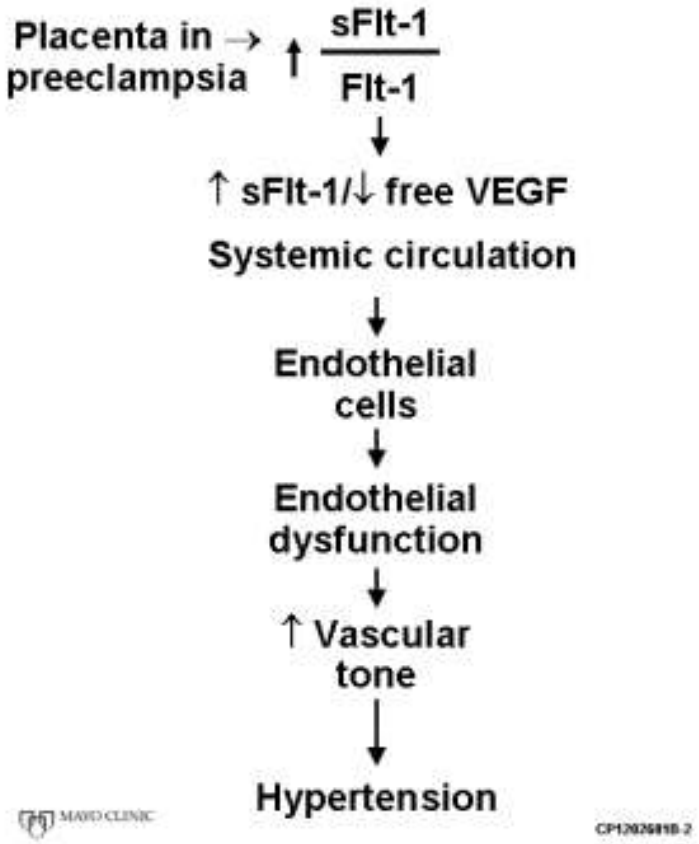
- Pregnancy-specific HTN disorder commonly associated with proteinuria
  - In the absence of proteinuria
  - Serum Cr >1.1 mg/dL or doubling
  - Thrombocytopenia < 100,000/μL
  - Elevated AST and ALT (2x normal)

ACOG, *Obstet Gynecol* 2013

- Endothelial dysfunction
  - A missing link between placental ischemia and maternal syndrome

Pathology of preeclampsia: contemporary view

sFlt-1 may be the missing link between placental ischemia and ED



Maynard et al. *JCI* 2003

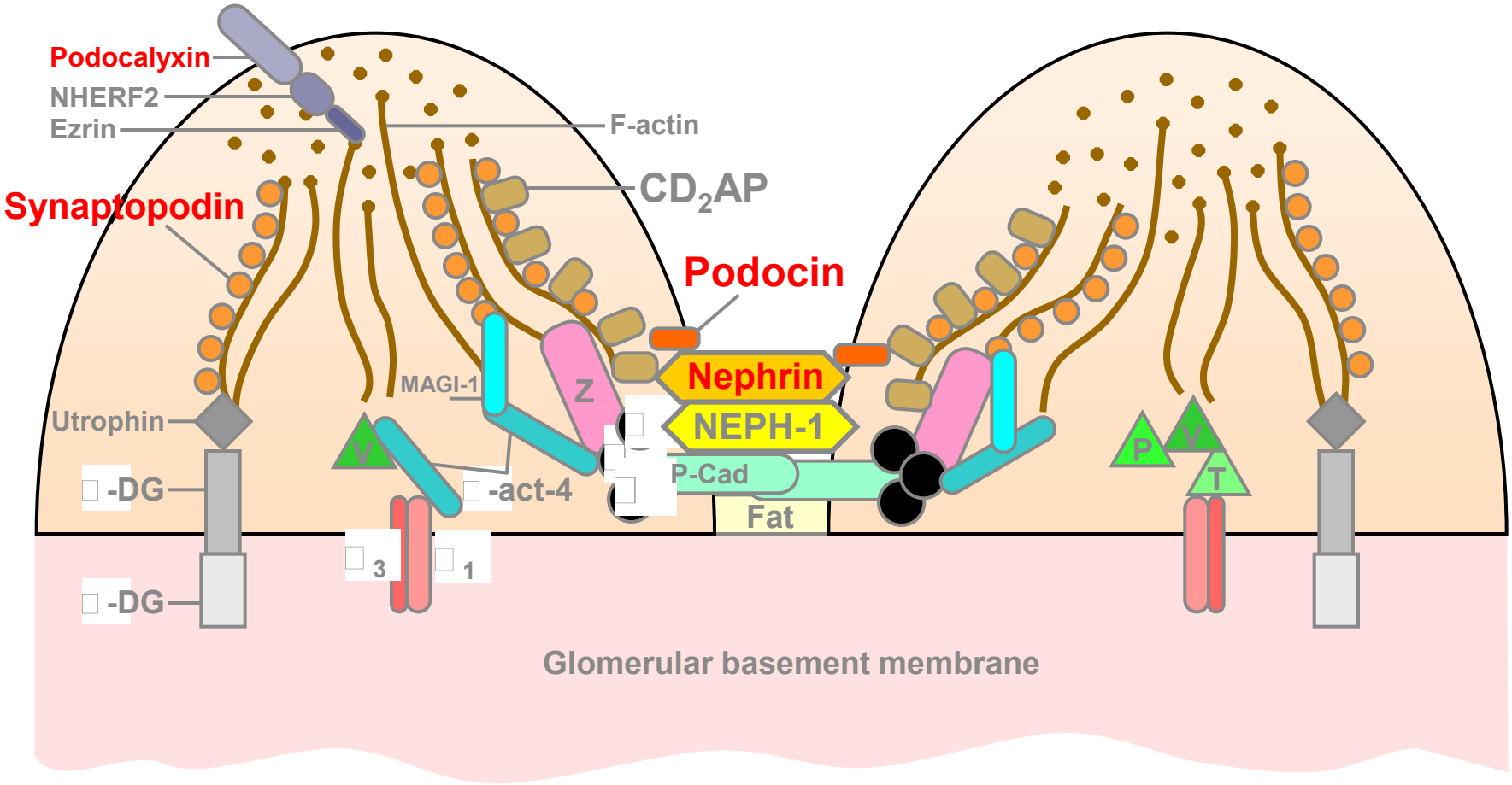
Angiogenic factors and preeclampsia

- Abnormal in early, severe preeclampsia, but not in late disease
- Angiogenic factor abnormalities
  - Abnormal placentation→ impaired angiogenesis→ early and severe preeclampsia (in < 50% of PE cases)
- Not informative for
  - term preeclampsia
  - postpartum preeclampsia

Endothelial dysfunction

- Dysregulation of angiogenic markers is related to endothelial dysfunction
- Provides a mechanism for hypertension
- ? Mechanism of early renal damage and proteinuria in preeclampsia

Podocytes: the ultimate barrier to proteinuria


Podocalyxin
NHERF2
Ezrin
F-actin
Synaptopodin
CD2AP
Podocin
Nephrin
NEPH-1
MAGI-1
Z
V
Utrophin
-DG
-act-4
P-Cad
Fat
3
1
-DG
P
V
T
Glomerular basement membrane

Inherited nephrotic syndromes and slit diaphragm proteins

- NPHS1 gene and down-regulation of nephrin →congenital nephrotic syndrome of Finnish type

Kestila et al. 1998

- NPHS2 gene and down-regulation of podocin →steroid-resistant nephrotic syndrome

Boute et al. 2000

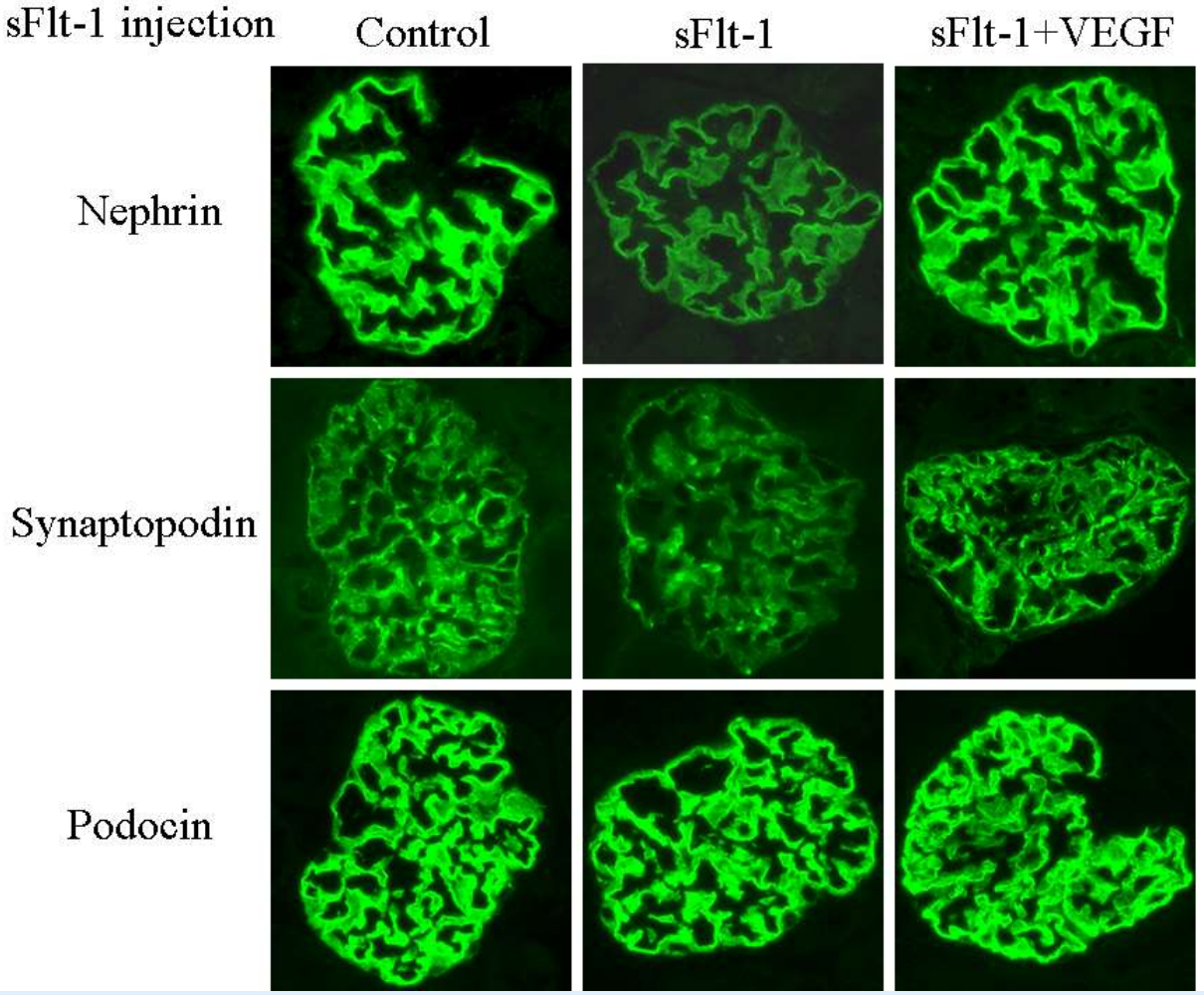
Preeclampsia: Down-regulation of slit diaphragm proteins

Glomerular expression of nephrin, synaptopodin and podocin in

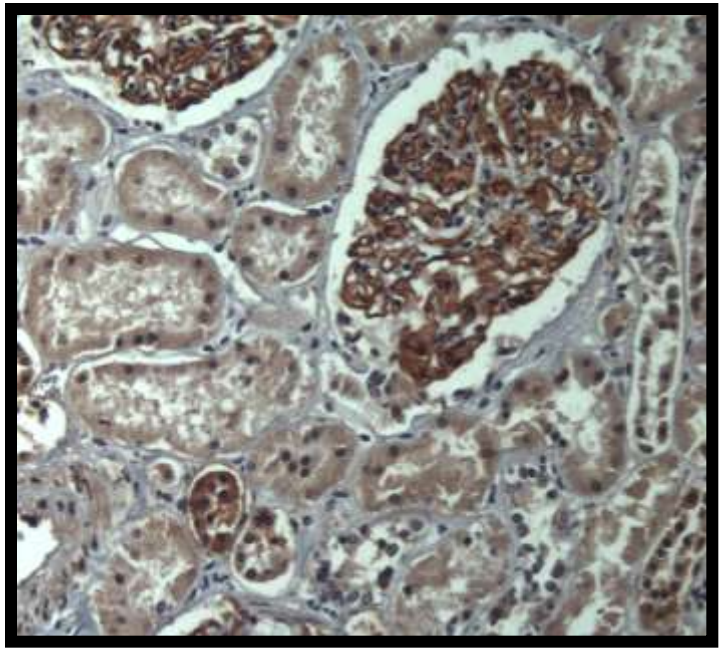
- In sFlt-1 injected mice
- In kidney sections from women with preeclampsia compared with control kidney sections (autopsy specimens)

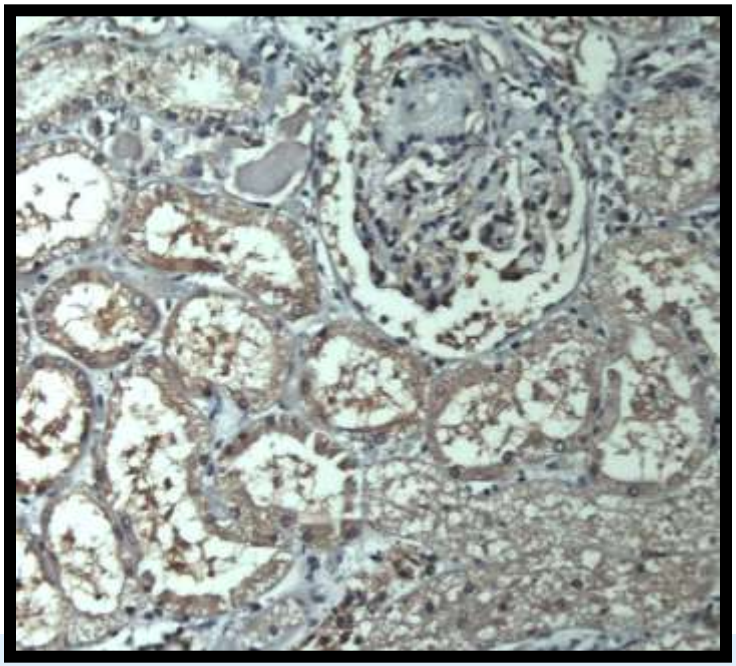
Garovic et al. 2007, NDT

Preeclampsia and podocyte protein expression

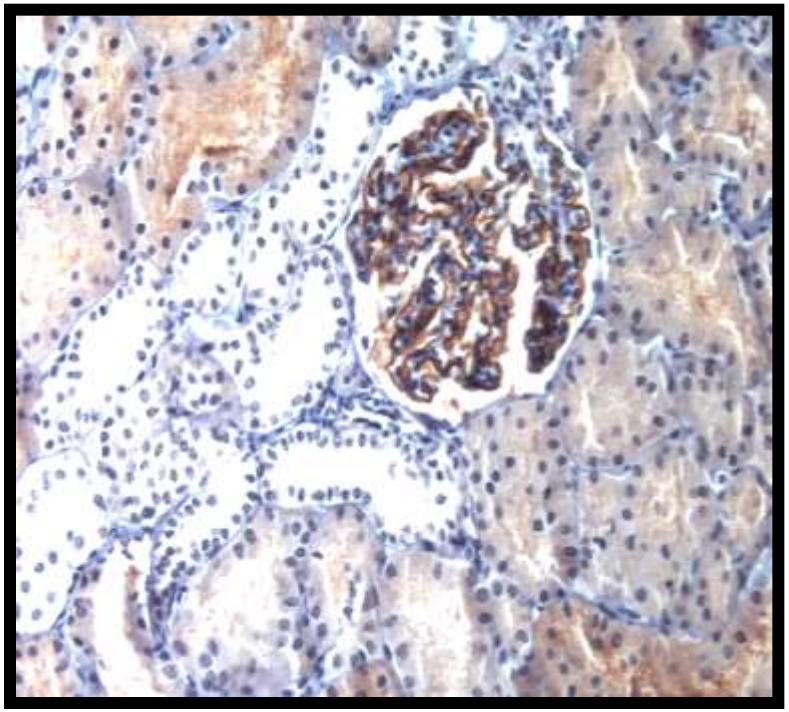


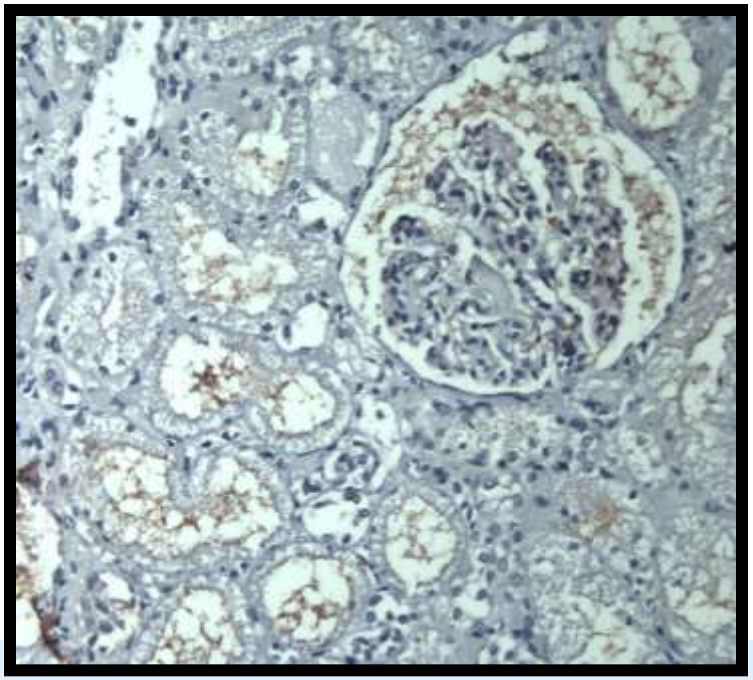
Nephrin Expression, Normal Pregnancy vs. Preeclampsia/HELLP



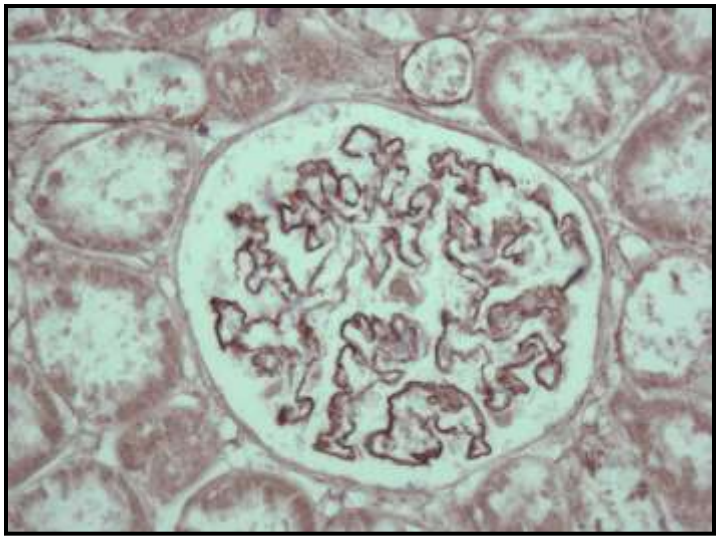


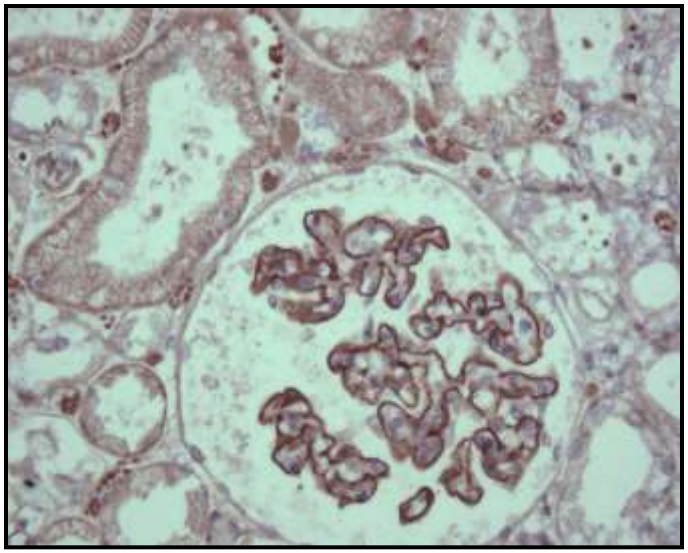
Synaptopodin Expression, Normal Pregnancy vs. Preeclampsia/HELLP





Podocin Expression, Normal Pregnancy vs. Preeclampsia/HELLP





Additional Data-LSU

	Preeclampsia (n=3) vs. non-HTN (n=5) and HTN (n = 2)
Nephrin **GLEPP-1** **Ezrin**	↓↓↓
VEGF **sFlt-1** **Nitrotyrosine**	↑↑↑
CuZn-SOD	↓↓↓

Zhao et al.
Rep Sci 2011

Preeclampsia and nephrin expression

- VEGF stimulates Src activity and promotes the Src-mediated nephrin Y1193 phosphorylation
- Reduced nephrin Y1193 phosphorylation promotes β-Arrestin 2-nephrin interactions, which lead to endocytosis of nephrin
- In preeclampsia, low free VEGF levels may down-regulate Src-mediated nephrin phosphorylation

Interaction between GEC and podocytes

- Endothelial cell swelling is the major alteration seen on biopsy in preeclampsia and in animals treated with sFlt-1
- Inhibition of VEGF by sFlt-1 may remove trophic signals that maintain a healthy endothelium, resulting in
  - endothelial cell swelling
  - loss of fenestrae
  - and release of substances that have direct or indirect effects on podocytes and the slit diaphragm

Interaction between GEC and podocytes

Cultured GEC treated with PE serum or anti-VEGF

- Increased preproendothelin-1 mRNA expression
- Increased endothelin-1

Podocytes exposed to endothelin-1, or to supernatant from endothelial cells exposed to preeclamptic serum

- Unchanged podocin expression
- Reduced synaptopodin expression
- Reduced nephrin expression

These changes were prevented by podocyte pre-treatment with endothelin-1 receptor antagonists

- Direct effects of endothelin-1 and its receptor activation

Collino et al. Am J Physiol Renal Physiol 2008

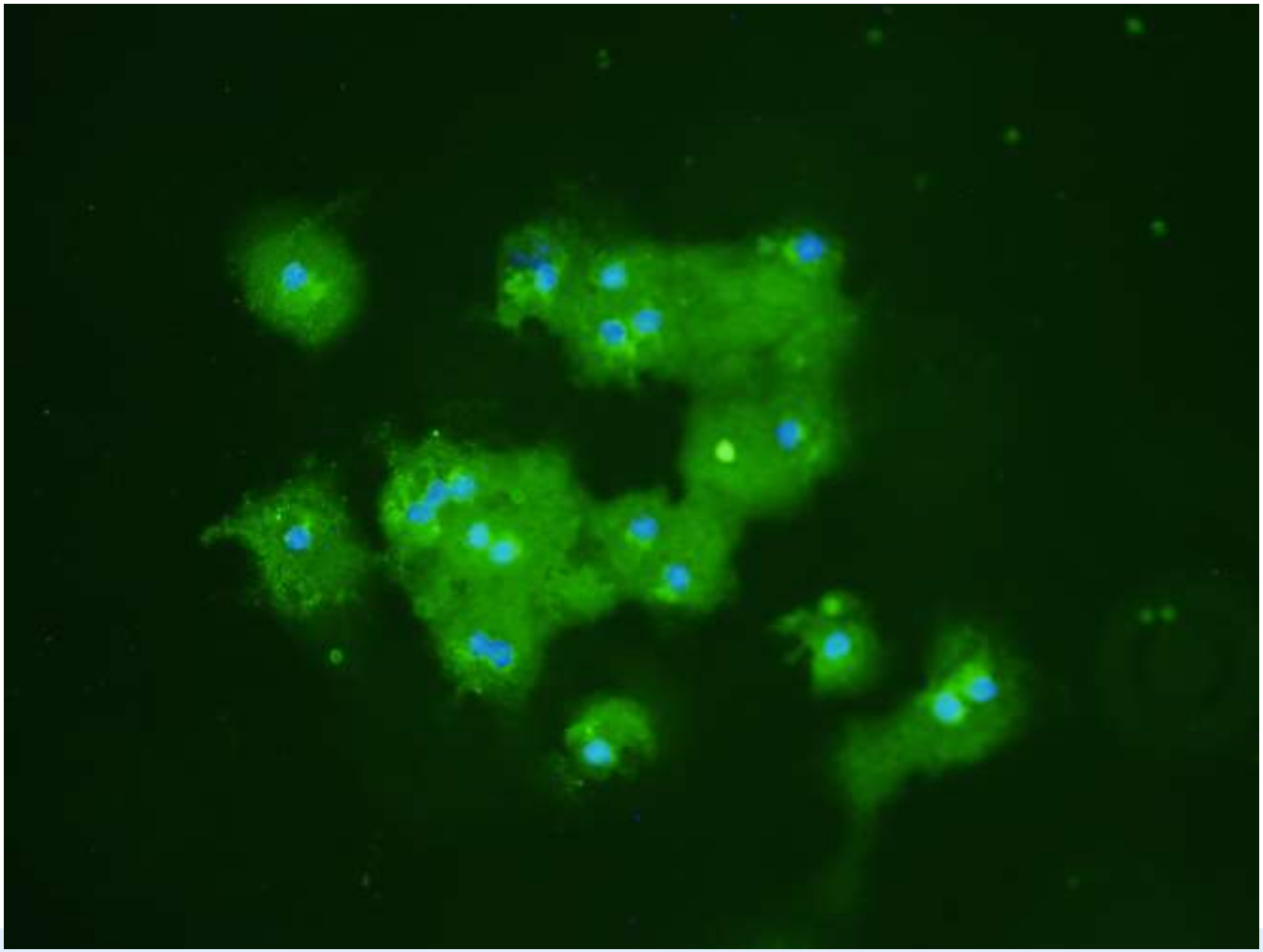
Podocyte loss-Podocytopenia

- Apoptosis
- Podocyturia: loss of podocytes in the urine
  - Corresponds to the active phase of disease
  - Potential diagnostic tool for detection of podocytopenia

Podocyturia assay

- Urine samples were plated on four-chamber slides and incubated at 37C
- Antibodies to one of four podocyte-specific proteins
  - Podocin
  - Podocalyxin
  - Synaptopodin
  - Nephrin
- FITC-labeled secondary antibody
- Podocytes: nucleated, positively stained cells

Podocyturia assay



Podocyturia

- Podocin staining
  - Present in 15 of 15 cases
  - Absent in 16 of 16 normotensive controls
  - Absent in 7 women with alternative causes of hypertension, proteinuria, or renal disease
- Podocalyxin, nephrin, and synaptopodin staining
  - Slightly less sensitive and specific than podocin

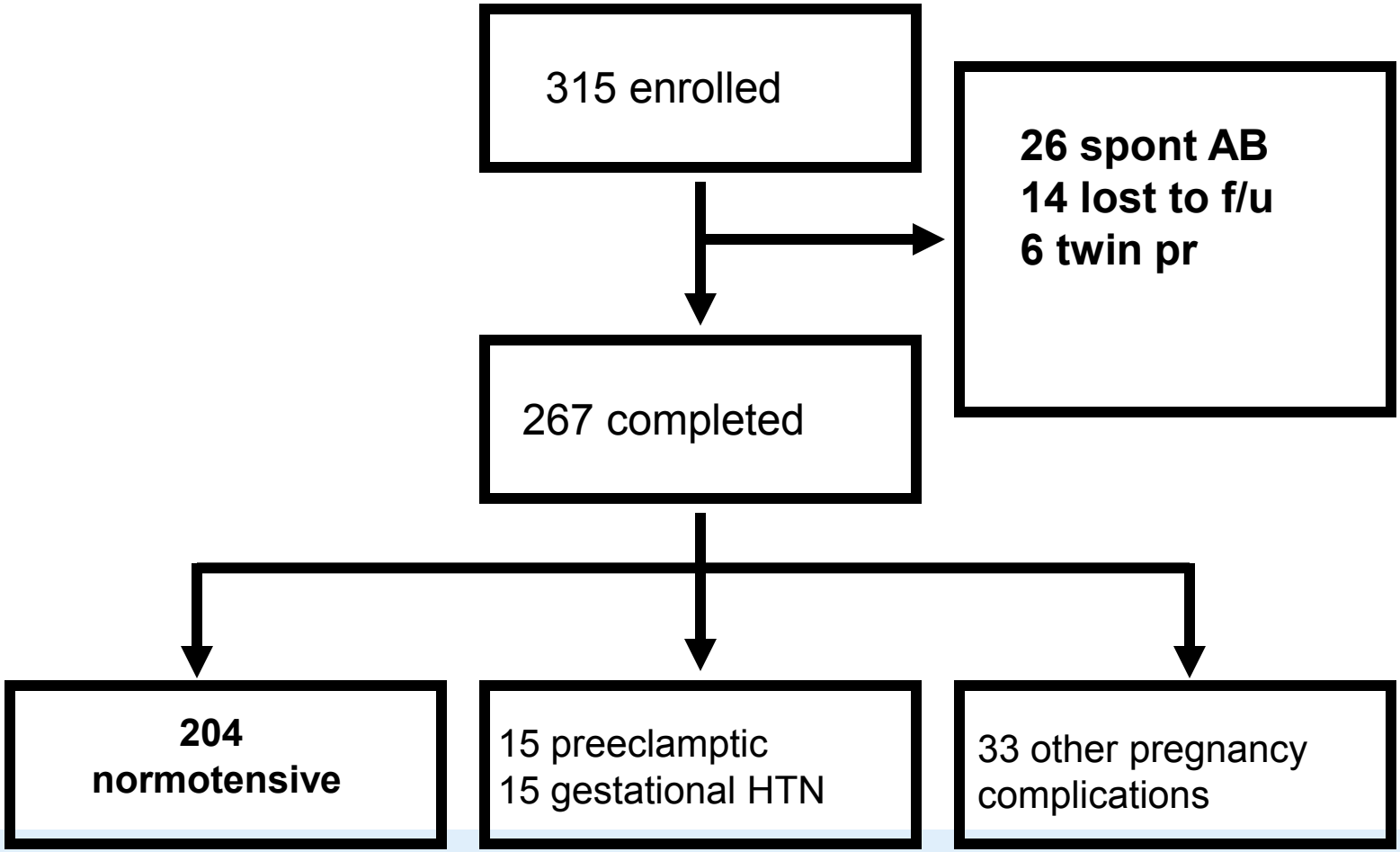
Garovic et al. AJOG, 2007

Podocyturia Predates Proteinuria and Clinical features of Preeclampsia Longitudinal Prospective Study

- Is podocyturia present before clinical evidence of preeclampsia?
- Does the presence of podocyturia correlate with proteinuria?
- Urine collection and podocyturia assay at
  - Presentation (8-12 weeks)
  - Mid gestation (22-26 weeks)
  - Delivery

Craici et al. *Hypertension*, 2013

Study population



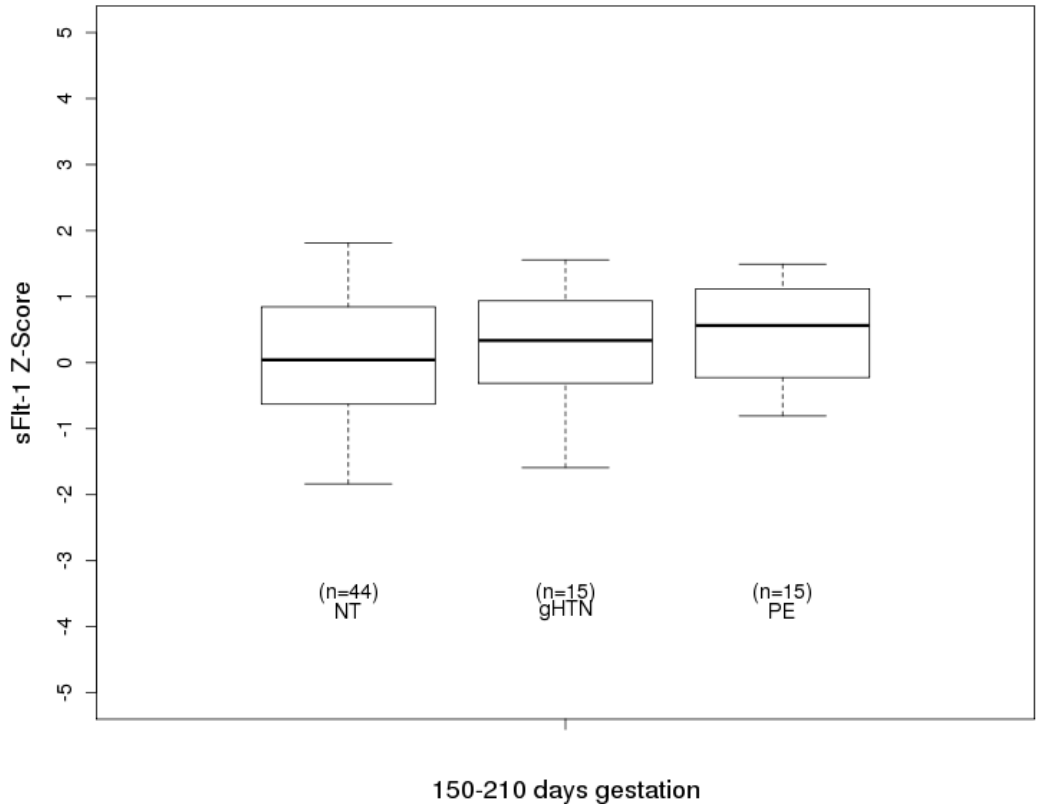
Podocyturia at delivery median 39.5 GW

	Podocyturia	SBP median	P/Cr Ratio
PE/HELLP n=15	15/15 0.77 cells/mg Cr	144	0.78
Gestational HTN n=15	1/15 0.04 cells/mg Cr*	141	0.07
Normotensive n=44	0/44 0 cells	120	0.09

Podocyturia at mid-gestation median 27 GW

	Podocyturia	SBP median	P/Cr Ratio
PE or HELLP n=15	15/15 0.28 cells/mg Cr	116	0.05
Gestational HTN n=15	0/15 0 cells	116	0.05
Normotensive n=44	0/44 0 cells	110	0.04

Angiogenic markers in normotensive pregnancies (NT), gestational hypertension (gHTN) or preeclampsia (PE)



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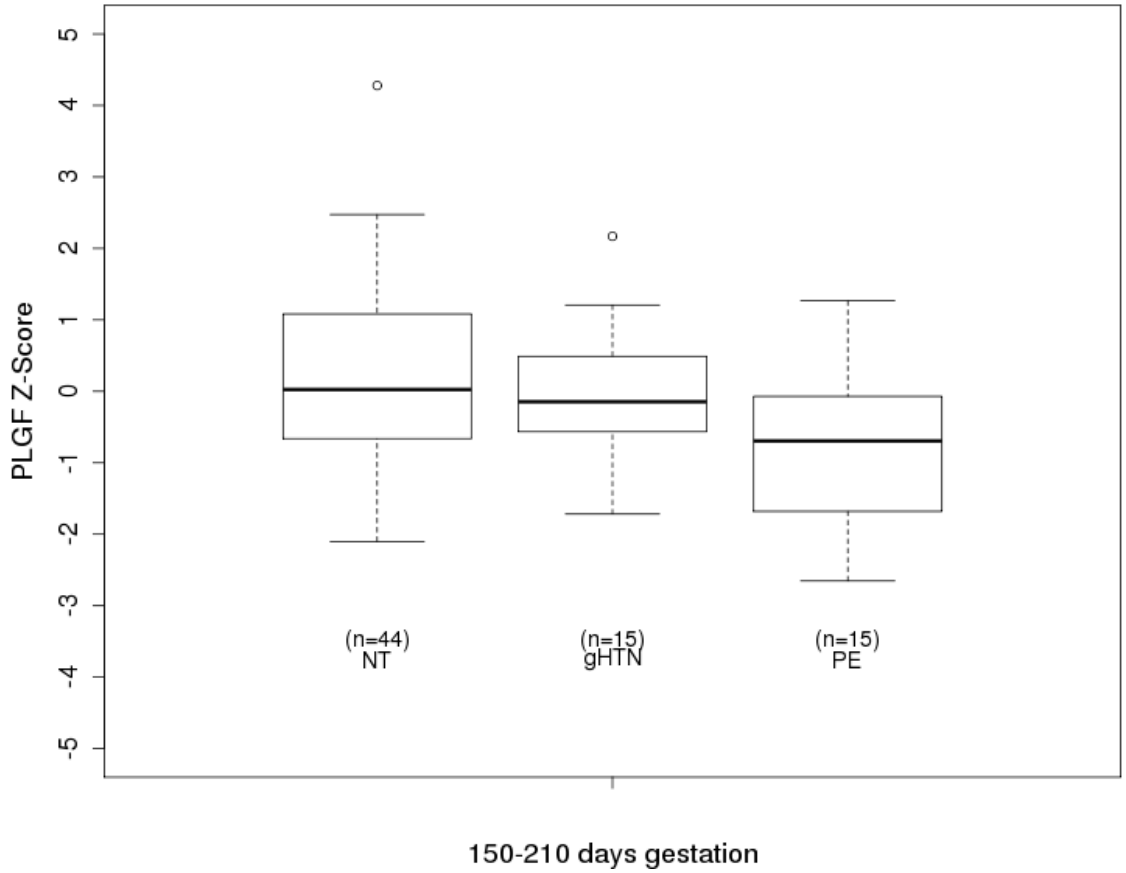
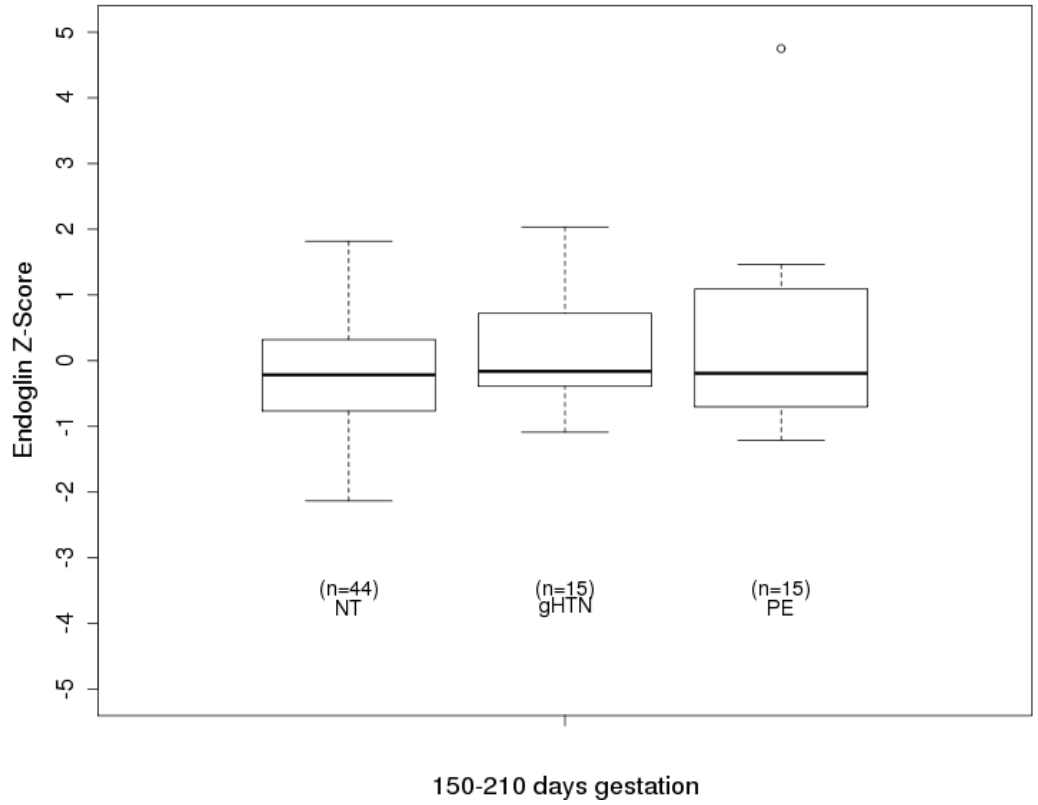


Angiogenic markers in normotensive pregnancies (NT), gestational hypertension (gHTN) or preeclampsia (PE)



Conclusions

- Podocyturia is present at delivery in preeclampsia and absent in normal controls and high-risk pregnancies
- In preeclampsia, podocyturia predates:
  - Proteinuria
  - Hypertension
- Podocyturia is a sensitive and specific test for the **diagnosis** and **prediction** of preeclampsia
- Positive correlation between the number of podocytes and the degree of proteinuria
  - Ongoing podocyte loss may be mechanistically related to the onset and severity of proteinuria.

Limitations

Questionable clinical utility of the test

- Technical complexity
- Length of time to obtain results
- Level of expertise and training required for interpretation
- Lack of standardized procedures

Alternative techniques that identify urinary podocytes and their components

- RT-PCR for nephrin and podocin
  - Elevated in PE compared to normotensive pregnant and non-pregnant women
    - *Kelder et al. 2012*
- ELISA for nephrin and podocalyxin (urinary supernatant)
  - Elevated in PE compared to normotensive pregnancy
    - *Wang et al. 2012; Son et al. 2013*
- Cytospin technique and staining for podocyte specific proteins

Author and year	Study groups	Time point(s)	Sample preparation	Podocyte detection method	Results
Garovic et al. (2007)	15 PE 16 NL	<24 h before delivery	Podocyte culture	IF for podocin	Present in 15/15 PE absent in 16/16 NL
Aita *et al.* (2009)	11 PE 45 NL	35 weeks 4 days post 1 month post	Cytospin	IF for podocalyxin	Podocyturia at 35 weeks and 4 days post in PE
Zhao *et al.* (2011)	16 severe PE 3 mild PE 7 NL	3rd trimester	Podocyte culture	IF for nephrin	Podocyturia present in all cases of severe PE
Kelder *et al.* (2012)	35 PE 5 GHTN 34 NL	31 to 36 weeks gestation	TRIzol RNA isolation	RT-PCR for nephrin, podocin	↑ mRNA for nephrin, podocin in PE vs NP
Wang *et al.* (2012)	20 PE 6 HTN 8 NL	3rd trimester	ELISA	ELISA for nephrin, podocalyxin	Urinary nephrin, podocalyxin ↑ in PE
Chen *et al.* (2013)	14 PE 14 GHTN 13 NL	<1 week before delivery	Cytospin	IF for podocalyxin	Number of podocytes higher in PE compared GHTN and NL
Son *et al.* (2013)	43 Severe PE 30 NL	<24 h before delivery	ELISA	ELISA for nephrin	higher in severe PE than in NL
Craici *et al.* (2013)	15 PE 15 GHTN 44 NL	2nd trimester	Podocyte culture	IF for podocin	Podocyturia sensitive and specific for later PE
Jim *et al.* (2012)	29 PE 9 GHTN and HTN 9 NL	<24 h before delivery	Cytospin	IF for synaptopodin	Sensitivity=38%, Specificity=70%

Limitations

- Lack of standardized procedures and use of alternative techniques that
- Identify different podocyte populations
- Identify different podocyte products
- Use different antibodies
- Lack of protocols that standardize urine processing and minimize pre-analytical variation
- Patient selection

Current Research

- Urinary loss of viable podocytes
  - Fast and observer-independent techniques
- A technique for the identification of urinary podocytes, based on the detection of podocyte-specific tryptic peptides by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS)
- Urinary microvesicles of podocyte origin

Renal injury after PE pregnancies

- History of PE
  - Increased risk for albuminuria
    - *Bar et al. NDT, 1996*
    - *Kattah et al. JCH, 2013*
  - Future kidney biopsy
    - *Vikse et al. JASN, 2006*
  - End-stage renal disease
    - *Vikse et al. NEJM, 2008*
  -

Renal injury after PE pregnancies

- Persistent podocyturia following PE s may reflect subclinical renal injury
  - 30% of patients with PE demonstrated persistent podocyturia 5-8 weeks postpartum
    - *White et al. PLoS One, 2014*
- Single episode of podocyte injury may result in glomerular destabilization and ongoing podocyte loss
    - *Wiggins et al. 2005*
- Dominant renal biopsy finding in women with PE and persistent proteinuria is FSGS
    - Heaton et al. J Pathol 1985

PE and Pregnancy HTN and Women's Health

- **2011 AHA Guidelines for the Prevention of CVD in women**
  - *Mosca et al. Circulation, 2011*
- **2011 AHA Guidelines for the Prevention of Stroke in women**
  - *Bushnell et al. Stroke, 2014*
- Preeclampsia may be an early indicator of CVD risk
- Future studies to identify women at risk and to determine the effectiveness of diagnostic and preventive interventions
- ? Guidelines for CKD/ESRD

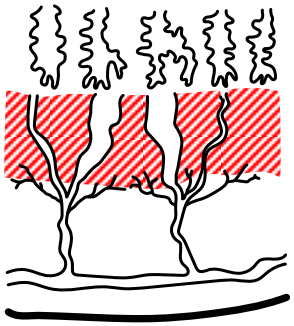
Future directives

- Development of standardized procedures
  - Urine processing
  - Development of highly reproducible techniques that are operator-independent
  - Identify different podocyte populations
- Longitudinal studies
- Patient selection

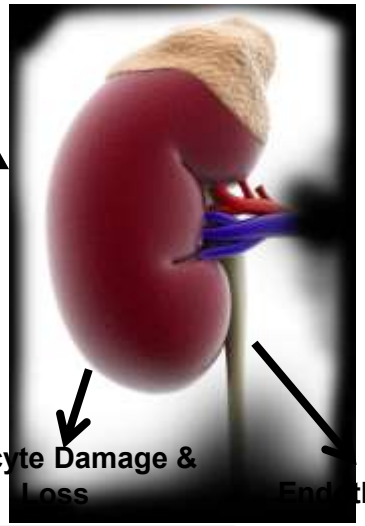
Questions?

Maternal preeclampsia
Endothelial dysfunction that predates pregnancy (hypertension, diabetes, and obesity)

Placental preeclampsia

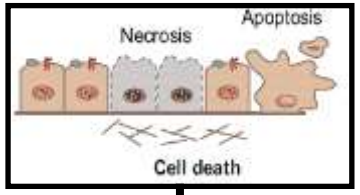


Impaired spiral arteries remodeling→damaged placenta→release of factors to maternal circulation that cause endothelial dysfunction



Podocyte Damage & Loss

Endotheliosis



Altered Myocardiac Wall

Damaged Cardiomyocytes

Cardiomyocyte reduction
Impaired contractility
Myocardial stiffness
Myocardial remodelling
Diastolic dysfunction

Sodium retention
Water retention
Hypertension

Glomerular & tubular damage
Renal mass reduction
↓ Renal plasma flow
↓ Glomerular filtration rate
Proteinuria

Heart Disease

Chronic Kidney Disease

Podocyturia and future risk for kidney disease

Urinary loss of viable podocytes

- 1.2 million glomeruli
- 300 podocytes/glomerulus (360M)
- Podocyte loss of 100/1mg Cr
- ½ podocytes lost in 5 years