Effects of childbirth on podocyturia in women with normotensive uncomplicated pregnancies

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Abstract

Changes in hemodynamics and blood pressure occur shortly before and after childbirth regardless of the mode of delivery. This study aimed to test the hypothesis that parturition induces a temporal increase in podocyturia monitored by podocyte-specific protein podocin mRNA expression levels (Pod-mRNA). A total of 105 urine specimens, consisting of 43 and 62 from 18 and 20 otherwise healthy women with vaginal (VD) and elective cesarean deliveries (ECS), respectively, were studied. Determination of urine protein and creatinine concentrations and quantitative analyses of Pod-mRNA, nephrin mRNA (Nep-mRNA), synaptopodin mRNA (Syn-mRNA), and aquaporin 2 mRNA expression (AQP2-mRNA) were performed using RT-PCR in pelleted urine samples. Levels of mRNA expression were corrected by urine creatinine concentration. Podocyturia increased significantly concomitant with significantly decreased Nep:Pod-mRNA ratio (NPR) in the urine collected immediately before or after childbirth regardless of the delivery mode compared to urine collected before commencement of labor or on postpartum day 3 or later. Podocyturia was significantly negatively correlated with NPR (correlation coefficient [r] = –0.614/–0.750 for VD/ECS women, respectively) as well as Syn:Pod-mRNA ratio. Systolic blood pressure exceeded 140 mmHg during labor in 50% of VD women and mean arterial pressure was significantly positively correlated with podocyturia during labor in VD women (r = 0.733). Thus, parturition induces a transient increase in urine podocytes with reduced Nep- and Syn-mRNA expressions. Glomerular podocytes with reduced Nep-mRNA and Syn-mRNA levels were suggested to be likely to detach from the glomerular basement membrane around childbirth.

INTRODUCTION

There are changes in hemodynamics during parturition regardless of the mode of delivery. Autotransfusion of 300 – 500 mL of blood occurs from the uterus into the systemic circulation immediately after each labor pain with uterine contraction in pregnant women undergoing vaginal delivery [25]. Blood pressure increases during labor, even in women with normotensive pregnancies [3, 17]. Fluid loading with or without vasopressors is usually given to protect against anesthesia-induced hypotension in women with cesarean deliveries [2]. Oxytocin used for uterine contraction immediately after cesarean delivery increases stroke volume and decreases blood pressure transiently [24]. These acute changes in hemodynamics occurring during parturition may cause increased glomerular hyperfiltration in pregnancy [11].

The podocytes are glomerular epithelial cells, located at the outermost layer of the glomerular basement membrane (GBM), the foot processes of which form tight interdigitating networks that regulate the filtration of circulating plasma proteins from the capillary lumen into Bowman's space [16, 29]. The podocytes are especially sensitive to pressure due to their location on the external surface of the glomerular capillaries [5, 23], detach from the GBM under stimuli including hyperfiltration and hypertension [21], and are excreted in the urine, resulting in podocyturia [31]. Indeed, increased podocyturia occurs in pregnancies complicated with hypertensive disorders of pregnancy (HDP) [4, 9, 13, 32] and proteinuria increases with increasing podocyturia in HDP women [8]. As parturition may be a stressful event for glomerular podocytes, podocyturia is expected to be increased during parturition, even in women with uncomplicated normotensive pregnancies. However, to our knowledge, there have been no studies addressing this issue to date.

Podocyturia can be monitored by urine levels of podocyte-specific protein mRNAs, including podocin (Pod-mRNA) and nephrin (Nep-mRNA) [13,32]. Urine synaptopodin protein mRNA (Syn-mRNA) was also suggested to be derived mainly from urine podocytes [32]. Aquaporin 2 (AQP2), expressed in the principal cells of the kidney connecting tubule and collecting duct, but not in podocytes, plays a critical role in regulation of body water balance [15] and AQP2-mRNA is also detectable in the urine [26].

The present study was performed to test the hypothesis that parturition causes a transient increase in podocyturia as monitored by podocyte-specific protein mRNAs among asymptomatic women until commencement of parturition.

METHODS

This study was conducted in accordance with the principles of the Declaration of

Helsinki and with the approval of the Institutional Review Board of Hokkaido University Hospital (013-3999, April 30, 2014). All women gave written informed consent prior to participation in this study.

Participants

A total of 38 healthy normotensive pregnant women in the third trimester that provided at least two urine samples during the perinatal period participated in this study (Table 1). Eighteen women underwent successful vaginal delivery (VD) and the remaining 20 underwent elective cesarean section (ECS) in the absence of labor pains mainly as repeat cesarean section. All 38 participants fulfilled the following two conditions: 1) normal blood pressure (defined as systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg) and no proteinuria (defined as spot urine protein:creatinine ratio [mg/mg] < 0.27) until commencement of labor or elective cesarean section; and 2) gave birth at Hokkaido University Hospital during the study period from May 2014 to June 2016. Thus, no women with HDP were included in this study. Blood pressure was measured approximately every 1 hour during labor for VD women and every 5 minutes during ECS. The blood pressure was measured repeatedly when hypertension developed during parturition.

Urine sampling

The 18 VD women provided a total 43 urine specimens collected during various perinatal stages, including antepartum on 1 – 14 days before VD in the absence of labor pains (AP, $n = 17$, all voided urine), during labor within 4 hours antepartum (DL, $n = 10$, all catheterized urine), postpartum day 3 – 7 (PD2, $n = 10$, all voided urine), and postpartum day 25 – 35 (PD3, $n = 6$, all voided urine) (Table 2). The 20 women with ECS provided a total 62 urine samples collected at AP ($n = 20$, all voided urine), immediately postpartum within 12 hours after ECS (IPP, $n = 19$, all catheterized urine), postpartum day 1 (PD1, $n = 5$, all voided urine), PD2 ($n = 12$, all voided urine), and PD3 ($n = 6$, all voided urine). Thus, all urine specimens collected at DL or IPP were sampled using sterilized catheters to avoid lochia contamination.

All 105 urine specimens were coded and processed within 2 hours of collection. Urine samples were centrifuged at 700 × g for 5 minutes. Urinary supernatant was stored at –20°C until measurement of protein and creatinine (Cr) levels. The pelleted urine samples were suspended in RNA*later* (Life Technologies, Carlsbad, CA) and stored at –20°C until isolation of RNA. Protein and Cr concentrations were measured using a Protein Assay Rapid Kit Wako and Laboassay Creatinine (Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively. Urine protein concentration was corrected by urine Cr and expressed as P/Cr (mg/mg).

Quantitative Real-time PCR assay

RNA isolation from the pelleted urine and reverse transcription reaction were performed as described previously [32]. The absolute Pod-, Nep-, Syn-, and AQP2-mRNA levels were quantified using an ABI Prism 7300 Sequence Detection System (Applied

Biosystems, Foster City, CA) with Power SYBR Green PCR master mix (Thermo Fisher Scientific Co. Ltd., Yokohama, Japan) and sample cDNA in a final volume of 15 µL per reaction. The following primers were used: podocin: forward 5'-AAGAGTAATTATATTCCGACTGGGACAT-3', reverse 5'-TGGTCACGATCTCATGAAAAGG-3'; nephrin: forward 5'-CAACTGGGAGAGACTGGGAGAA-3', reverse 5'-AATCTGACAACAAGACGGAGCA-3'; synaptopodin: forward 5'-AAGTCACATCCAGCTCCTTC-3', reverse 5'-CTTCTCCGTGAGGCTAGTG-3'; aquaporin-2: forward 5'-TGGGCCATATGTGCTATGGAGA-3', reverse 5'-AAGGACACTCAGGTGCCAGGA-3'. The thermal cycling conditions were 95°C for 10 minutes, followed by 40 cycles of 15 s at 95°C and 1 minute at 60°C. All data were constructed from 0.5-µL samples analyzed in triplicate. The PCR product of each gene was used as a standard, and the standard curve was established with 10-fold serial dilution of the product. The transcript numbers were determined from linear regression of these standard curves. The detection limit for Pod-, Nep-, Syn-, and AQP2-mRNA expression was 100 copies/reaction. Therefore, we assumed that samples with undetectable levels of Pod-, Nep-, or Syn-mRNA, but a detectable level of AQP2-mRNA expression, contained 100 copies/reaction of the target. The urine samples with undetectable Pod-mRNA were not used for analyses of nephrin:podocin mRNA expression ratio (NPR) and synaptopodin:podocin mRNA expression ratio (SPR).

Culture of urine sediment and immunofluorescence staining

Aliquots of the urine samples were centrifuged for 5 min at 700 × g at room temperature, and the pellets were rinsed twice with human diploid fibroblast (HDF) solution. The pellets were resuspended in 400 uL Dulbecco's modified eagle's medium containing 10% fetal bovine serum supplemented with antibiotics (penicillin, streptomycin, fungizon), and cultured in a collagen-coated 8-well culture slide. Following overnight incubation at 37°C in 5% CO_2, slides were gently rinsed and washed with phosphate-buffered saline (PBS), and thoroughly dried and frozen at -80°C until immunofluorescence staining. Slides were fixed in 4% paraformaldehyde at room temperature for 10 min and permeabilized for 10 min with 0.25% Triton X-100. After fixation and permeabilization, slides were processed following a standard protocol for indirect immunofluorescence labelling. Incubation with primary antibodies was carried out in a humidity chamber overnight at 4°C. The following day slides were incubated with secondary antibodies in a humidity chamber for 30 min at room temperature. Primary antibodies included rabbit anti-human podocin (Sigma-Aldrich, St.Louis, MO , #P0372) and guinea pig anti-human nephrin intracellular domain (Progen Biotechnik, Heidelberg, Germany, #GP-N2). Secondary antibodies included FITC-labelled goat anti-rabbit IgG (Sigma-Aldrich, #F0382) and Alexa Fluor 555 conjugated goat anti-guinea pig IgG (Abcam, Cambrige, MA, #ab150186). Slides were mounted with Vectashield mounting media with DAPI (Vector Laboratories, Burlingame, CA) to facilitate the differentiation of nucleated whole cells from cell fragments. Nucleated cells having podocin and or nephrin staining were considered to be podocytes. Podocyte density was corrected by urine creatinine concentration.

Data expression

All assays were performed against standards to allow data to be expressed in terms of copies/ng of extracted RNA. As the urine sample volume varied from 3 to 130 mL, we first expressed data per milliliter of urine to compensate for urine concentration, and expressed data as per milligram of urine Cr. All data are therefore expressed as copies per mg Cr.

Statistical analyses

Data are presented as the median (range). Statistical analyses were performed using the JMP10© statistical software package (SAS, Cary, NC). The Kruskal–Wallis test with Bonferroni correction was used for comparisons between three or more groups. Differences in frequencies were examined using Fisher's exact test. The Spearman's rank order correlation was used to test associations between two variables. In all analyses, $P < 0.05$ was taken to indicate statistical significance. However, a significant finding regarding a linear correlation between two variables was defined as that meeting both $P < 0.05$ and correlation coefficient (r) > 0.2.

RESULTS

The highest systolic and diastolic pressures recorded during parturition were significantly higher in VD than ECS women (Table 1). A considerable number of women with vaginal deliveries developed hypertension transiently during labor (Table 1). Vasopressors were used in 70% (14/20) of ECS women to increase blood pressure during anesthesia. Oxytocin was used in 50% (9/18) of VD women to augment labor pains during labor and 100% (20/20) of ECS women immediately after childbirth to facilitate uterine involution.

AQP2-mRNA expression was detectable in all 105 urine specimens, but Pod-mRNA, Nep-mRNA, and Syn-mRNA expression were undetectable in 6 (5.7%), 8 (7.6%), and 20 (19%) of the 105 urine specimens, respectively. Pod-mRNA expression was undetectable in 2 of 37 specimens at AP (5.4%), 0 of 10 specimens at DL (0.0%), 0 of 19 specimens at IPP (0.0%), 1 of 5 specimens at PD1 (20%), 2 of 22 specimens at PD2 (9.1%), and 1 of 12 specimens at PD3 (8.3%).
In the 18 VD women, urine Pod- and Syn-mRNA expression levels increased significantly at DL and decreased to levels seen at AP until PD2. However, neither Nep- nor AQP2-mRNA exhibited a significant perinatal change (Fig. 1). In the 20 ECS women, urine Pod-mRNA expression increased significantly immediately postpartum (at IPP) and decreased to levels seen at AP until PD2, while neither Nep, Syn-, nor AQP2-mRNA exhibited significant perinatal changes (Fig. 2).

These observations suggested that podocyturia increased transiently during and after parturition regardless of the mode of delivery, but these podocytes excessively excreted in the urine showed lower levels of Nep-mRNA expression compared to those on or

after AP and PD2. To confirm this, urine NPR and SPR were analyzed. Indeed, the NPR showed a transient significant decrease at DL/IPP in women with VD/ECS, respectively and returned to the level seen at AP until PD2 (Fig. 3). The SPR exhibited a similar change to that of NPR in ECS women, but not in VD women confirming that urine podocytes collected immediately after ECS expressed less Syn-mRNA as well as Nep-mRNA.

As expected based on Figs. 1 – 3, the urine Pod-mRNA level was significantly negatively correlated with urine NPR regardless of the mode of delivery (Fig. 4A, 4C), again confirming that podocyturia increased with decreasing Nep-mRNA in the urine podocytes. The urine Pod-mRNA level was also significantly negatively correlated with urine SPR regardless of the delivery mode (see legend for Fig. 4). Approximately half of VD women showed transient hypertension during labor. The possible association between podocyturia and blood pressure was analyzed (Fig. 4B, 4D). In the urine collected at AP and DL in VD women, the Pod-mRNA expression level was significantly positively correlated with mean arterial pressure (MAP), while there were no such significant correlations in the urine from ECS women. The correlation coefficient (r) improved when only urine collected during labor was used for the analysis in VD women (see legend for Fig. 4).

Paired AP and IPP urine samples from 7 ESC women were cultured for 24 hours, cells attached to the culture slide during incubation were stained with fluorescence, and number of nucleated cells with fluorescence specific for podocin and or nephrin was counted on microscopy to determine whether viable podocytes were present in the urine and whether number of viable podocytes increased after parturition (Fig. 5). We considered that nucleated cells with fluorescence for podocin and or nephrin were viable podocytes. Number of viable podocytes was significantly greater in the IPP than AP urines and correlated significantly positively with that of urine Pod-mRNA copies (Fig. 5, E).

DISCUSSION

This study demonstrated for the first time that podocyturia monitored by Pod-mRNA expression level was significantly increased in the urine collected around childbirth compared to that collected antepartum in the absence of labor pains or on postpartum day 3 or later. This was consistent with the results of a previous study [1] in which the number of urine podocytes on postpartum day 4 was increased in 20% (9/45) of women with uncomplicated pregnancies. It was speculated that the increased podocyturia would have been seen in a larger number of women if urine collected at an earlier stage of puerperium than postpartum day 4 were examined in the study by Aita *et al*. [1].

Blood pressure increases during labor even in women not complicated with HDP [3,17]. The blood pressure increased from 112/67 mmHg at the last prenatal visit to 136/86 mmHg during labor of the 18 VD women in this study, consistent with an earlier finding in which corresponding blood pressure levels were 119/74 and 135/81 mmHg,

respectively [3]. This increase in blood pressure during labor may have been a factor responsible for the transient increase in podocyturia during labor in VD women based on the present observation that MAP during labor was significantly positively correlated with urine Pod-mRNA level during labor in VD women (see legend for Fig. 4).

However, factors other than the blood pressure not specified in this study may have been responsible for the transient increase in podocyturia immediately after cesarean section within 12 hours postpartum. Very acute changes in mechanical stress are speculated to occur in the glomerular podocytes of ECS women; in women with cesarean section, fluid loading is usually given before introduction of anesthesia to prevent anesthesia-induced hypotension [2], a further increase in the circulating blood occurs just after childbirth because the blood that perfused the enlarged uterus and placenta returns to the general circulation with involution of the uterus and expulsion of the placenta, and oxytocin given to facilitate uterine involution after childbirth increases stroke volume [24]. Vasopressors and oxytocin were used during ECS in 70% and 100% of women, respectively in this study. It was speculated that these factors were associated with the transient increase in podocyturia immediately after ECS within 12 hours after childbirth.

In this study, the transient increase in podocyturia at DL/IPP was concomitant with the transient decrease in NPR (Fig. 3) and podocyturia was significantly negatively correlated with NPR (Fig. 4A, 4D). These observations suggested that parturition acted to reduce glomerular podocyte Nep-mRNA expression, glomerular podocytes with reduced Nep-mRNA level were likely to detach from the GBM, and abundant podocytes with reduced Nep-mRNA level occurred in the urine collected at DL/IPP. These observations were consistent with the results of previous animal and human studies [7, 10, 26, 32, 33]. Persistent proteinuria was shown to be associated with decreased Nep-mRNA expression in urine podocytes in an animal model [7] and decreased NPR in the urine of animal models and in urine from patients with SLE-associated glomerular disease [26]. Expression of nephrin, but not podocin, is reduced in kidney biopsy specimens from women with preeclampsia [10] in whom podocyturia is increased [4, 9, 13, 32], nephrin expression was shown to be reduced in the kidneys of women that died from preeclampsia [33], and Nep-mRNA expression is reduced in the urine podocytes recovered from women with preeclampsia [32]. All of these observations indicated that phenotypic alteration of glomerular podocytes can occur in response to various stimuli and podocytes with reduced Nep-mRNA expression are likely to detach from the GBM.

The mRNA expression level appears to respond promptly to various stimuli; for example, the level of endothelin-1 mRNA from bovine aortic endothelial cells grown in vitro shows a rapid (within 1 hour of exposure) and significant (fivefold) decrease in response to fluid shear stress of physiological magnitude [14]. Length of labor for VD and fluid shear stress occurring in ECS may be long and strong enough, respectively, to induce downregulation of Nep-mRNA in glomerular podocytes. The urine Syn-mRNA was suggested to originate from urine podocytes [32]. Podocyturia monitored by urine Pod-mRNA level exhibited significant negative correlations with not only NPR but also SPR (Fig. 4). These observations suggested that glomerular podocyte Syn-mRNA

expression was also reduced in response to stimuli brought about by parturition.

Proteinuria increased transiently during labor and immediately postpartum (see legend for Fig. 1 for VD women). Albuminuria increases during parturition in women with uncomplicated pregnancies [6]. As the podocytes together with the GBM determine the permselectivity of plasma proteins [16, 29], parturition accompanied by the transient increase in podocyturia may have caused the transient increase in proteinuria. The AQP2-mRNA expression level did not show significant changes in this study. AQP2 is expressed in the principal cells of the kidney connecting tubule and collecting duct [15]. The cells expressing AQP2-mRNA were considered to be relatively insensitive to various changes occurring during parturition.

Number of viable podocytes increased transiently after parturition in this study. Increase in urine viable:apoptotic podocyte ratio is positively associated with disease activity in patients with lupus nephritis [19], suggesting that viable podocytes are likely to detach from the GBM in pathological process. As podocytes are terminally differentiated cells [16] and their turnover rate is very low [18, 22], their detachment from the GBM causes a long-lasting decrease in number of podocytes in the kidneys [20].

Limitation of our study included following two: factors responsible for increased podocyturia immediately after cesarean section were not specified; and it was unknown whether reduced nephrin expression actually occurred in the glomerular podocytes during parturition. Experiments using animal models are required to elucidate these issues.

In this study, podocyturia monitored by Pod-mRNA expression increased with increasing MAP in VD women. As low degree of endotheliosis is seen even in women with normotensive pregnancies [27], glomerular endotheliosis is not pathognomonic for preeclampsia. Preeclampsia accompanies hypertension, proteinuria, and marked podocyturia [32], may be the extreme of the adaptational process, rather than a separate abnormal condition [27], and is a prominent risk factor for end-stage renal disease (ESRD) [30]. Degree of endotheliosis may be associated with degree of phenotypic alteration of glomerular podocytes with respect to reduced nephrin expression and or podocyte loss in the kidney. Aging is associated with a decrease in number of podocytes in the kidney [12] and the risk of ESRD increases with age [28]. The present study indicated markers of podocyte injury were present even in the urines of women with normotensive pregnancies. However, it remains to be studied whether this contribute to future renal disease.

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Disclosure

The authors declare no conflicts of interest.

Author contributions

IF, TZ, and HM participated in discussion on this study design. IF, TZ, TU, SI, KN, TK, TY, and MM collected urine specimens from participants. IF and TZ measured urine variables and IF performed the statistical analysis and drafted the manuscript. HM coordinated the study and helped to draft the manuscript. All authors have read and approved the final manuscript.

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Table 1. Demographic characteristics of 38 women

	Vaginal delivery (VD)	Caesarean delivery (ECS)
No. of women	18	20
Maternal age (years)	32.5 (22 – 41)	33 (21 – 45)
≥ 35	6 (33%)	9 (45%)
Nulliparous	5 (28%)	13 (65%)*
Body height (m)	1.61 (1.54 – 1.71)	1.60 (1.48 – 1.70)
Pre-pregnancy weight (kg)	51 (44 – 68)	53 (37 – 68)
Body mass index (kg/m^2)	20.5 (17.0 – 26.1)	21.0 (16.8 – 25.0)
≥ 25	1 (5.6%)	0 (0.0%)
Blood pressure (mmHg)		
SBP at the last prenatal visit	112 (88 - 137)	106 (80 - 126)
DBP at the last prenatal visit	67 (52 - 84)	65 (45 - 79)*
During parturition		
Highest SBP	136 (118 - 205)	126 (104 - 144)*
≥ 140	9 (50%)	1 (5.0%)*
Highest DBP	86 (56 - 121)	78 (61 - 101)*
≥ 90	5 (28%)	1 (5.0%)
Use of vasopressors	0 (0.0%)	14 (70%)*
Use of oxytocin	9 (50%)	20 (100%)*
GW at delivery	39.2 (36.1 – 41.3)	38.0 (36.0 – 39.0)*
< 37	1 (5.6%)	1 (5.0%)
Infant birthweight (kg)	2.86 (2.11 – 3.64)	2.94 (2.00 – 3.58)

Data are presented as the median (range). *, $P < 0.05$ vs. women with vaginal deliveries; DBP, diastolic blood pressure; GW, gestational week; SBP, systolic blood pressure.

Table 2. Methods and timing for urine sampling

	Vaginal delivery (VD)	Caesarean delivery (ECS)
No. of women	18	20
Urine sampling		
Total no. of urine samples	43	62
No. of urine samples/person	2 (2 - 4)	3 (2- 5)
Antepartum (AP)	17 (94%)	20 (100%)
During labor (DL)†	10 (56%)	0 (0.0%)
Immediately postpartum (IPP)†	0 (0.0%)	19 (95%)
Postpartum day 1 (PD1)	0 (0.0%)	5 (25%)
Postpartum day 3 – 7 (PD2)	10 (56%)	12 (60%)
Postpartum day 25 – 35 (PD3)	6 (33%)	6 (30%)

†, All collected at these stages were catheterized urine specimens and all collected at other stages were voided urine specimens. AP, 1 – 14 days before delivery in the absence of labor pains at the last prenatal visit; DL, during labor within 4 hours before vaginal delivery; IPP, immediately postpartum within 12 hours after caesarean section.

FIGURE LEGENDS

Figure 1 Perinatal changes in various urine mRNA expressions and urine protein:creatine ratio (P/Cr) in women with vaginal delivery

*, $P < 0.05$ between two values. Serial changes in individual data and median values are presented on the left and right, respectively. In 9 women with paired AP and DL samples, Pod-mRNA, Nep-mRNA, Syn-mRNA, and AQP2-mRNA levels and P/Cr increased at DL in 8 (89%), 4 (44%), 9 (100%), 5 (56%), and 7 (78%) women, respectively. The median P/Cr (mg/mg) increased significantly from 0.038 at AP to 0.062 at DL in 9 paired specimens ($P = 0.0209$).

Figure 2 Perinatal changes in expression of various urine mRNAs and urine protein:creatine ratio (P/Cr) in women with elective cesarean delivery

*, $P < 0.05$ between two values. Serial changes in individual data and median values are presented on the left and right, respectively. In 19 women with paired AP and IPP samples, Pod-mRNA, Nep-mRNA, Syn-mRNA, and AQP2-mRNA levels and P/Cr increased at IPP in 19 (100%), 11 (58%), 17 (89%), 10 (53%), and 18 (95%) women, respectively. The median P/Cr (mg/mg) increased significantly from 0.046 at AP to 0.124 at IPP in 19 paired specimens ($P = 0.0002$).

Figure 3 Perinatal changes in Nep-mRNA:Pod-mRNA ratio (NPR) and Syn-mRNA:Pod-mRNA ratio (SPR)

*, $P < 0.05$ between two values. Urine samples were similar to those in Fig. 1 and Fig. 2, but six samples with undetectable Pod-mRNA level were excluded from this analysis. In VD women, median NPR was 0.713, 0.069, 1.456, and 2.217, and median SPR was 0.235, 0.203, 0.499, and 0.628 at AP, DL, PD2, and PD3, respectively. In ECS women, median NPR was 0.516, 0.026, 0.305, 2.492 and 0.979, and median SPR was 0.243, 0.017, 0.258, 0.721 and 0.723 at AP, IPP, PD1, PD2, and PD3, respectively.

Figure 4 Correlations of podocyturia monitored by Pod-mRNA with Nep-mRNA:Pod-mRNA ratio (NPR) and mean arterial pressure (MAP)

In these analyses, 25 urine samples with detectable Pod-mRNA collected at AP ($n = 15$) and DL ($n = 10$) were used for VD women and 39 urine samples with detectable Pod-mRNA collected at AP ($n = 20$) and IPP ($n = 19$) were used for ECS women. MAP ≥ 100 mmHg occurred during parturition in 8 of 10 VD women (80%) and 4 of 19 ECS women (21%). Pod-mRNA was also significantly negatively correlated with SPR in both VD and ECS women ($r = -0.401$, $P = 0.0496$; $r = -0.691$, $P < 0.0001$, respectively) (data not shown).

The correlation coefficient (r) of MAP for Pod-mRNA level improved to 0.733 with P = 0.0278 when 10 urine samples collected at DL only were used for VD women. The r of MAP for Pod-mRNA was –0.311 (P = 0.1876) when 19 urine samples collected at IPP only were used for ECS women. Thus, blood pressure was correlated with podocyturia during labor in VD women, but not in ECS women.

Figure 5 Urine podocytes detected by immunofluorescence staining and correlation between numbers of urine podocytes and urine podocin mRNA copies

A and B, immunofluorescence staining for podocin (green) and nephrin (red) with a blue nuclear counterstain (DAPI) to count number of podocytes; C and D, podocytes stained with fluorescence in the IPP urine from a participant that showed 14.8 podoctytes attached to the culture slide (per mg creatinine) during overnight incubation as well as 44.4 Pod-mRNA copies (per mg creatinine); E, correlation between number of Pod-mRNA copies and that of podocytes stained with fluorescence in 7 ECS women with paired AP and IPP samples. In the 7 women, number of viable podocytes increased significantly from 0.3 (0.0 – 0.6)/mg creatinine in the AP urines to 2.6 (0.2 – 31.9)/mg creatinine in the IPP urines (P = 0.028) and number of Pod-mRNA copies increased significantly from 1.5 (1.1 – 15.3)/ mg creatinine in the AP urines to 52.3 (4.4 - 159)/mg creatinine in the IPP urines (P = 0.018).

Fig.1

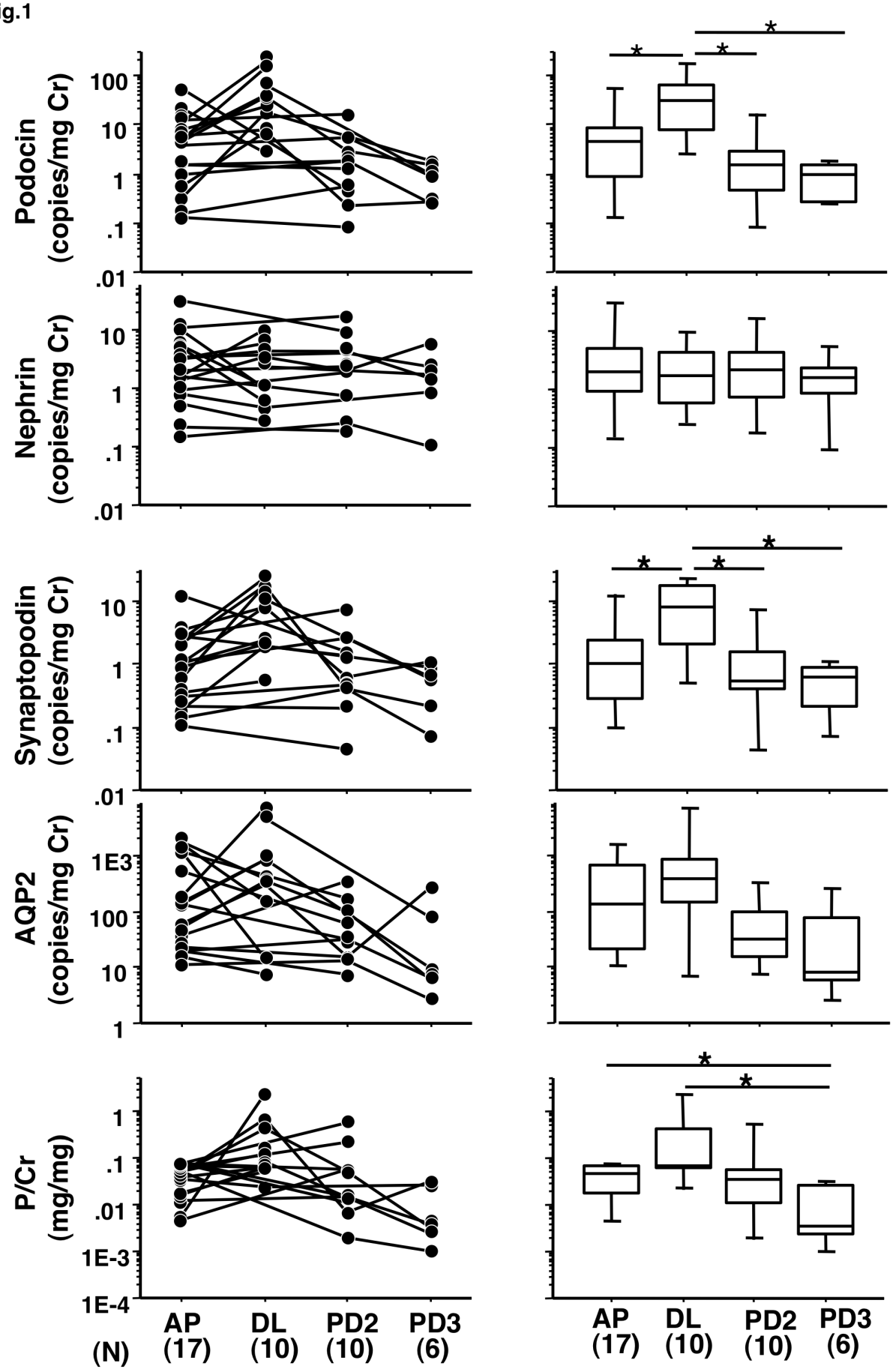

Podocin (copies/mg Cr)
100
10
1
.1
.01
Nephrin (copies/mg Cr)
10
1
.1
.01
Synaptopodin (copies/mg Cr)
10
1
.1
.01
AQP2 (copies/mg Cr)
1E3
100
10
1
P/Cr (mg/mg)
1
.1
.01
1E-3
1E-4
*
*
*
AP (17)
DL (10)
PD2 (10)
PD3 (6)
(N)

Fig.2

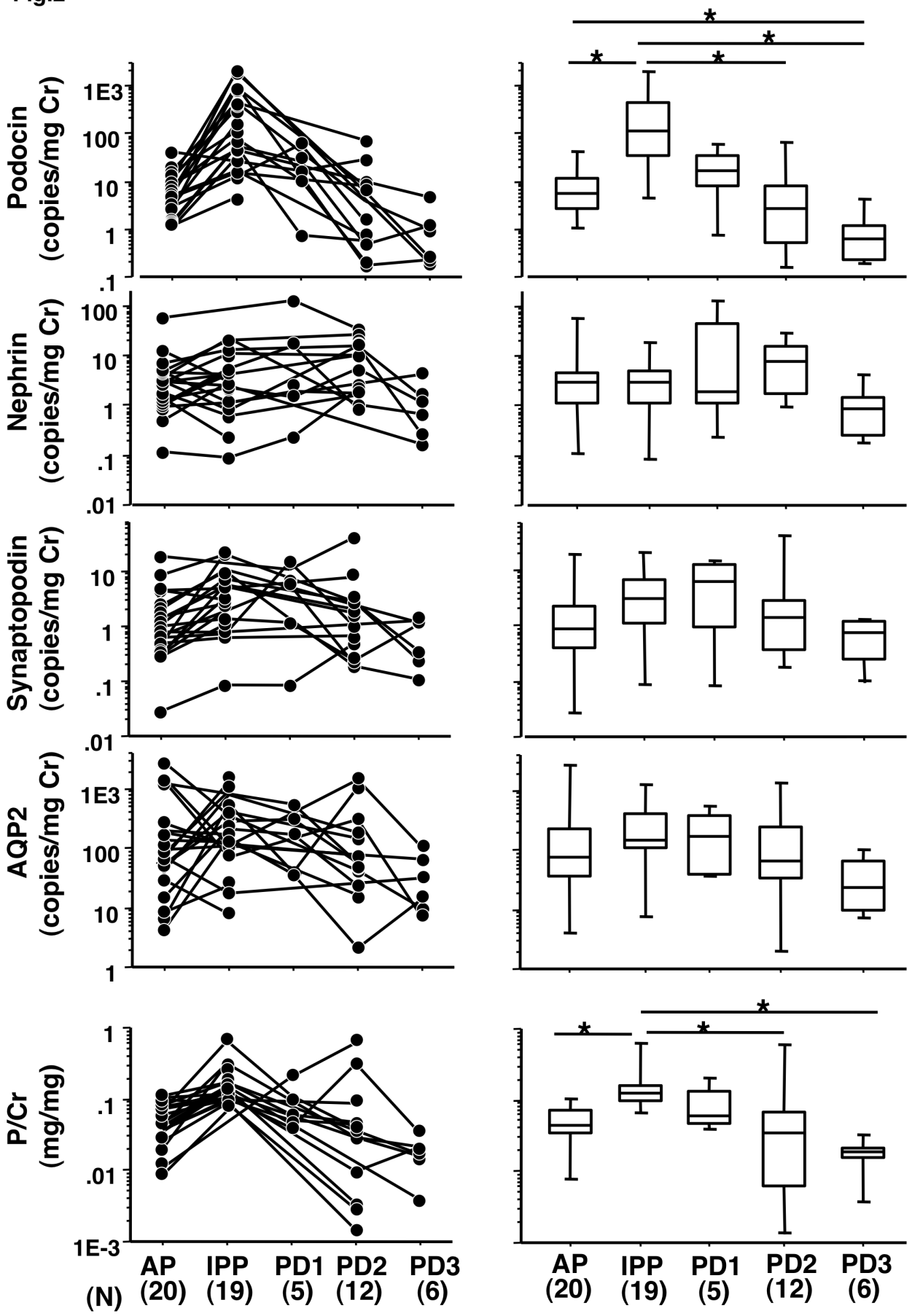

Podocin (copies/mg Cr)
Nephrin (copies/mg Cr)
Synaptopodin (copies/mg Cr)
AQP2 (copies/mg Cr)
P/Cr (mg/mg)
1E3
100
10
1
.1
.01
1E-3
*
AP (20)
IPP (19)
PD1 (5)
PD2 (12)
PD3 (6)
(N)

Fig.3

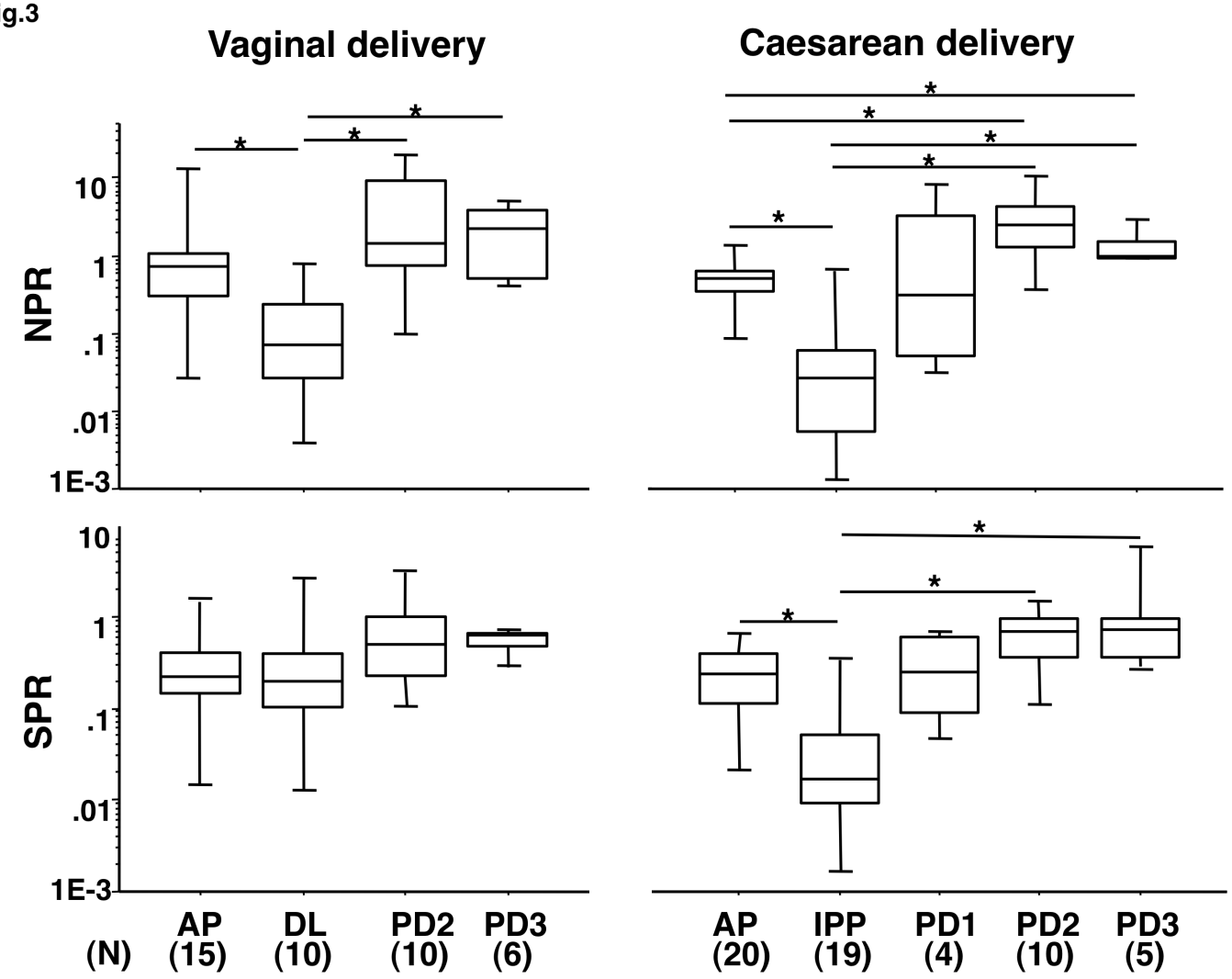

Vaginal delivery
Caesarean delivery
NPR
SPR
10
1
.1
.01
1E-3
*
(N)
AP (15)
DL (10)
PD2 (10)
PD3 (6)
AP (20)
IPP (19)
PD1 (4)
PD2 (10)
PD3 (5)

Fig. 4

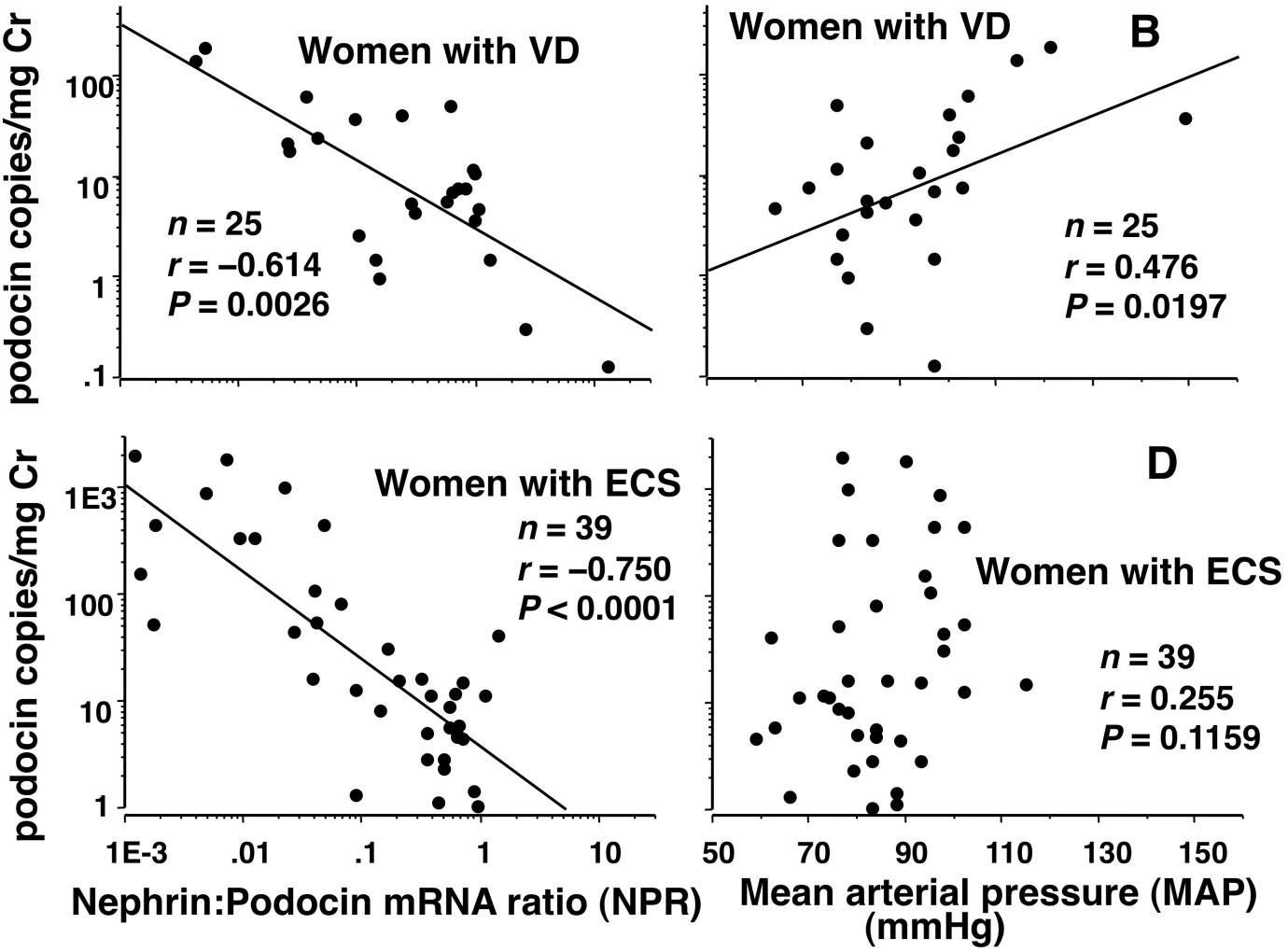
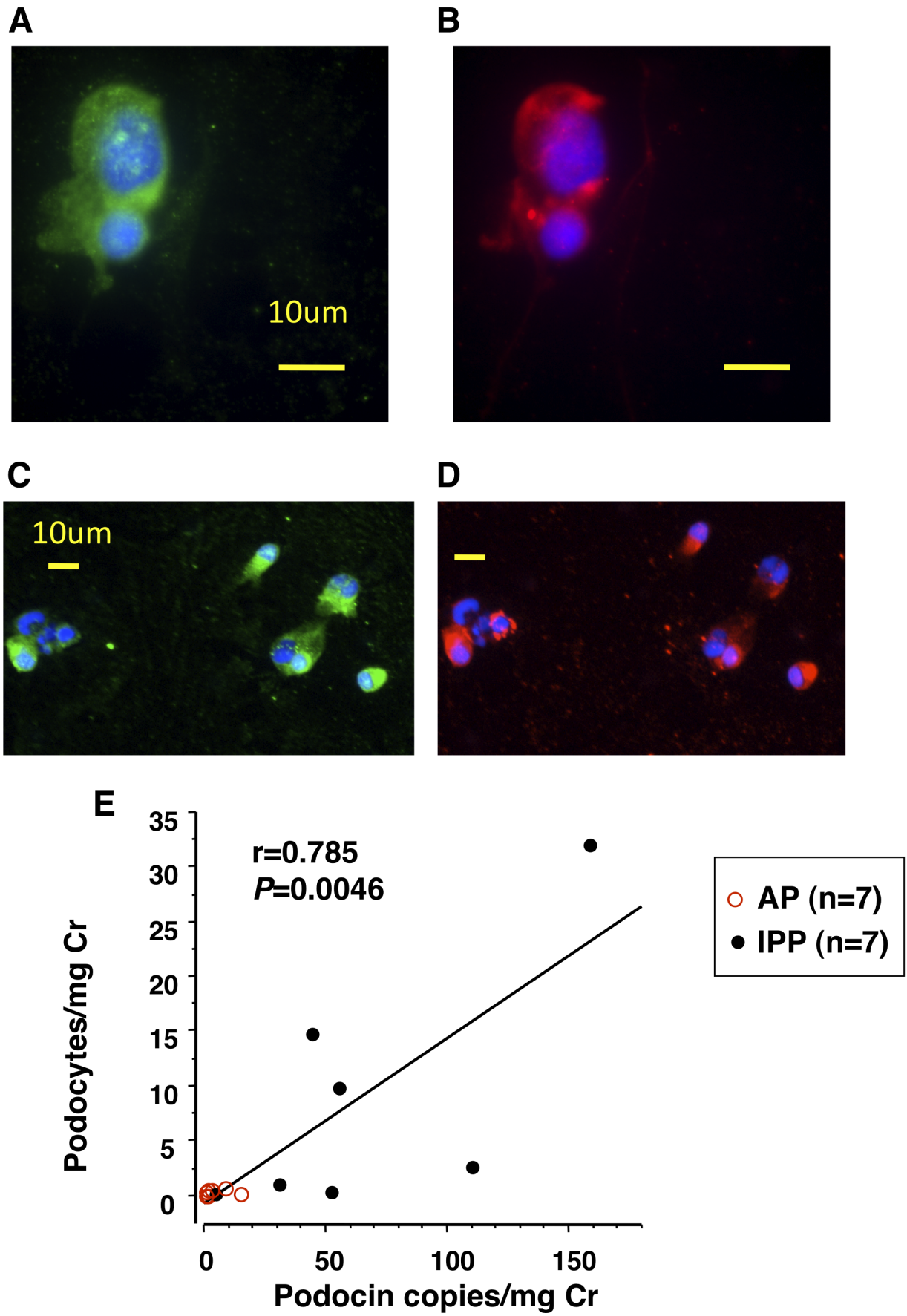

Women with VD
n = 25
r = –0.614
P = 0.0026
B
Women with VD
n = 25
r = 0.476
P = 0.0197
Women with ECS
n = 39
r = –0.750
P < 0.0001
D
Women with ECS
n = 39
r = 0.255
P = 0.1159
podocin copies/mg Cr
podocin copies/mg Cr
100
10
1
.1
1E3
100
10
1
1E-3
.01
.1
1
10
50
70
90
110
130
150
Nephrin:Podocin mRNA ratio (NPR)
Mean arterial pressure (MAP) (mmHg)

Fig.5


A
B
10um
C
D
10um
E
r=0.785
P=0.0046
AP (n=7)
IPP (n=7)
Podocytes/mg Cr
Podocin copies/mg Cr
0
5
10
15
20
25
30
35
0
50
100
150