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1 **Effects of childbirth on podocyturia in women with normotensive uncomplicated**
2 **pregnancies**

3

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18 **Key words:** aquaporin, podocin, nephrin, podocyte injury, proteinuric disease

19 **Abstract**

20 Changes in hemodynamics and blood pressure occur shortly before and after childbirth
21 regardless of the mode of delivery. This study aimed to test the hypothesis that
22 parturition induces a temporal increase in podocyturia monitored by podocyte-specific
23 protein podocin mRNA expression levels (Pod-mRNA). A total of 105 urine specimens,
24 consisting of 43 and 62 from 18 and 20 otherwise healthy women with vaginal (VD)
25 and elective cesarean deliveries (ECS), respectively, were studied. Determination of
26 urine protein and creatinine concentrations and quantitative analyses of Pod-mRNA,
27 nephrin mRNA (Nep-mRNA), synaptopodin mRNA (Syn-mRNA), and aquaporin 2
28 mRNA expression (AQP2-mRNA) were performed using RT-PCR in pelleted urine
29 samples. Levels of mRNA expression were corrected by urine creatinine concentration.
30 Podocyturia increased significantly concomitant with significantly decreased
31 Nep:Pod-mRNA ratio (NPR) in the urine collected immediately before or after
32 childbirth regardless of the delivery mode compared to urine collected before
33 commencement of labor or on postpartum day 3 or later. Podocyturia was significantly
34 negatively correlated with NPR (correlation coefficient $[r] = -0.614/-0.750$ for VD/ECS
35 women, respectively) as well as Syn:Pod-mRNA ratio. Systolic blood pressure
36 exceeded 140 mmHg during labor in 50% of VD women and mean arterial pressure was
37 significantly positively correlated with podocyturia during labor in VD women ($r =$
38 0.733). Thus, parturition induces a transient increase in urine podocytes with reduced
39 Nep- and Syn-mRNA expressions. Glomerular podocytes with reduced Nep-mRNA and
40 Syn-mRNA levels were suggested to be likely to detach from the glomerular basement
41 membrane around childbirth.

42 INTRODUCTION

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

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

66 Podocyturia can be monitored by urine levels of podocyte-specific protein mRNAs,
67 including podocin (Pod-mRNA) and nephrin (Nep-mRNA) [13,32]. Urine synaptopodin
68 protein mRNA (Syn-mRNA) was also suggested to be derived mainly from urine
69 podocytes [32]. Aquaporin 2 (AQP2), expressed in the principal cells of the kidney
70 connecting tubule and collecting duct, but not in podocytes, plays a critical role in
71 regulation of body water balance [15] and AQP2-mRNA is also detectable in the urine
72 [26].

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

77 METHODS

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

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

82 ***Participants***

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

96 ***Urine sampling***

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

107 All 105 urine specimens were coded and processed within 2 hours of collection. Urine
108 samples were centrifuged at $700 \times g$ for 5 minutes. Urinary supernatant was stored at
109 -20°C until measurement of protein and creatinine (Cr) levels. The pelleted urine
110 samples were suspended in RNAlater (Life Technologies, Carlsbad, CA) and stored at
111 -20°C until isolation of RNA. Protein and Cr concentrations were measured using a
112 Protein Assay Rapid Kit Wako and Laboassay Creatinine (Wako Pure Chemical
113 Industries, Ltd., Osaka, Japan), respectively. Urine protein concentration was corrected
114 by urine Cr and expressed as P/Cr (mg/mg).

115 ***Quantitative Real-time PCR assay***

116 RNA isolation from the pelleted urine and reverse transcription reaction were performed
117 as described previously [32]. The absolute Pod-, Nep-, Syn-, and AQP2-mRNA levels
118 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 $700 \times g$ at room temperature,
 and the pellets were rinsed twice with human diploid fibroblast (HDF) solution. The
 pellets were resuspended in 400 µL 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% CO₂, 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, Cambridge, 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.

163 **Data expression**

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

169 **Statistical analyses**

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

178 **RESULTS**

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

186
187 AQP2-mRNA expression was detectable in all 105 urine specimens, but Pod-mRNA,
188 Nep-mRNA, and Syn-mRNA expression were undetectable in 6 (5.7%), 8 (7.6%), and
189 20 (19%) of the 105 urine specimens, respectively. Pod-mRNA expression was
190 undetectable in 2 of 37 specimens at AP (5.4%), 0 of 10 specimens at DL (0.0%), 0 of
191 19 specimens at IPP (0.0%), 1 of 5 specimens at PD1 (20%), 2 of 22 specimens at PD2
192 (9.1%), and 1 of 12 specimens at PD3 (8.3%).
193 In the 18 VD women, urine Pod- and Syn-mRNA expression levels increased
194 significantly at DL and decreased to levels seen at AP until PD2. However, neither Nep-
195 nor AQP2-mRNA exhibited a significant perinatal change (Fig. 1). In the 20 ECS
196 women, urine Pod-mRNA expression increased significantly immediately postpartum
197 (at IPP) and decreased to levels seen at AP until PD2, while neither Nep, Syn-, nor
198 AQP2-mRNA exhibited significant perinatal changes (Fig. 2).

199
200 These observations suggested that podocyturia increased transiently during and after
201 parturition regardless of the mode of delivery, but these podocytes excessively excreted
202 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,

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

249 However, factors other than the blood pressure not specified in this study may have
250 been responsible for the transient increase in podocyturia immediately after cesarean
251 section within 12 hours postpartum. Very acute changes in mechanical stress are
252 speculated to occur in the glomerular podocytes of ECS women; in women with
253 cesarean section, fluid loading is usually given before introduction of anesthesia to
254 prevent anesthesia-induced hypotension [2], a further increase in the circulating blood
255 occurs just after childbirth because the blood that perfused the enlarged uterus and
256 placenta returns to the general circulation with involution of the uterus and expulsion of
257 the placenta, and oxytocin given to facilitate uterine involution after childbirth increases
258 stroke volume [24]. Vasopressors and oxytocin were used during ECS in 70% and
259 100% of women, respectively in this study. It was speculated that these factors were
260 associated with the transient increase in podocyturia immediately after ECS within 12
261 hours after childbirth.

262 In this study, the transient increase in podocyturia at DL/IPP was concomitant with the
263 transient decrease in NPR (Fig. 3) and podocyturia was significantly negatively
264 correlated with NPR (Fig. 4A, 4D). These observations suggested that parturition acted
265 to reduce glomerular podocyte Nep-mRNA expression, glomerular podocytes with
266 reduced Nep-mRNA level were likely to detach from the GBM, and abundant podocytes
267 with reduced Nep-mRNA level occurred in the urine collected at DL/IPP. These
268 observations were consistent with the results of previous animal and human studies [7,
269 10, 26, 32, 33]. Persistent proteinuria was shown to be associated with decreased
270 Nep-mRNA expression in urine podocytes in an animal model [7] and decreased NPR
271 in the urine of animal models and in urine from patients with SLE-associated
272 glomerular disease [26]. Expression of nephrin, but not podocin, is reduced in kidney
273 biopsy specimens from women with preeclampsia [10] in whom podocyturia is
274 increased [4, 9, 13, 32], nephrin expression was shown to be reduced in the kidneys of
275 women that died from preeclampsia [33], and Nep-mRNA expression is reduced in the
276 urine podocytes recovered from women with preeclampsia [32]. All of these
277 observations indicated that phenotypic alteration of glomerular podocytes can occur in
278 response to various stimuli and podocytes with reduced Nep-mRNA expression are
279 likely to detach from the GBM.

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

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

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

299 Number of viable podocytes increased transiently after parturition in this study. Increase
300 in urine viable:apoptotic podocyte ratio is positively associated with disease activity in
301 patients with lupus nephritis [19], suggesting that viable podocytes are likely to detach
302 from the GBM in pathological process. As podocytes are terminally differentiated cells
303 [16] and their turnover rate is very low [18, 22], their detachment from the GBM causes
304 a long-lasting decrease in number of podocytes in the kidneys [20].

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

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

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326 **Disclosure**

327 The authors declare no conflicts of interest.

328 **Author contributions**

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

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441

442 **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)

465

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

468

469

470 **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%)

482

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

487

488 **FIGURE LEGENDS**

489 **Figure 1 Perinatal changes in various urine mRNA expressions and urine** 490 **protein:creatinine ratio (P/Cr) in women with vaginal delivery**

491 *, $P < 0.05$ between two values. Serial changes in individual data and median values are
492 presented on the left and right, respectively. In 9 women with paired AP and DL
493 samples, Pod-mRNA, Nep-mRNA, Syn-mRNA, and AQP2-mRNA levels and P/Cr
494 increased at DL in 8 (89%), 4 (44%), 9 (100%), 5 (56%), and 7 (78%) women,
495 respectively. The median P/Cr (mg/mg) increased significantly from 0.038 at AP to
496 0.062 at DL in 9 paired specimens ($P = 0.0209$).

497 **Figure 2 Perinatal changes in expression of various urine mRNAs and urine** 498 **protein:creatinine ratio (P/Cr) in women with elective cesarean delivery**

499 *, $P < 0.05$ between two values. Serial changes in individual data and median values are
500 presented on the left and right, respectively. In 19 women with paired AP and IPP
501 samples, Pod-mRNA, Nep-mRNA, Syn-mRNA, and AQP2-mRNA levels and P/Cr
502 increased at IPP in 19 (100%), 11 (58%), 17 (89%), 10 (53%), and 18 (95%) women,
503 respectively. The median P/Cr (mg/mg) increased significantly from 0.046 at AP to
504 0.124 at IPP in 19 paired specimens ($P = 0.0002$).

505 **Figure 3 Perinatal changes in Nep-mRNA:Pod-mRNA ratio (NPR) and** 506 **Syn-mRNA:Pod-mRNA ratio (SPR)**

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

513 **Figure 4 Correlations of podocyturia monitored by Pod-mRNA with** 514 **Nep-mRNA:Pod-mRNA ratio (NPR) and mean arterial pressure (MAP)**

515 In these analyses, 25 urine samples with detectable Pod-mRNA collected at AP ($n = 15$)
516 and DL ($n = 10$) were used for VD women and 39 urine samples with detectable
517 Pod-mRNA collected at AP ($n = 20$) and IPP ($n = 19$) were used for ECS women. MAP
518 ≥ 100 mmHg occurred during parturition in 8 of 10 VD women (80%) and 4 of 19 ECS
519 women (21%). Pod-mRNA was also significantly negatively correlated with SPR in
520 both VD and ECS women ($r = -0.401$, $P = 0.0496$; $r = -0.691$, $P < 0.0001$,
521 respectively) (data not shown).

522 The correlation coefficient (r) of MAP for Pod-mRNA level improved to 0.733 with P
523 = 0.0278 when 10 urine samples collected at DL only were used for VD women. The r
524 of MAP for Pod-mRNA was -0.311 ($P = 0.1876$) when 19 urine samples collected at
525 IPP only were used for ECS women. Thus, blood pressure was correlated with
526 podocyturia during labor in VD women, but not in ECS women.

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

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

Fig.1

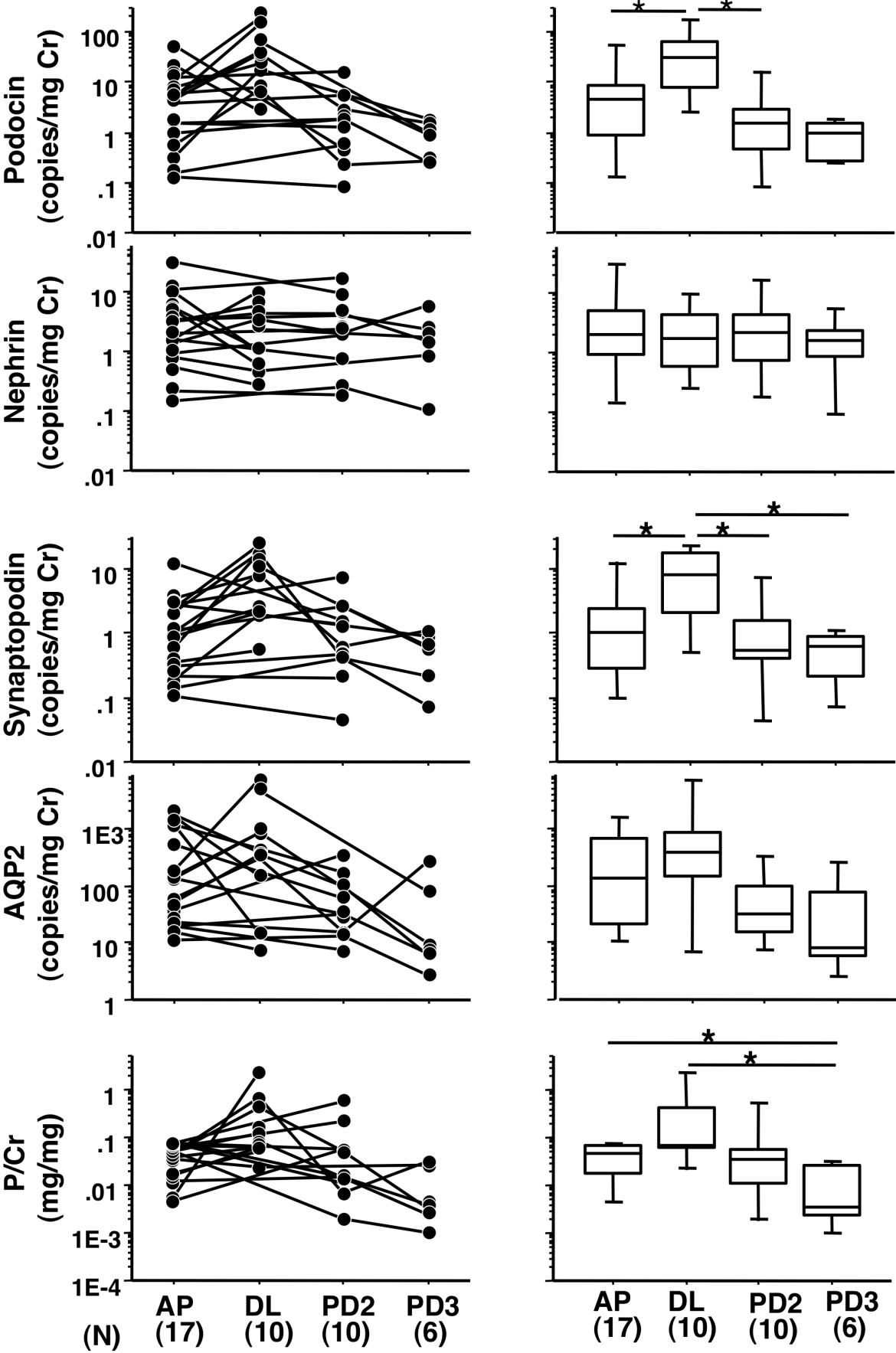


Fig.2

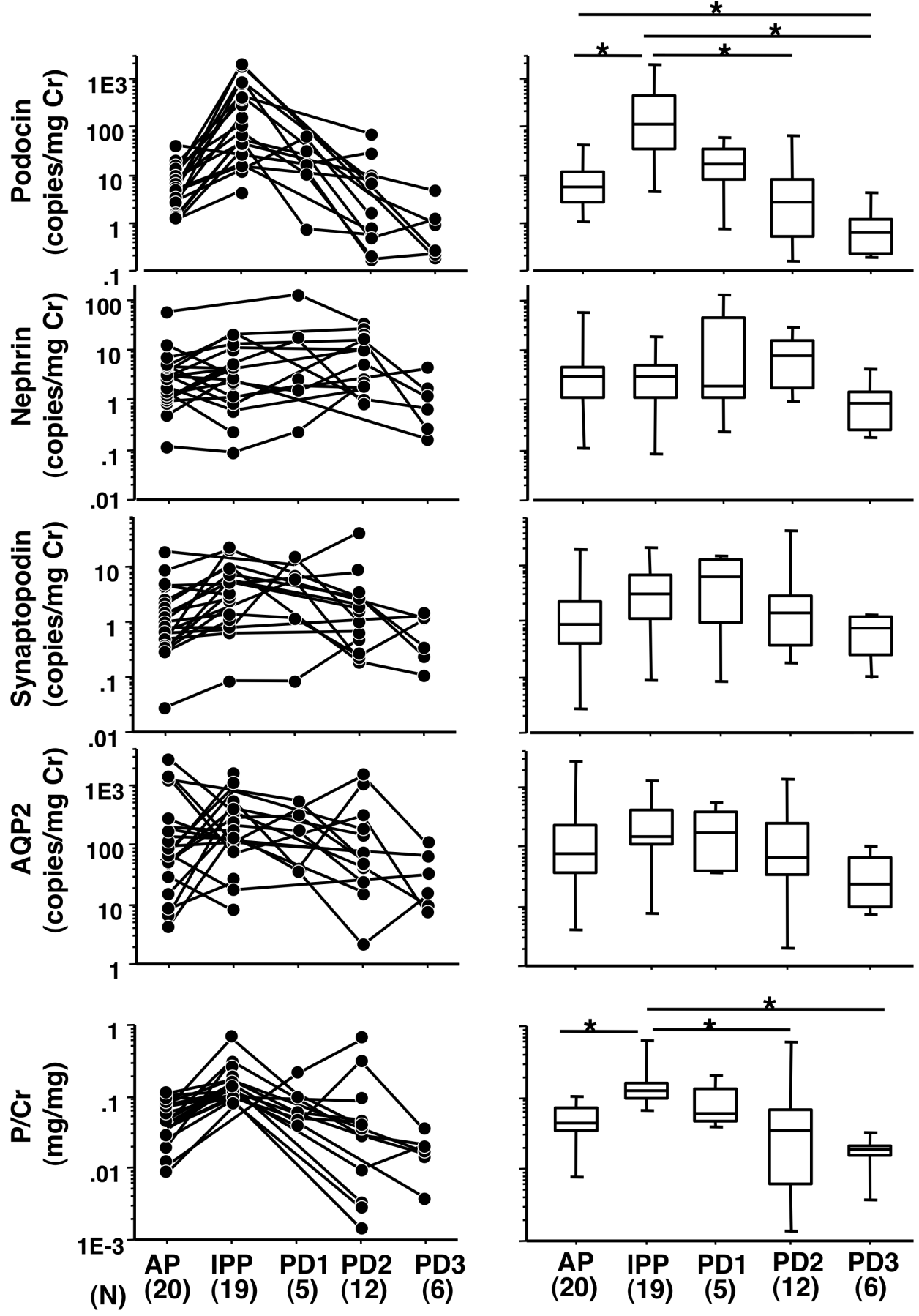


Fig.3

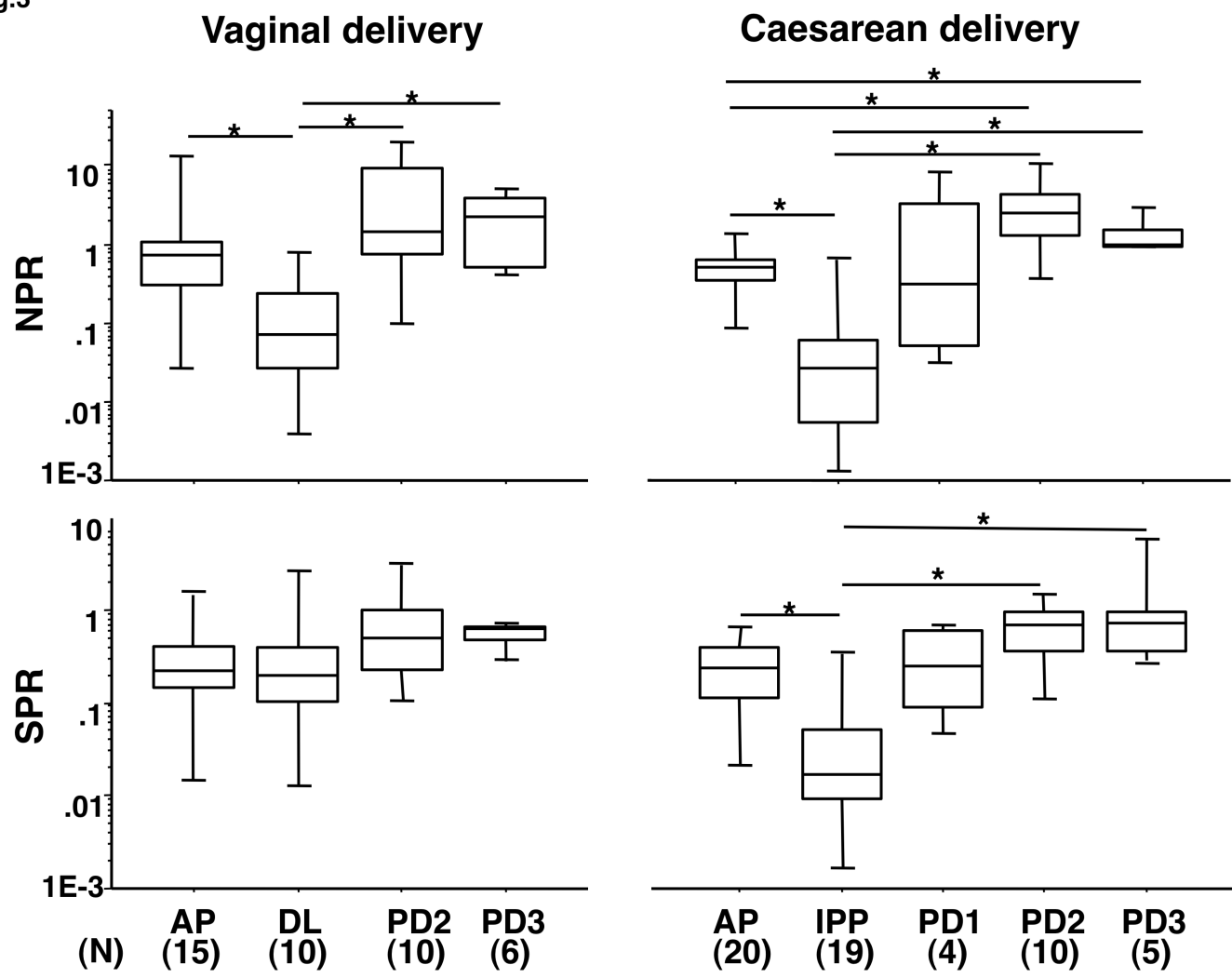


Fig. 4

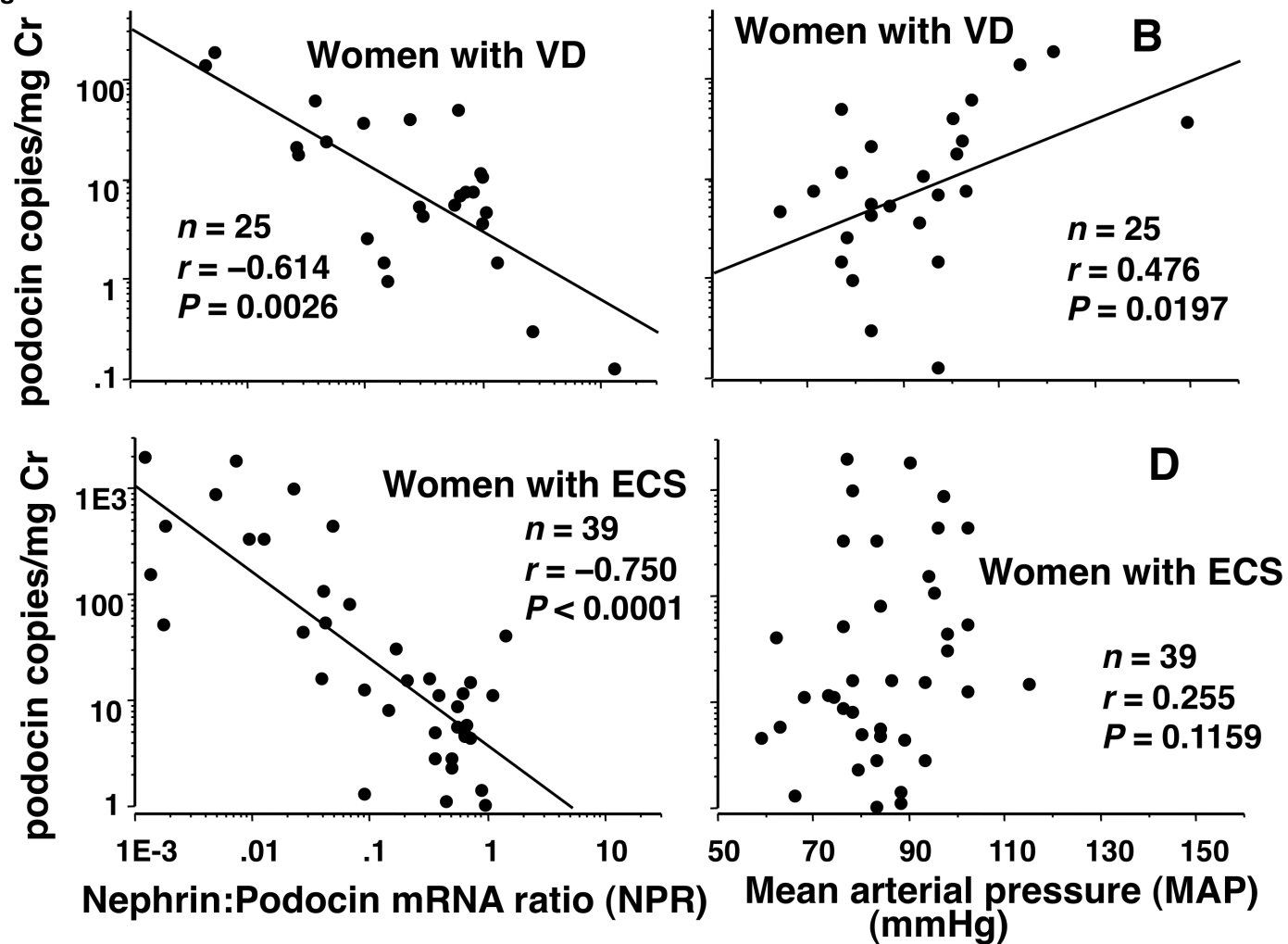


Fig.5

