Colocalization of the (Pro)renin receptor/Atp6ap2 with H+-ATPases in mouse kidney but prorenin does not acutely regulate intercalated cell H+-ATPase activity

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Abstract: The (Pro)renin receptor (P)RR/Atp6ap2 is a cell surface protein capable of binding and non-proteolytically activate prorenin. Additionally, (P)RR is associated with H+-ATPases and alternative functions in H+-ATPase regulation as well as in Wnt signalling have been reported. Kidneys express very high levels of H+-ATPases which are involved in multiple functions such as endocytosis, membrane protein recycling as well as urinary acidification, bicarbonate reabsorption, and salt absorption. Here, we wanted to localize the (P)RR/Atp6ap2 along the murine nephron, examine whether the (P)RR/Atp6ap2 is coregulated with other H+-ATPase subunits, and whether acute stimulation of the (P)RR/Atp6ap2 with prorenin regulates H+-ATPase activity in intercalated cells in freshly isolated collecting ducts. We localized (P)PR/Atp6ap2 along the murine nephron by qPCR and immunohistochemistry. (P)RR/Atp6ap2 mRNA was detected in all nephron segments with highest levels in the collecting system coinciding with H+-ATPases. Further experiments demonstrated expression at the brush border membrane of proximal tubules and in all types of intercalated cells colocalizing with H+-ATPases. In mice treated with NH4Cl, NaHCO3, KHCO3, NaCl, or the mineralocorticoid DOCA for 7 days, (P)RR/Atp6ap2 and H+-ATPase subunits were regulated but not co-regulated at protein and mRNA levels. Immunolocalization in kidneys from control, NH4Cl or NaHCO3 treated mice demonstrated always colocalization of PRR/Atp6ap2 with H+-ATPase subunits at the brush border membrane of proximal tubules, the apical pole of type A intercalated cells, and at basolateral and/or apical membranes of non-type A intercalated cells. Micropuncture of isolated cortical collecting ducts and luminal application of prorenin did not acutely stimulate H+-ATPase activity. However, incubation of isolated collecting ducts with prorenin non-significantly increased ERK1/2 phosphorylation. Our results suggest that the PRR/Atp6ap2 may form a complex with H+-ATPases in proximal tubule and intercalated cells but that prorenin has no acute effect on H+-ATPase activity in intercalated cells.

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Developmental evidence for obstetric adaptation of the human female pelvis

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The bony pelvis of adult humans exhibits marked sexual dimorphism, which is traditionally interpreted in the framework of the “obstetrical dilemma” hypothesis: Giving birth to large-brained/large-bodied babies requires a wide pelvis, whereas efficient bipedal locomotion requires a narrow pelvis. This hypothesis has been challenged recently on biomechanical, metabolic, and biocultural grounds, so that it remains unclear which factors are responsible for sex-specific differences in adult pelvic morphology. Here we address this issue from a developmental perspective. We use methods of biomedical imaging and geometric morphometrics to analyze changes in pelvic morphology from late fetal stages to adulthood in a known-age/known-sex forensic/clinical sample. Results show that, until puberty, female and male pelves exhibit only moderate sexual dimorphism and follow largely similar developmental trajectories. With the onset of puberty, however, the female trajectory diverges substantially from the common course, resulting in rapid expansion of obstetrically relevant pelvic dimensions up to the age of 25–30 y. From 40 y onward females assume a mode of pelvic development similar to males, resulting in significant reduction of obstetric dimensions. This complex developmental trajectory is likely linked to the pubertal rise and premenopausal fall of estradiol levels and results in the obstetrically most adequate pelvic morphology during the time of maximum female fertility. The evidence that hormones mediate female pelvic development and morphology supports the view that solutions to the obstetrical dilemma depend not only on selection and adaptation but also on developmental plasticity as a response to ecological/nutritional factors during a female’s lifetime.

Significance

The obstetrical dilemma hypothesis states that the human female pelvis represents a compromise between designs most suitable for childbirth and bipedal locomotion, respectively. This hypothesis has been challenged recently on biomechanical, metabolic, and biocultural grounds. Here we provide evidence for the pelvis’ developmental adaptation to the problem of birthing large-headed/large-bodied babies. We show that the female pelvis reaches its obstetrically most adequate morphology around the time of maximum fertility but later reverts to a mode of development similar to that of males, which significantly reduces the dimensions of the birth canal. These developmental changes are likely mediated by hormonal changes during puberty and menopause, indicating “on-demand” adjustment of pelvic shape to the needs of childbirth.

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Females and males of most mammalian species differ in various morphological characteristics, such as the size and shape of the body as a whole and of soft and hard tissue structures (1). Sex-specific differences are also well documented in humans and nonhuman primates, particularly in the pelvis, and various hypotheses have been proposed to explain how pelvis sexual dimorphism evolves and develops (2–11). There is general agreement that the female pelvis is under obstetric selection to be adequately capacious for childbirth. However, the exact nature of selective pressures and developmental mechanisms yielding female and male pelvic phenotypes is still largely unknown, and whether obstetric adaptations involve trade-offs with other aspects of pelvic function, such as locomotor efficiency and abdominal stabilization, continues to be debated (12, 13).

One key hypothesis discussed in this context is Washburn’s obstetrical dilemma (OD) (14). In its original form (14), the OD hypothesis posits a conflict between the evolution of bipedal locomotion (selection for biomechanically efficient, narrow pelves) and of large brains (selection for large-brained neonates, and obstetrically efficient, wide pelves). According to Washburn, the dilemma is “solved by delivery of the fetus at a much earlier stage of development” (ref. 14, p. 74) than in our closest living relatives, the great apes. Although the OD hypothesis thus primarily seeks to explain the early timing of birth and human altriciality (15), it also provides an explanation for pelvic sexual dimorphism: Selection favored wider female pelves to reduce the risks involved in birthing large-brained/large-bodied babies, but did so at the expense of locomotor efficiency (2, 5, 7). According to this hypothesis, the tight fit between the neonate head and maternal pelvis (obstetric constraints) and the high prevalence of obstructed labor in humans (16–18) reflect a trade-off between obstetric and locomotor selection pressures on the female pelvis.

Over the past years, the OD hypothesis has been reexamined extensively and has been challenged on various grounds (10–13, 19–22). The energetics of gestation and growth (EGG) hypothesis (12, 20, 23) provides a new perspective, proposing that the timing of birth is constrained by the limited metabolic output of the mother rather than by spatial limitations of her pelvis. Furthermore, inverse-dynamics models and experimental data indicate that a wide pelvis does not reduce bipedal locomotor efficiency (12, 13). Because these studies effectively falsify a major tenet of the OD, the tight fit between neonate head and maternal pelvis and the high prevalence of obstructed labor require alternative explanations. It has been proposed that solutions to the OD can be renegotiated (11) through ecologically

pelvis | development | evolution | obstetrical dilemma | sex steroids
mediated phenotypic plasticity of pelvic and fetal dimensions but that rapid changes in environmental conditions may result in fetopelvic mismatch (10,11,23). Obstructed labor thus would be a consequence of a mismatch between maternal and neonatal development (23,24) or of biocultural factors (22) rather than an evolutionary trade-off between obstetrics and locomotion.

On the other hand, indirect evidence for gene-mediated constraints on fetopelvic proportions comes from a recent study demonstrating that mothers with large heads (who, because of the high heritability of cranial dimensions, are likely to have large-headed babies) tend to have obstetrically more favorable pelvic dimensions than mothers with small heads (25). However, correlation between head size and these pelvic dimensions is also present in males (25), although the correlation is less pronounced than in females. Thus the extent to which the observed patterns represent female-specific obstetric selection, sex-neutral genetic–developmental integration, and/or developmental plasticity remains to be clarified.

Somatic sexual dimorphism such as that of the pelvis is largely the result of hormonally regulated sex-biased gene expression (26,27). Previous research on the development of pelvic sexual dimorphism in mammals reveals a wide variety of modes of divergence. Several studies in rodents (28–30) suggest that the pubertal developmental trajectory of the male pelvis deviates from the prepubertal mode shared by both sexes, presumably under testosterone influence. This hypothesis also was proposed for humans and for other primates (6,31). Other studies suggest that estrogen effects are crucial for female pelvic development during puberty (4,32).

Here we reevaluate the evidence for the OD and alternative hypotheses from a developmental perspective. We propose the developmental obstetric dilemma (DOD) hypothesis, which posits that pelvic morphology reflects changing obstetric needs (versus other, possibly locomotor, needs) during a female’s lifetime. Given that female fertility (measured as birth rate per year) reaches its peak around the age of 25–30 y (33,34) and declines toward 40–45 y, the DOD hypothesis predicts that (i) sex-specific differences in human pelvic morphology become pronounced after puberty; (ii) the female pelvis reaches its obstetrically most adequate morphology around the age of highest fertility; (iii) during postmenopausal life, the female pelvis reverts to an obstetrically less adequate morphology, which is probably most adequate for locomotion and other functions; (iv) the male pelvis does not show these developmental changes.

To test the DOD hypothesis, we track pelvic development from late fetal stages to late adulthood in an anonymized known-age and known-sex forensic/clinical sample (n = 275) (Materials and Methods). The bony elements constituting the pelvis fuse relatively late during development, so that the 3D morphology of the pelvis critically depends on the presence of ligaments and other soft tissue structures. Thus computed tomography (CT) was used to analyze pelvic morphology in the context of surrounding tissues. Pelvic size and shape were quantified with a total number of k = 377 3D anatomical landmarks. Sex-specific patterns of shape variation during development were analyzed and visualized with methods of geometric morphometrics (GM) (Materials and Methods).

Results

Fig. 1 graphs sex-specific trajectories of pelvic shape change along the first three principal components (PCs) of shape space and visualizes actual pelvic morphologies at six developmental stages from birth to late adulthood. Fig. 2 graphs the temporal course of pelvic size and shape change. Pelvic growth trajectories (i.e., age-related increase in size) of females and males are largely similar (Fig. 2A). PC1, which accounts for 45% of the total shape variation in the sample, captures a shared male/female mode of shape change (Fig. 2B). It is closely correlated with increase in size (females: r² = 0.91; males: r² = 0.92) and thus represents ontogenetic allometry (i.e., growth-related change in shape). PC2 (accounting for 11% of the total shape variation) and PC3 (accounting for 10%) track the development of sex-specific differences in pelvic shape. Female and male trajectories diverge early during infancy (see PC3 in Figs. 1B and 2D) and exhibit further separation during late childhood (see PC2 in Figs. 1A and 2C), resulting in moderate but significant sexual dimorphism at the onset of puberty (age 10–12 y) (Fig. 2E and Table S1). These findings confirm previous studies on
the early development of sexual dimorphism in pelvic substructures (7, 35–37).

From the age of ~10 y onward the female trajectory changes its direction substantially, whereas the male trajectory continues its earlier course (Figs. 1B and 2D and Table S2). Around the age of 40–45 y, the female trajectory changes again, assuming a direction that is largely parallel to that of the male trajectory (Figs. 1B and 2D and Table S2). Overall, the mean difference between male and female pelvic shapes (i.e., pelvic sexual dimorphism) reaches a peak during early adulthood and is reduced during later adult life (Fig. 2E), as has been observed earlier (38).

Fig. 3 visualizes the corresponding modes of sex-specific change in pelvic shape (for additional visualizations and animations, see Fig. S1 and Movies S1–S6). In males, pelvic development from ~15 y to young adulthood (~25 y) is characterized by a relative reduction of anteroposterior and superoinferior dimensions (Fig. 3A and Movies S1–S3). During this process the superior portion of the sacrum is tilted ventrally, and the greater sciatic notch becomes narrower.

Development of the female pelvis during the same period (~15 to ~25 y) (Fig. 3A and Movies S1–S3) differs substantially from the male mode (Table S2). The sacrum and the ischiopubic region undergo substantial eversion, and the iliac blades undergo inversion. As a result, the anteroposterior dimensions of the pelvic midplane and outlet and the transverse dimensions of the pelvic inlet and outlet become larger (Figs. 3A and 4). Also, the subpubic angle (Fig. 4A) and the angle formed by the greater sciatic notch become wider. As an additional effect, the biacetabular distance becomes relatively wider, and bi-iliac width is relatively reduced (Fig. 4D). Overall, these developmental changes result in a wide, obstetrically favorable birth canal.

It should be noted that the contrasting patterns of male and female pelvic development from puberty to young adulthood visualized here (Fig. 3) were described in part by Coleman (39), who used anteroposterior radiographs and a precursor of GM methods to track pelvic development in a longitudinal sample of the Fels Longitudinal Study begun in 1929. Using the same sample, a multivariate analysis of linear pelvic dimensions yielded similar results (7).

Around the age of 40–45 y, the female pelvis resumes a mode of shape change which is similar to that of males (Figs. 2 and 3B,
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earlier (4), testosterone may be involved in the maintenance of the human male pelvic morphology throughout development but does not lead to developmental divergence during puberty.

Referring to recent hypotheses on ecological and nutritional factors influencing the OD (23), we postulate that the female pelvis is highly sensitive to in vivo modification via environmental modulation of hormone levels. As proposed in the framework of human reproductive ecology (23), and specifically by the predictive adaptive response hypothesis (54), an individual’s developmental trajectory may be modified according to the environmental conditions “expected” (i.e., likely) during its reproductive phase. The relationship between the term fetus and its mother’s pelvic morphology thus might be mediated via estrogen levels, which in turn are sensitive to the current state of ecological parameters relevant for prenatal and postnatal development.

Based on the evidence presented here, the DOD hypothesis predicts that higher levels of estrogen in females during puberty/young adulthood result in development/maintenance of an obstetrically more favorable pelvic morphology, which facilitates the delivery of larger babies. The relationships between sex hormone levels, maternal pelvic morphology, fetal size, and pre/postnatal development are complex and are topics of intense research (10, 11, 23, 55, 56). For example, it has been shown that females who are large at birth have comparatively high estradiol levels during adulthood (57). Estradiol levels also are influenced by diet and nutritional status (58–60) and are good predictors of fertility (61), and, likely, of adult pelvic shape (this study). Given this network of cause and effect, there is ample opportunity for in vivo feedback between ecological/nutritional conditions, sex hormone levels, neonate size, and maternal body and pelvic dimensions. For instance, the observed within-subject correlation of pelvic obstetric dimensions with body size and head size (25) could partly be an effect of higher estradiol levels in larger females (57), resulting in obstetrically more favorable morphologies of their pelves.

Evidence for estradiol-mediated female-specific patterns of pelvic development in humans (this study), nonhuman primates (4), and rodents (32) may indicate either evolutionarily conserved or convergent developmental mechanisms of sexual dimorphism in mammalian species exhibiting obstetric constraints. Because pelvic width does not correlate with locomotor efficacy (52), the question remains why the female pelvis did not evolve and/or does not develop wider obstetric dimensions, which would significantly reduce the existing perinatal risks for the mother and the infant. Pelvic size might be limited by nutritional conditions, which impose global constraints on body growth (11). The high prevalence of obstructed labor thus might largely represent a modern phenomenon resulting from a mismatch between secular increases in neonate size and maternal size. However, additional factors must be advanced to explain both the limited expansion of female pelvic dimensions during pubertal development and the reversal to more constricted dimensions during postmenopausal development. One conspicuous feature of the female expansion/reversal pattern is the widening/shortening of the distance between the ischial spines (Figs. 3 and 4C). The ischial spines are larger in humans than in nonhuman primates, because they constitute important attachment sites for the ligaments and fasciae forming the pelvic floor (62). The spines and associated ligamentous structures substantially constrain the birth canal dimensions, but they provide support for the abdominal and pelvic organs and contribute to sagittal stabilization of the sacrum (62–64). Intraradial hydrostatic pressure reaches high peak values during walking and running (65), and although that pressure positively influences the stability of the lumbar spine, it results in high strains in the pelvic floor (66). Pelvic floor strains thus might represent a limiting factor of birth canal dimensions, and this hypothesis receives support from the observation that wider dimensions correlate with a higher prevalence of pelvic floor disorders (67).

Based on these considerations, we hypothesize that the evolutionary and developmental dilemma of the female pelvis reflects a trade-off between obstetrics and abdominopelvic stability. During a female’s lifetime, the dilemma is alleviated first in one direction, by widening the birth canal during the time of highest fertility, and then in the other, by restricting its dimensions during postmenopausal life. Although our data provide support for the obstetric side of the dilemma, testing its locomotor side will require a shift of focus from bipedal locomotor economy toward locomotion-related abdominopelvic stability. It remains to be clarified whether the female postmenopausal reversal to more constricted birth canal dimensions evolved under selective pressures acting on postproductive life (68) or whether it represents a proximate effect of reduced estrogen levels and developmental plasticity. Also, when during human evolution the developmental mode of the female pelvis started to diverge from the male mode remains to be investigated.

Materials and Methods

The study is based on an anonymized known-age and known-sex forensic clinical sample of nonsymptomatic humans (n = 275) ranging from late fetal stages to late adulthood (Table S4). Data sources are the Collections of the Anthropological Institute, the Vrto偃 database of the Institute of Forensic Medicine of the University of Zurich, Children’s Hospital of Zurich, the Institute of Diagnostic and Interventional Radiology of the University of Zurich, the digital autopsy database of the Catholic University of Leuven, Belgium (KU Leuven), and clinical datasets freely available from the OsiriX web-page (www.osirix-viewer.com).

Volumetric data were acquired with medical CT (beam collimation 128 × 0.6 mm; in-plane pixel size 0.2 × 0.2–0.7 × 0.7 mm², slice increment 0.2–1.0 mm), 3D surface models of the bony pelvis were generated with Avizo 6.3.1 (FEI Visualization Sciences Group), and subsequent mesh cleaning was performed with Geomagic XOS (3D Systems). Only well-preserved pelves were used. Several specimens (n = 9 with ages <8 y, n = 5 with ages 12–15 y, and n = 14 with ages 50–80 y) required minor virtual reconstruction (69, 70).

The shape of the pelvis was quantified with a total number of k = 377 3D anatomical landmarks, which denote locations of biological and/or geometric homology among specimens of the sample. These comprise fixed landmarks (LMs) (k = 63), curve semi-landmarks (SLMs) (k = 90), and surface SLMs (k = 224) (Fig. S2 and Tables S5 and S6). The fixed-LM set comprises 14 LM pairs, which eventually fuse during pelvic development. For geometric morphometric analyses, the mean position was calculated for each pair, resulting in k̄ = 49 fixed LM and a total of k = 363 LM points. Surface SLMs were generated from an arbitrary specimen’s point cloud, and iterative SLM sliding procedures were applied as described in ref. 71. SLM sliding was performed relative to the symmetrized mean configuration, using the minimum bending energy criterion. These data were submitted to generalized Procrustes analysis. All procedures were performed with the R package Morpho, version 2.3.1.1 (72).

Principal component analysis was used to reduce the dimensionality of shape space and visualize major patterns of shape variation in the sample. Sex-specific moving averages of PC scores, centroid size, and angular and linear pelvic dimensions were calculated to explore patterns of morphological change along developmental trajectories. To test for differences between group-specific pelvic shapes (Tables S1 and S3), Procrustes ANOVA was performed using the R package geomorph, version 3.0.0-1 (73). Directions of developmental trajectories through shape space were compared using the methods proposed in ref. 74 (Table S2).

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