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Adrien Levasseur, Typhanie Dumontet, Antoine Martinez

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Review

“Sexual dimorphism in adrenal gland development and tumorigenesis”

Adrien Levasseur, Typhanie Dumontet and Antoine Martinez

Abstract

Sexual dimorphism not only is a matter of gonadal sex or secondary sexual characteristics but also deals with slight differences, the consequences of which may be important because, as we know, the devil is in the detail. Sex determination leads to male or female genetic programming that will influence many biological and/or pathological processes. The adrenal gland is a good example of a nonreproductive sexually dimorphic organ, and most adrenal lesions occur in females. Little is known about the mechanism behind this prevalence, but recent publications suggest the involvement of gonadal hormones and the potentially protective role of androgens. The scarcity of aggressive adrenal lesions has lead researchers to develop animal models able to recapitulate female prevalence to investigate the mechanism hidden behind this dimorphism. Males and females evolve in the same way and give rise to two not so different individuals, and in this review, we will focus on the small differences that could have deleterious effects on adrenal gland physiopathology, with a primary focus on females.

Corresponding authors: Martinez, Antoine (antoine.martinez@uca.fr); Levasseur, Adrien

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Sex determination and differentiation

In mammalian species, after gamete fertilization and depending on the chromosomal content of the zygote, the developing embryo will be genetically a male (heterogametic XY) or female (homogametic XX). Primary sex determination and gonadal differentiation depend on specific patterns of gene expression, leading to the ovaries or testes through the balance between genetic pathways that promote one fate and repress the other [1]. Embryonic life is a complex and dynamic process during which all the organs are formed, and

among them, the gonads will be responsible for sex-specific hormonal production that will define the secondary sex characteristics. From development to adulthood, ovarian estrogens/progestogens and testicular androgens are potent regulators of many physiological functions such as reproduction, behavior, metabolism, and so on. [2]. Although primary and secondary sex characteristics are well acknowledged, more subtle dimorphisms that occur in nearly all organs are often ignored. Differences between females and males can be observed either in physiology or pathology. Indeed, sexual dimorphism as a source of disease has been largely disregarded for nonreproductive organs, and a retrospective analysis of the biomedical literature revealed that the vast majority of studies performed on mice are either analyzing one sex (usually a male) or do not specify which sex has been used [3]. For instance, a recent review showed that the complex interplay between sex chromosome-encoded information and sex hormones can promote gender bias in cancer incidence and mortality, not only by altering the immediate environment of cancer cells or general organ homeostasis but also by directly influencing cancer initiation [4].

Gender bias in adrenocortical tumors

Adrenocortical carcinoma (ACC) is a rare malignant tumor that affects children and adults. Childhood ACC occurs during the first 5 years of life, and a female:male ratio of 1.7:1 is observed in patients younger than 3 years. A smaller second peak of ACC appears during adolescence, and for patients older than 13 years, females are also more affected than males, with a ratio of 6.2:1 [5,6]. In adults, the same gender bias is observed with a ratio of 1.5–2.5:1 in ‘favor’ of females, leading to the hypothesis that sex hormones and/or information encoded by sex chromosomes could be important players in the outcome of ACC [7,8].

Benign adrenocortical tumors responsible for constitutive (ACTH-independent) production of cortisol can result in Cushing syndrome in patients. These tumors originate from either unilateral adrenocortical adenomas (ACAs) or bilateral lesions termed micronodular hyperplasia (primary pigmented nodular adrenocortical disease [PPNAD]) and primary bilateral macronodular adrenal hyperplasia (PBMAH). Cushing syndrome in patients with ACA and PPNAD is frequently the

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consequence of sporadic or inherited mutations leading to constitutive activation of the cAMP/PKA signaling pathway. The most prevalent defects affecting PKA signaling are sporadic activating mutations of the catalytic subunit alpha (*PRKACA*) in ACA and germline inactivating mutations of the regulatory subunit alpha (*PRKARIA*) in PPNAD [9,10]. Cortisol-producing ACAs are more frequent in females than in males, with a 4–8:1 ratio [9]. Female prevalence is also found in PPNAD, which is commonly diagnosed in a familial predisposition syndrome to endocrine tumors, Carney complex (CNC). Follow-up in families with CNC provides insight into the nature of the gender predilection of PPNAD. Despite a sex ratio of 0.5:1 throughout childhood, this ratio changes, and females are more affected than males after 12 years of age, reaching 2–3:1 in adults, whereas sex prevalence tends to fade during menopause [9,11]. Thus, the clinical evidence for PPNAD in females with CNC during sexual maturity suggests that the gender predilection seems influenced by gonadal hormones rather than chromosomal sex in this disease. Interestingly, no sex bias at diagnosis was observed for any other tumoral manifestations of CNC, suggesting that PKA-regulated organ homeostasis should be particularly sensitive to sex hormones in the human adrenal cortex. Although some alterations of PKA signaling activity have been involved in PBMAH, most familial cases relied on mutations of the tumor suppressor ARMC5 [12] and have a female: male ratio of 2–3:1 [9].

PBMAH, PPNAD, and ACC are rare adrenal diseases with a prevalence of only a few individuals per million, which make analysis and sample availability complicated [7,9,11,13]. To further the understanding of the pathological process of adrenal lesions and try to provide mechanistic responses to the sex-biased prevalence, the use of animal models is crucial. Some mouse models recapitulate female-biased prevalence of adrenal lesions such as the Δ Cat (*Akr1b7-Cre::Ctnnb1^{lox(ex3)}*) mouse model in which constitutive activation of β -catenin is restricted to steroidogenic cells within the adrenal cortex [14]. In this model, the uncontrolled activation of β -catenin represents an oncogenic event that may trigger adrenal carcinoma development in aging female mice, but not in males [14]. The DAdKO (*AS^{Crel} +::Prkar1a^{fl/fl}*) mouse model developed by the same team showed the importance of regulation of the PKA signaling pathway in adrenal cortex homeostasis [15]. This study demonstrates clear sex differences in adrenal cortex homeostasis and disease onset/progression. DAdKO females develop ACTH-independent Cushing syndrome by 3 months of age, whereas 7–12 months are necessary for mutant males. The onset of Cushing syndrome and cortex alterations are delayed by

androgens as shown by castration and hormone supplementation experiments in DAdKO males. Furthermore, this study revealed that testicular androgens increase WNT signaling that antagonizes PKA, leading to decrease in the adrenocortical cell turnover that is 3 times slower in males [15]. The DAdKO mice represent a powerful model to address mechanisms of sexual dimorphism in female prevalence of adrenal lesions. However, this prevalence is not obvious in all mouse models. In ACC, overexpression of insulin-like growth factor 2 (IGF2) and constitutive activation of the Wnt/ β -catenin signaling pathway have been involved in the vast majority of sporadic ACCs [8,16]. Two studies using two different approaches to overexpress IGF2 and β -catenin signaling in the adrenal, APC KO–H19^{ADMD} and Δ Cat;AdIgf2, demonstrated that in this context, activation of both signaling pathways results in a mild promoting effect on tumor progression [17,18]. Although females are more prone to develop adrenal tumors in Δ Cat;AdIgf2, no sexual dimorphism has been highlighted in the APC KO–H19^{ADMD} model, contrary of what is observed in humans. Another frequent alteration in patients with ACC is characterized by inactivation of the TP53/RB pathway [8,16]. In a transgenic mouse model of p53/Rb inhibition, which induces metastatic ACC, males and females are both affected, but rapid development of endometrial tumors has hampered the follow-up of adrenal tumor progression in females [19]. Mouse models are very useful tools to study the onset and progression of adrenal lesions, and when female/male phenotypes were compared, most of them recapitulate the sex bias observed in humans.

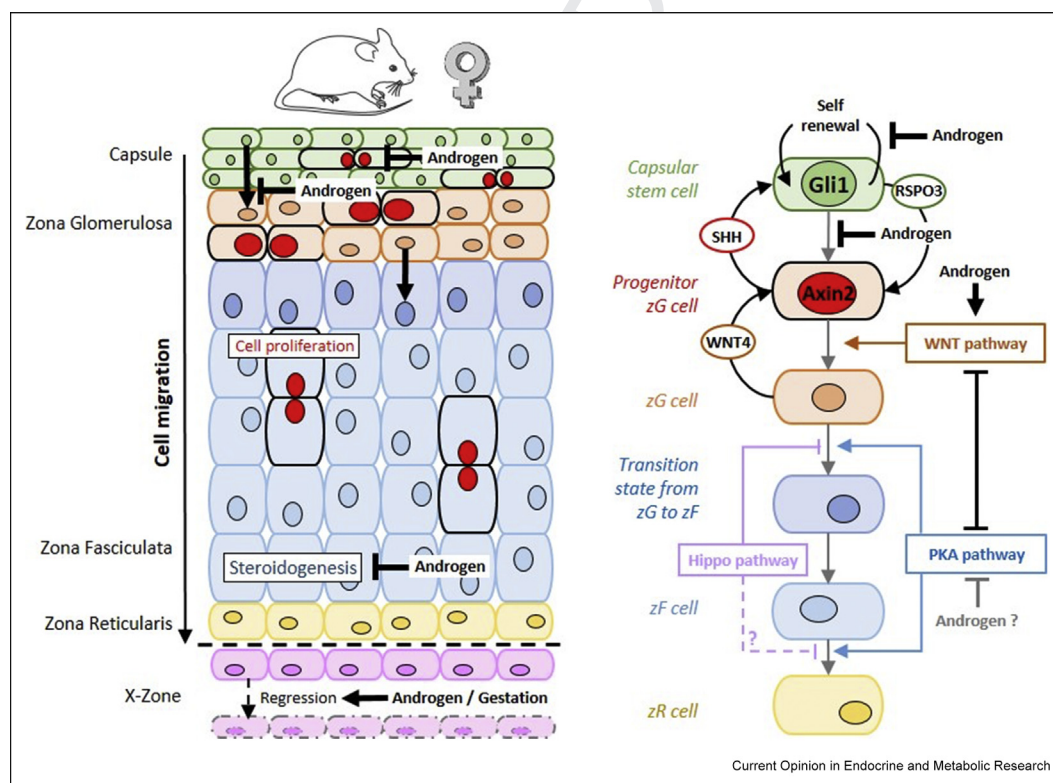
Influence of sex on adrenal cortex morphology and endocrine function

Mouse adrenal glands are sexually dimorphic. Indeed, female adrenal glands displayed a higher weight than those of males, and function (glucocorticoid production) is markedly influenced by gender and age [20]. Accordingly, this dimorphism essentially relies on increased cellularity in the female zona fasciculata. Furthermore, the X-zone, a transient cortical region located at the cortical–medullary boundary, considered a reminiscent fetal zone [21,22], regresses at puberty in males and during the first gestation in females. In a recent review, Huang and Kang [23] listed factors that affect the X-zone clearance, and among them, androgen action remains the most efficient inducer of regression, although participation of other sex steroids cannot be excluded. In 2013, a transcriptomic analysis of adrenal sexual dimorphism in mice was performed to address if there was a specific signature in gene expression and to investigate the effects of androgen privation in males (by castration) or supplementation in females (by testosterone

administration) [24]. Hierarchical clustering revealed 269 genes differentially expressed in males vs females, 71 of which were regulated by changes in the androgenic milieu, suggesting that in addition to male sex hormones, other potential factors including sex chromosome-encoded information could explain those differences. In coherence with these early studies, gene expression data obtained from wild-type and mutant mice developing Cushing's syndrome confirmed that samples from castrated males clustered close to female samples. This indicates that in mice, androgens should be the main determinant of adrenal sex dimorphism in both physiology and disease [15]. The time points of sexual dimorphism in gene expression can be seen until embryonic day 14.5 for *Cyp17*, which is weakly expressed in males and intensively in females. By embryonic day 18.5, *Cyp17* is no longer expressed in male adrenals but is still highly expressed in those of females [25]. The role of this early dimorphic expression is not well

understood because *Cyp17* expression is lost in adult adrenals. *Nr0b1/Dax1*, which encodes important players of adrenogonadal development and function, has sexually dimorphic expression from postnatal day 14 to adulthood in mouse adrenals [26]. *Nr0b1* is highly expressed in female adrenals and shows androgen-dependent down-regulation in those of males [24,26]. Owing to its role in steroidogenesis, dimorphic expression of *Nr0b1* was proposed to participate in sex differences in steroid hormone production [27]. In other rodent models, sexual dimorphism may not have the same expression. Rats are similar to mice, having larger female adrenal glands [28,29], whereas hamsters have the opposite [30]. The adrenal gland is highly sensitive to stress and will produce glucocorticoids after ACTH stimulation. Adrenal responsiveness to ACTH is sexually dimorphic in rats [31] and mice, and the better responsiveness in females relies on sexually dimorphic function of the adrenal molecular clock that may be lost under an androgenic environment

Figure 1



Adrenal cortex renewal in female mice and interplay with androgens (adapted from Dumontet et al [15] and Grabek et al [48]). Scheme of female adrenal cortex cell renewal dynamics: *Gli1*+ stem cells and *Axin2*+/*Shh*+ progenitor cells residing within the capsule and zG, respectively, that can either proliferate or be recruited to ensure cell renewal of the definitive/adult cortex. Centripetal migration and zonal differentiation result from the antagonistic interplay between WNT and PKA signaling pathways, with the WNT pathway promoting zG identity, whereas the PKA pathway stimulates cell turnover and favors conversion to zF identity and eventually to zR identity. In the zF, the Hippo pathway exerts a repressive role in zF differentiation/maintenance and therefore potentially in that of the zR. The X-zone regresses during the mid-gestation in females and at puberty in males. The androgenic milieu tones down cortex replenishment by yet unclear mechanisms. Indeed, testicular androgens impede capsular stem cell recruitment and proliferation that only occur in females or prepubescent males. Moreover, androgens sustain the WNT pathway, which restrains the PKA pathway, leading to lower cortical cell renewal in males than in females. Steroidogenic cells in the zF could also be negatively affected by androgens, resulting in lower steroidogenic activity in males than in females.

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[32]. In humans, few studies have investigated the adrenal gland size in adults. Stein et al. [33] revealed that in the Canadian population, adrenal weight (around 15 gg) was similar in both genders, whereas a study on the Chinese population showed a statistical difference ($p < 0.001$) in adrenal weight between men (12.4 g) and women (10.7 g) [34]. Finally, Ludescher et al. [35] reported a bigger adrenal absolute volume in men (9.84 ml vs 4.91 ml), but after normalizing to body volume, this difference was lost. Concerning adrenarche, which refers to the onset of increased production of adrenal androgens during childhood, adrenarche in girls occurs earlier than that in boys [36]. However, this dimorphism in adrenarche dynamic setup might be explained by sex-dependent differences in peripheral androgen metabolism. Concerning adrenal androgen production, in prepubescent children, no significant sex differences in DHEAS or androstenedione were found. Differences appear with age where adult males show a higher level of DHEAS than females from the age of 17 to 69 years [37].

Mechanisms influencing adrenal sexual dimorphism

Adrenal sex dimorphism seems to rely on a complex interplay between adrenal homeostatic maintenance and responsiveness to gonadal steroids. Steroid hormone receptors, including androgen receptors (ARs), estrogen receptors (α/β), are expressed in both mouse and human adrenals, suggesting their participation in the gland's function [38–40]. Besides, adult cortex homeostasis is ensured by tissue renewal from capsular subcapsular progenitor cells (expressing markers *GLI1* and *SHH*) that undergo centripetal migration and successive conversion into the different zonal cell types to replenish the cortex [41–43].

Evidence for androgenic control in mouse cortex homeostasis was first experienced for the transient cortex using male castration and/or female androgen administration. Indeed, the male X-zone regresses at puberty, and castration leads to development of a secondary X-zone [44]. The keen interest to prove participation of the AR in the adrenal gland is on the way, as illustrated by preliminary results presented during scientific meetings. Two different teams developed mouse models with AR inactivation in the adrenal cortex. These models would definitively prove that the AR is essential for regression of the X-zone and for limiting the adult cortex size in males, the loss of the AR being sufficient to abolish any sexual dimorphism [45,46].


Various mechanisms downstream to AR signaling should be involved in the gender-specific regulation of

definitive cortex homeostasis (Figure 1). The low androgenic milieu found in females was shown to favor PKA-prone cortex cell renewal, whereas in males, androgens limit this action by sustaining the RSPO/Wnt/ β -catenin pathway [15]. Disruption of the Hippo signaling pathway in adrenocortical cells leads to progressive atrophy of the adrenal cortex (affecting mainly the zona fasciculata) only in male mutant mice [47]. One part of the explanation to this phenotype could be provided by a recent study combining the lineage-tracing experiment of adrenal stem cells with gonadectomy and DHT treatments. This study revealed that after puberty, sex-specific stem cell activity is driven by male hormones that repress *Glil*-positive stem cells from the capsule and cell proliferation, leaving only one progenitor compartment (subcapsular *Shh*+ cells) available for tissue renewal [48]. In contrast, females can recruit within two different populations of stem cells situated in the capsular and steroidogenic compartments (*Glil*+ and *Shh*+), leading to a 3-fold higher turnover than males. These data show a higher potential for cortical cell renewal in female mouse adrenals attested by 2 complementary genetic models (15, 48), which may help explain the overrepresentation of adrenocortical diseases/tumors in women.

Conclusion, issues, and perspectives

Mice and humans are different, but animal models are a powerful tool to thoroughly study biological processes that cannot be dissected finely in humans because of ethics, diseases rareness, timing onset of the disease, and so on. Nevertheless, mouse models can not only recapitulate observations made in clinics regarding adrenal lesions but also provide insight into adrenal physiology [49,50]. The challenge in research now is to decipher how the identified mechanisms are interconnected with androgenic signaling and whether these rules are applicable to human adrenals. Furthermore, sexual dimorphism not only is limited to regulation of cell turnover but also may influence metabolic activities dealing with cholesterol and steroid synthetic pathways [15]. Grabek et al. [48] made a great demonstration that cell proliferation and recruitment in the adrenal cortex is more active in females and regulated by androgens, and as the authors said, “Future research will need to focus on how these differences translate into sex-specific diseases, which, in the long run, may pave the way for development of sex-specific treatment options.” Sexual dimorphism does not stand only for reproductive organs; taking into account both sexes individually in future experiments will improve our general understanding on sexual dimorphism in every organ, and do not let females apart in studies because their hormones could be considered as ‘a problem’ [51].

Conflict of interest statement

 Nothing declared.

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