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Human Adrenal Cortex: Epigenetics and Postnatal Functional Zonation

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Abstract
The human adrenal cortex, involved in adaptive responses to stress, fluid homeostasis, and secondary sexual characteristics, arises from a tightly regulated development of a zone and cell type-specific secretory pattern. However, the molecular mechanisms governing adrenal zonation, particularly postnatal zona reticularis development, which produce adrenal androgens in a lifetime-specific manner, remain poorly understood. Epigenetic events, including DNA and histone modifications as well as regulation by noncoding RNAs, are crucial in establishing or maintaining the expression pattern of specific genes and thus contribute to the stability of a specific differentiation state. Emerging evidence points to epigenetics as another regulatory layer that could contribute to establishing the adrenal zone-specific pattern of enzyme expression. Here, we outline the developmental milestones of the human adrenal cortex, focusing on current advances and understanding of epigenetic regulation of postnatal functional zonation. Numerous questions remain to be addressed emphasizing the need for additional investigations to elucidate the role of epigenetics in the human adrenal gland. Ultimately, improved understanding of the epigenetic factors involved in adrenal development and function could lead to novel therapeutic interventions.

Introduction

The human adrenal cortex emerges from a tightly regulated development of a zone and cell type-specific secretory pattern. The developmental program that gives rise to the adrenal gland begins early during embryogenesis and continues into adult life [1]. The adrenogonadal primordium is first distinguished at 4 weeks post-conception (wpc) in the form of a group of cells that expresses the essential transcription factor SF-1. The subset of cells with higher SF-1 expression migrate dorsomedially to develop into adrenal primordium [1]. By approximately 7 wpc, the developing adrenal becomes encapsulated with the formation of a fibrous layer over the developing cortical cells. By the 8th week of gestation, the human fetal

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adrenal consists of the inner fetal zone, the outer capsule, and the newly emerging definitive zone between them that later develops into the adult cortex [1, 2].

The ontogeny of expression of steroidogenic enzymes determines the zonal differential steroidogenic activity and its onset. The fetal zone expresses 17α-hydroxylase/17,20-lyase, P450c17 *(CYP17A1)* and cytochrome b5 (*CYB5A*) and produces dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) used by the placenta as estrogen precursors, essential for the maintenance of pregnancy [2]. During weeks 9–10 of gestation, transient adrenal expression of 3β-hydroxysteroid dehydrogenase/Δ4–5 isomerase, 3βHSD2 *(HSD3B2)* enables cortisol production [3]. It is presumed that this transient suppression of the hypothalamic-pituitary-adrenal axis inhibits adrenal androgen secretion to prevent virilization of the female fetus [3]. After 23–24 wpc, cortisol synthesis starts again from the transitional zone, a third cortical zone between the definitive zone and the fetal zone [2, 3]. After birth, a strong remodeling of the adrenal gland occurs: the medullary islands coalesce to form the medulla, the fetal zone regresses by the 3rd postnatal month and the definitive and transition zones develop and differentiate through the process of zonation into the adult adrenal [2]. These morphological changes are accompanied by a rapid drop in DHEA and DHEAS production due to the involution of the fetal zone. In preadrenarche children, the zona glomerulosa (ZG) and the zona fasciculata (ZF) are clearly present but only focal islands of reticularis cells, insufficient to influence serum DHEAS levels, can be identified. Adrenal production of DHEA and DHEAS resumes at adrenarche (ages 6–8 years) when a continuous layer of reticularis cells develops and thickens forming the zona reticularis (ZR) [1, 4–6]. Peak levels of DHEA and DHEAS occur between ages 20 and 25 and decline thereafter. There appears to be a reduction in the width of the ZR with aging (adrenopause), without overall changes in the width of the adrenal cortex. The origin and maintenance of the adrenocortical zones have been debated over the years. Nevertheless, evidence from animal studies favors the progenitor cell proliferation/migration hypothesis [7]. This theory proposes that the cortical cells originate from a stem cell population in the outer cortex or capsule, and migrate centripetally becoming functionally differentiated in the appropriate zonal environment [1, 5, 7]. In agreement with the migration model, the cell proliferation index is highest in the outer ZG of human adrenal tissues from early infancy to late puberty [8]. Genes encoding a number of transcription and growth factors as well as polypeptide hormones have been linked to adrenocortical cell development and to modulation of steroidogenic function [6, 8–11]. However, the exact molecular mechanisms governing human adrenal zonation and ZR development remain poorly understood.

It is now clear that, in addition to genetic factors, non-DNA sequence modifications in the genome, collectively referred to as epigenetics, are key players in human development. Epigenetic events, including modifications of DNA by DNA methylation and modifications of the histone proteins by acetylation, methylation, ubiquitination, SUMOylation, and phosphorylation, as well as regulation of gene expression mediated by small noncoding RNAs (sncRNA), are crucial in establishing the correct pattern of gene expression. They can potentially cause long-lasting changes in how the genome is read by transcription factors (TFs) and therefore, consolidate cellular differentiation and allow for cell-type specific, stable functioning [12]. The goal of this review is to provide an overview of current advances and understandings of epigenetics in the regulation of human adrenal gland development and functional zonation.

Functional Zonation of the Human Adrenal Cortex

In the adult adrenal, the outer cortex differentiates through the process of zonation into three functionally and morphologically distinct zones: ZG, ZF, and ZR. These zones are responsible for the production of aldosterone, cortisol, and DHEA/DHEAS, respectively. The cleavage of cholesterol to pregnenolone is the first, rate-limiting and hormonally regulated step in the biosynthesis of steroid hormones common to all steroidogenic cells [13, 14]. The cholesterol side chain cleavage enzyme, P450scc *(CYP11A1)*, supported by its electron transport system consisting of NADPH, adrenodoxin reductase (*FDXR*), and adrenodoxin (*FDX1*), catalyzes the 20α-hydroxylation, 22-hydroxylation, and cleavage of the 20–22 carbon bond of cholesterol to yield pregnenolone in the inner mitochondrial membrane [13]. The steroid acute regulatory protein, as part of a multiprotein complex, facilitates the movement of cholesterol from the outer to the inner mitochondrial membrane, thus providing the substrate for steroid hormone biosynthesis [13, 15]. The specific repertoire of enzymes distal to P450scc in a cell determines the fate of pregnenolone metabolism and defines the function of that cell [13].

The coexpression of 3βHSD2 and aldosterone synthase, P450c11AS *(CYP11B2)* in the ZG leads to aldoste-

rone production regulated by the renin/angiotensin system. The ZF does not express angiotensin II receptors or P450c11AS, but instead expresses the ACTH receptor MC2R and 11β-hydroxylase, P450c11 *(CYP11B1)*. The coexpression of 3βHSD2 and P450c17 along with P450c11 in the ZF results in the production of cortisol under the influence of ACTH. By contrast, the ZR expresses relatively very little 3βHSD2 and large amounts of P450c17 and cytochrome b5, which selectively activate the 17,20-lyase activity of P450c17 and DHEA-sulfotransferase *(SULT2A1)* [6, 13]. Consequently, the C19 steroid DHEA is produced, much of which is sulfated to DHEAS [6, 13]. This Δ^5 pathway is the preferred route to C19 steroid production in humans as the 17,20-lyase activity of human P450c17 does not efficiently convert 17α-hydroxyprogesterone (17OHP) to androstenedione (A4) [6, 13]. Androgen production in the human adrenal cortex is zonally and developmentally regulated. The 3βHSD2 deficient ZR is indistinct during infancy but a continuous layer of reticularis cells starts to develop and thicken around 4–5 years [4, 5]. This process, known as adrenarche, is followed by a rise in circulating concentrations of DHEAS, with clinical signs physiologically observed between the ages of 6 and 10 years [4, 5, 16]. The human adrenal gland also makes little A4 and testosterone (T). It has been proposed that A4 is produced from DHEA in a layer of cells in the interface of the ZF and ZR which expresses P450c17, cytochrome b5, and 3βHSD2 [17]. The expression of aldo-keto reductase 1C3 *(AKR1C3)*, also known as 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5), in the ZR would be responsible for the conversion of DHEA and A4 to androstenediol and T, respectively [18].

The advent of comprehensive analyses of steroid profiles by liquid chromatography and gas chromatography-tandem mass spectrometry has revealed alternative adrenal androgen synthesis pathways [19–24]. 17OHP is also a substrate for an alternative, so-called backdoor pathway to androgen synthesis, which generates 5α-dihydrotestosterone (DHT) without the intermediacy of DHEA, A4, or T [13]. This pathway depends on the 5α and 3α reduction of 17OHP to produce 17-hydroxyallopregnenolone, followed by 17,20-lyase activity of P450c17 and 17βHSD dehydrogenase activity, yielding androstanediol, and finally a 3α oxidation step to DHT [13]. In contrast to the classical pathway, human P450c17 has a very high affinity for the backdoor pathway intermediate, 17-hydroxyallopregnenolone, which is an excellent substrate for its 17,20-lyase activity, not dependent on cytochrome b5, leading to androsterone production [13]. Recent evidence has shown that the human adrenal cortex would express the enzymes to complete all the steps in the backdoor pathway to DHT [25–27] and that this pathway would contribute to the androgen production in pathological states in which 17OHP accumulates [21, 22, 24]. Moreover, recent studies have demonstrated that the human adrenal cortex also produces a unique set of 11-oxygenated C19 steroids [19, 20, 23]. The first step of the 11-oxygenated androgen pathway is dependent on the adrenal *CYP11B1*-catalyzed 11β-hydroxylation of A4 to 11β-hydroxyandrostenedione, which is a major product of adrenal steroidogenesis [19, 20]. The human adrenal cortex also produces small amounts of the 11-oxygenated C19 steroids including 11-ketoandrostenedione, 11β-hydroxytestosterone, and the potent androgen 11-ketotestosterone [19, 20].

Even though the biochemical pathways leading to the formation of adrenal steroids have been elucidated, the primary signal(s) that drive postnatal zonation of the human adrenal cortex remain(s) unknown.

Mechanisms of Epigenetic Regulation

Epigenetics refers to heritable regulation of gene expression that is not encoded in the underlying DNA sequence. The main mechanisms responsible for mediating epigenetic effects are: (a) DNA methylation, (b) histone modifications, and (c) RNA-based mechanisms, such as small noncoding RNAs or inhibitory RNAs [12, 28]. Epigenetic regulation is a complex phenomenon that consists of a variety of different processes such as gene silencing, X-chromosome inactivation, and imprinting [28, 29]. Genomic imprinting is a complex form of epigenetic inheritance that plays a key role in maintaining normal embryogenesis, and prenatal and postnatal growth. Perturbations in parental epigenetic asymmetry can lead to the development of known malformation disorders, such as IMAGE and Beckwith-Wiedemann syndromes.

DNA Methylation

DNA methylation involves the transfer of a methyl group to the C5 position of cytosines within CpG dinucleotides by DNA methyltransferases. CpG dinucleotides tend to cluster as CpG islands. CpG island methylation is associated with transcriptional repression [30], especially when these methylated sites involve promoter or other gene regulatory regions, such as imprinting control regions. Many imprinting control regions contain differentially methylated regions that direct parent-specific regu-

lation of imprinted clusters of genes in a chromosomal region [29]. Hypermethylation leads to the binding of methyl-CpG-binding domain proteins, transcription repressors, and/or histone deacetylases, which alter the chromatin structure to form a co-repressor complex, thereby repressing gene transcription [30]. Otherwise, methylation-free DNA allows binding of TFs and is transcriptionally active. DNA methylation may, however, be activating if it prevents binding or limits expression of transcriptional repressors. 5-Hydroxymethylation of 5-methyl cytosines through TET family proteins leads to CpG de-methylation and thereby re-expression of genes silenced by DNA methylation [31].

Histone Modifications

Posttranslational modifications such as acetylation, methylation, phosphorylation, deamination, β-N-acetylglucosamine, ADP ribosylation, ubiquitination, and SUMOylation occur at specific residues in histones N-terminal tails. These modifications can change the charge of histones and affect the structure of chromatin to upregulate or downregulate gene expression. For example, the acetylated state of histone neutralizes its positive charges, hence facilitating chromatin relaxation and increasing the accessibility of TFs to their target genes [32]. Specific enzymes that include histone acetyltransferases, histone deacetylases, and histone methyltransferases modify histones. Specific combinations of histone modifications occurring on the same histone tail or on another tail confer the overall expression status of a DNA region [33].

Small Noncoding RNA

The majority of the human genome (≈95% in the human) is transcribed and processed into large or sncRNAs. These sncRNAs, consisting of 17–250 nucleotides in length, are associated with diverse effector complexes to affect gene expression at either transcriptional or post-transcriptional levels. Many sncRNA species have been identified, including miRNAs, piwi-interacting RNAs, small nuclear RNAs, endogenous small interfering RNAs, and small nucleolar RNAs [34]. Of these, miRNAs have been the most extensively studied. miRNA genes are clustered and located within intergenic regions of the genome or in introns or exons of protein-coding genes. RNA polymerase II/III transcribes individual miRNA genes, generating long primary transcripts (pri-miRNAs). These pri-miRNAs are processed in the nucleus by the RNase III-type enzyme Drosha, yielding hairpin precursors (pre-miRNA). After being actively exported to the cytoplasm, the pre-miRNA hairpins are further processed into 19–25 nt miRNA duplex structures by the RNase III protein Dicer [35]. The miRNA duplex is unwound into the mature single-strand form and incorporated into a multiple-protein nuclease complex, the RNA-induced silencing complex, which guides the complex to bind a complementary sequence within the 3′-untranslated region of the target mRNA. Depending on the extent of the miRNA-mRNA pairing, binding of the miRNA to its target mRNA induces negative regulation of gene expression through mRNA cleavage and/or translational repression. Short and/or imperfect base pairing favors translational repression [36].

Evidence for the Role of Epigenetics in Human Adrenal Cortex Physiology

The human *CYP11B2* and *CYP11B1* gene promoters possess a number of target sites for DNA methylation that are closely associated with gene silencing [37–40]. The *CYP11B1* gene promoter was found to be predominantly unmethylated in ZF while completely methylated in both ZG and ZR. In contrast, the *CYP11B2* gene promoter is hypomethylated in ZG compared with that in ZF and ZR, suggesting that DNA methylation patterns exert adrenal zone-specific expression patterns of CYP11B2 and CYP11B1 enzymes [39].

Typically, CpG islands are associated with regions that are involved in transcriptional regulation [30]. The absence of a CpG island in the human *CYP17A1* [41] and *HSD3B2* [42] genes suggests that direct epigenetic regulation of the *CYP17A1* and *HSD3B2* promoters is not essential. In humans, expression of *CYP17A1* is driven by a complex interaction of TFs [43]. Indirect evidence of epigenetic regulation of human *CYP17A1* is tied to the induction of the GATA-4 and GATA-6 TFs by the histone methyltransferase inhibitor 5-aza-2′-deoxycytidine (5-aza-dC) in human adrenocortical NCI-H295A cells [44, 45]. These TFs were shown to be required for *CYP17A1* expression.

Experiments using 5-aza-dC to alter DNA methylation patterns in different steroidogenic cell lines provided some conflicting data regarding *HSD3B2* expression [46, 47]. Adrenarche is the consequence of a process of postnatal organogenesis in which a new zone of the adrenal cortex, the ZR, develops [1, 4, 5]. A major characteristic of steroidogenesis at adrenarche is a specific ZR downregulation of *HSD3B2* expression [4, 5]. The orphan nuclear receptor NR4A1 (nuclear receptor subfamily 4

Table 1. Overview of the available evidence on epigenetic regulation of steroidogenic enzymes, transcription factors, growth factors and nuclear receptors related to human adrenal zonation

Gene	Epigenetic mechanism			Tissue/cell	Reference
	DNA methylation	miRNA	histone modification		
Steroidogenic enzymes					
CYP11B1	yes	miR-24, miR-125a-5p, miR-125b-5p	unknown	HAT, aldosteronomas, H295R	37, 39, 51, 52
CYP11B2	yes	miR-24	unknown	HAT, CPA, H295R	38, 39, 51
CYP21A1	unknown	yes	unknown	H295R	52
CYP17A1	no	miR-320a-3p	unknown	H295R	41, 44, 52
HSD3B2	no	unknown	unknown	HAT	42
Transcription factors					
NR4A1	no	unknown	histone H3 acetylation	HAT, human leucocytes	42
GATA4	yes	unknown	unknown	H295R	44
GATA6	yes	unknown	unknown	H295R	44
Tyrosine quinase receptors					
IGF1R	unknown	miR-99a, miR-100	unknown	childhood ACT	68
Nuclear receptors					
ESR2	yes	unknown	unknown	human ovarian cell lines	57

HAT, normal human adrenal tissue; CAP, cortisol-producing adenoma; H295R, human adrenocortical carcinoma NCI H295R cell line.

group A member 1, also known as NGFIB or Nur77) has emerged as an essential transcription factor for human *HSD3B2* expression [10, 48]. Within the adult and fetal adrenal cortex, *NR4A1* expression is also zone-specific, paralleling the expression of *HSD3B2* [10]. However, the mechanisms regulating this zonal expression pattern are unknown. A recent study has shown that the *NR4A1* promoter was virtually completely unmethylated with no developmental changes in methylation status in human adrenal tissues from early infancy to late puberty [42]. Moreover, similar unmethylated profiles in laser-microdissected ZR and ZF cells were observed, suggesting that the regulation of human adrenal *HSD3B2* would not be tied to a DNA methylation-mediated ZR-specific downregulation of *NR4A1* [42]. A recent study showed that human adrenal corticocarcinoma NCI-H295R cells grown under starvation conditions acquire a hyperandrogenic steroid profile by repressing *HSD3B2* and *RARB* mRNA expression [49]. The authors also provided evidence that RARB regulated *HSD3B2* transcription in cooperation with NR4A1 [49]. However, no ZR-specific decrease in RARB mRNA levels at the age when adrenarche occurs was observed [42], suggesting that this experimental starved tumor cell model would not be a good model for understanding adrenal functional zonation during human development. Further studies are required to evaluate if epigenetic mechanisms other than DNA methylation are associated with human adrenal cell-specific transcript regulation underlying developmental androgen production. Furthermore, it might be interesting to consider a role of ncRNAs in the regulation of adrenal androgen production.

Recent studies have suggested a role for miRNAs in adrenal development and function. The end product of the renin-angiotensin system, angiotensin II, upregulates the expression of miR-21 in human adrenocortical H295R cells. miR-21 overexpression in H295R cells resulted in increased aldosterone secretion and proliferation, suggesting that this miRNA can downregulate the expression of genes responsible for inhibiting aldosterone secretion and cell proliferation [50]. Microarray miRNA profiling in normal human adrenal followed by bioinformatic analysis identified many miRNA-binding sites in the 3′ untranslated region of *CYP11B1*, *CYP11B2*, *CYP21A1*, and *CYP17A1* mRNAs involved in the corticosteroidogenic pathway [51, 52]. miR-24 has been shown to direct-

ly target *CYP11B1* and *CYP11B2* leading to a decrease in aldosterone and cortisol production [51]. Manipulation of individual miRNA levels in human adrenocortical H295R cells demonstrated a direct effect of miR-125a-5p and miR-125b-5p on *CYP11B2* and of miR-320a-3p on *CYP11A1* and *CYP17A1* mRNAs [52].

Relevant data was also obtained from studies carried out in animal models. Recent studies involving adrenocortical-specific Dicer knockout mice have shown that Dicer is required for normal mouse adrenal cortex development and suggested a role for miRNA-mediated regulation of a subset of essential genes for normal adrenal organogenesis and homeostasis [53, 54]. Hu et al. [55] have demonstrated that hormones can regulate miRNAs in rat adrenal glands. Chronic ACTH treatment in vivo altered the levels of many miRNAs. Significant differences were observed in the expression levels of 163 miRNAs between control adrenals and adrenals from estradiol-treated rats. Dexamethasone treatment caused changes in miRNA levels as well [55]. Thus, miRNAs may be involved in the posttranscriptional/posttranslational regulation of steroidogenesis.

Table 1 summarizes current knowledge on the role of epigenetics in regulating the expression of steroidogenic enzymes, TFs, and nuclear receptors related to human adrenal cortex development and functional zonation.

Perspectives, Future Directions and Challenges

The adrenal cortex is a highly dynamic organ. In addition to the establishment of zonation, there is constant centripetal migration of cells under normal conditions and the cortex rapidly responds to requirements of hormonal production by altering the relative sizes of the zones (remodeling). Based on current knowledge, it is conceivable to propose that epigenetic mechanisms might provide the coordination and transcriptional plasticity in the control of zonation and remodeling. However, many questions remain to be answered underscoring the need for additional work on this subject.

Zonation and constant remodeling of the adrenocortical zones require the precise control of progenitor cell proliferation in the periphery, as well as the subsequent migration and differentiation into the appropriate zone-specific steroidogenic cell [1] (Fig. 1). It is reasonable to hypothesize the existence of epigenomic marks that are laid down in the progenitor cells or as positional cues during centripetal migration to give rise to the different cell types and their expression patterns. A role for estrogens in ZR functional differentiation through ZR-specific expression of estrogen receptor β (ERβ) has been suggested [8, 56]. DNA methylation has been shown to control *ERβ* gene expression in a human ovarian cell line [57]. However, the contribution of DNA methylation to *ERβ* zone-specific expression in the human adrenal cortex has not been analyzed. Furthermore, estrogens regulate miRNA transcription through ERα and β in a tissue-specific and cell-dependent manner [58]. To consider a role for miRNAs in adrenal functional zonation, more information regarding the regulation of their own transcription is required.

Epigenetic factors that lead to different phenotypes in nonclassic 21OHD patients with identical genotype have been suggested [59]. Intra-adrenal backdoor pathway metabolites might have physiological significance as paracrine/autocrine regulators of adrenocortical function through inhibition of P450 CYP17A1 [60]. However, the mechanisms that regulate backdoor pathway enzyme expression are unknown.

Epidemiological studies support the hypothesis that malnutrition in prenatal life leads to metabolic programming of the fetal organs with subsequent development of insulin resistance, premature and/or exaggerated adrenarche and increased risk of metabolic syndrome in adulthood and polycystic ovary syndrome in women [61]. Evidence has recently emerged suggesting that epigenetic changes may occur as a consequence of developmental programming and result in permanent changes in the expression patterns of particular genes. Such epigenetic modifications may be responsible for an increased susceptibility to disease. Hyperinsulinism has been suggested as the origin of premature adrenarche [62]. Further research in this field, particularly as to whether epigenetic changes to genes involved in insulin resistance and ZR development occur, is essential to understand the underlying mechanisms of developmental programming of disease.

Adrenocortical tumors (ACT) in children possess several distinct pathological features compared to ACT in adults. The expression of *HSD3B2* and *NR4A1* is markedly lower in ACT that arise in young children than in normal adrenal cortex, supporting the hypothesis that the tumors originate from deregulation of either the fetal zone during embryogenesis or the developing ZR during the first few years of life [63]. Consistently, more than 90% of pediatric patients present with a hyperandrogenic steroid profile with virilizing features [64]. Over the last few years, several studies have shown that ACT are frequently associated with aberrant DNA methylation and

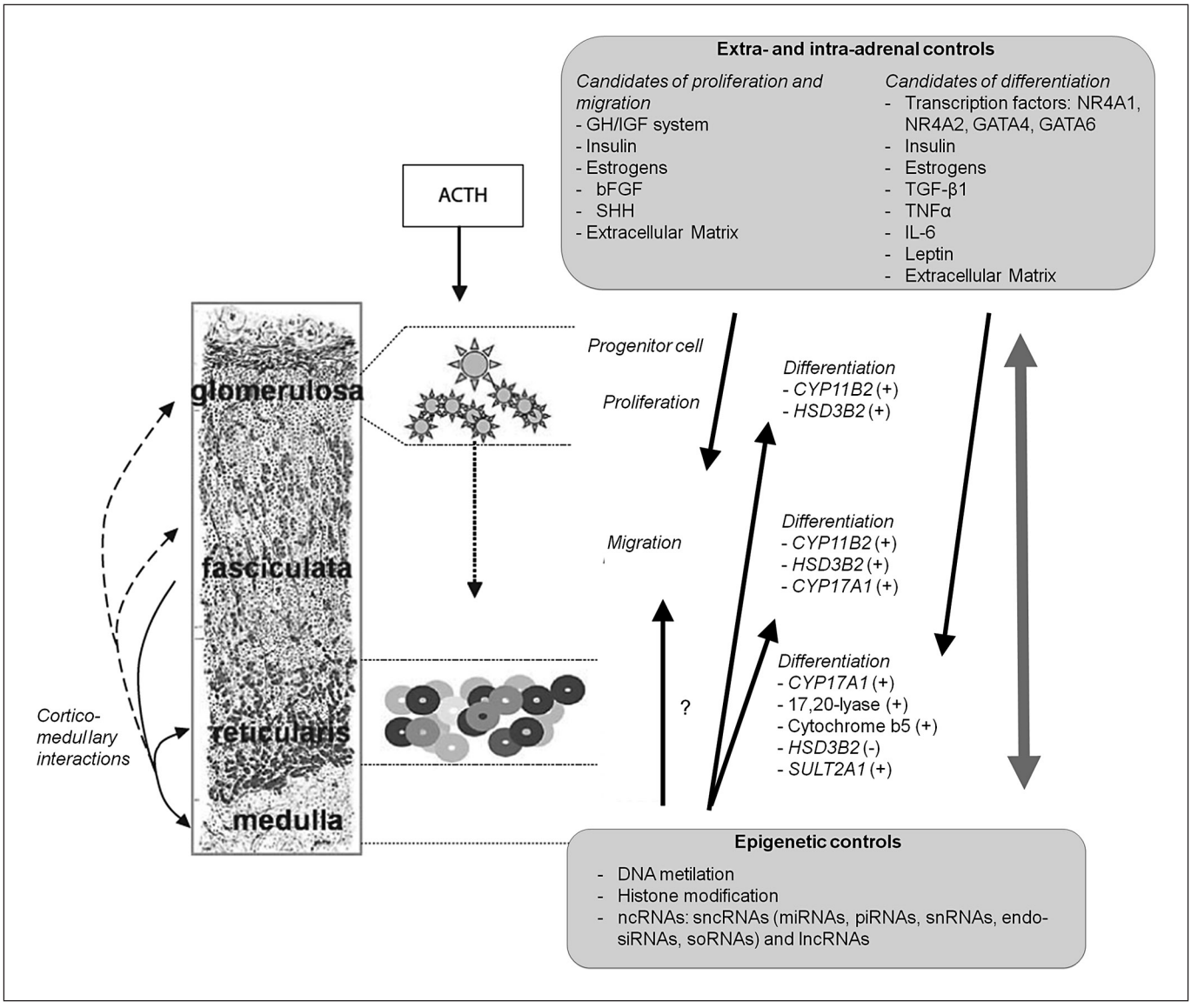

Fig. 1. Model for growth and differentiation (zonation) of the adult adrenal gland. The scheme depicts the progenitor cell differentiation/migration and its putative regulation. Under the permissive action of ACTH, progenitor cell proliferation in the periphery, as well as subsequent migration toward the center of the gland, might be stimulated by several factors. In each zone, cell type arises from a carefully orchestrated enzyme expression pattern that produces an exclusive specialized cell phenotype. Several extra- and intra-adrenal factors, as well as transcription factors were suggested to be involved in cell type-specific differentiation. Emerging evidence suggest that epigenetic regulation may contribute in establishing the zone-specific pattern of enzyme expression. It is reasonable to hypothesize the existence of epigenomic marks that are laid down in the progenitor cells or as positional cues during centripetal migration to give rise to the different cell types and their expression patterns. Besides, there is a reciprocal interplay between hormones and transcription factors and epigenetic regulation.

deregulation of a specific set of miRNAs [65–68]. However, most studies were performed in adults and results have not been analyzed based on the hormonal secretion profile of the tumor. Similar unmethylated CpG-rich NR4A1 promoter profiles were evidenced in both virilizing childhood ACT and normal adrenal tissues, indicating that promoter methylation could not account for the downregulation of *NR4A1* mRNA expression in hyperandrogenic childhood ACT [42]. Pediatric ACT seem to produce excess androgens through the classic and/or the

alternative backdoor pathways [27]. Pathways responsible for tissue and cell development and the pathology of cancer often coincide. In this regard, CYP11B2-regulating miRNAs and *CYP11B2* promoter methylation are deregulated in aldosterone producing adenoma tissues [38, 40, 51, 52]. However, no information regarding epigenetic changes in childhood ACT and their association with the steroid profile is available.

miR-99a and miR-100 were found to be highly downregulated in childhood ACT compared to normal adrenal cortex. Functional analysis showed that they play an important role in growth signaling through the regulation of *IGF-1R* expression by 3′UTR-binding [68]. We have proposed a role of IGFs in the postnatal mechanism of human progenitor adrenal cell proliferation and migration [8, 69]. However, a role of miRNAs in cell type-specific *IGF-1R* expression in normal human adrenal cortex has not been determined.

Given that epigenome, adrenal zonation, and steroid biosynthesis are highly species specific, a major challenge is that the use of common small animal models is not appropriate. Studies are limited to human tissues. The relevance of in vitro studies using cell culture is debatable, especially in the epigenetic field, as microenvironment and culture conditions differ dramatically from in vivo conditions and can modify the cell chromatin profile. Additionally, the strict zonal constraints on function and gene expression of the intact gland are absent. The use of precise tissue microdissection techniques together with next-generation sequencing technologies for human adrenal zone-specific miRNA and DNA methylation profiling will give rise to promising paths to deepen our understanding of epigenetics in adrenal zonation.

Final Comments

The specific mechanisms governing the fascinating zonation and remodeling of the human adrenal cortex remain undetermined. This is particularly true for the ZR, which develops continuously after birth and produces adrenal androgens in a lifetime-specific fashion that is found to be similar only in higher primates. Emerging evidence points to epigenetics as another regulatory layer that could contribute to the modulation of both local adrenal zonation and systemic metabolic signals. Although further investigations and stronger evidence are required, these reports open the door for research in epigenetic regulation of developmental stage- and cell type-specific human adrenal function. The discovery of novel players controlling these processes would help to elucidate the causes of hyperandrogenic disorders, adrenal insufficiency, and adrenocortical cancer, and provide targets for preventive, diagnostic, and therapeutic uses.

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Disclosure Statement

The authors have no conflicts of interest.

References

1 Xing Y, Lerario AM, Rainey W, Hammer GD. Development of adrenal cortex zonation. Endocrinol Metab Clin North Am. 2015 Jun;44(2):243–74.
2 Ishimoto H, Jaffe RB. Development and function of the human fetal adrenal cortex: a key component in the feto-placental unit. Endocr Rev. 2011 Jun;32(3):317–55.
3 Goto M, Piper Hanley K, Marcos J, Wood PJ, Wright S, Postle AD et al. In humans, early cortisol biosynthesis provides a mechanism to safeguard female sexual development. J Clin Invest. 2006 Apr;116(4):953–60.
4 Auchus RJ. The physiology and biochemistry of adrenarche. Endocr Dev. 2011;20:20–7.
5 Belgorosky A, Baquedano MS, Guercio G, Rivarola MA. Adrenarche: postnatal adrenal zonation and hormonal and metabolic regulation. Horm Res. 2008;70(5):257–67.
6 Turcu A, Smith JM, Auchus R, Rainey WE. Adrenal androgens and androgen precursors: definition, synthesis, regulation and physiologic actions. Comprehensive physiology. Hoboken: Wiley; 2014. p. 1369–81.
7 Vinson GP. Functional Zonation of the Adult Mammalian Adrenal Cortex. Front Neurosci. 2016 Jun;10:238.
8 Belgorosky A, Baquedano MS, Guercio G, Rivarola MA. Expression of the IGF and the aromatase/estrogen receptor systems in human adrenal tissues from early infancy to late puberty: implications for the development of adrenarche. Rev Endocr Metab Disord. 2009 Mar;10(1):51–61.
9 Hammer GD, Parker KL, Schimmer BP. Minireview: transcriptional regulation of adrenocortical development. Endocrinology. 2005 Mar;146(3):1018–24.
10 Bassett MH, Suzuki T, Sasano H, De Vries CJ, Jimenez PT, Carr BR et al. The orphan nuclear receptor NGFIB regulates transcription of 3beta-hydroxysteroid dehydrogenase. implications for the control of adrenal functional zonation. J Biol Chem. 2004 Sep;279(36):37622–30.

11 Lavoie HA, King SR. Transcriptional regulation of steroidogenic genes: STARD1, CYP11A1 and HSD3B. Exp Biol Med (Maywood). 2009 Aug;234(8):880–907.
12 Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. Nature. 2004 May; 429(6990):457–63.
13 Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev. 2011 Feb;32(1):81–151.
14 Rainey WE, Nakamura Y. Regulation of the adrenal androgen biosynthesis. J Steroid Biochem Mol Biol. 2008 Feb;108(3-5):281–6.
15 Rone MB, Fan J, Papadopoulos V: Cholesterol transport in steroid biosynthesis: role of protein–protein interactions and implications in disease states. Biochim Biophys Acta 2009;1791:646–658.
16 Remer T, Boye KR, Hartmann MF, Wudy SA. Urinary markers of adrenarche: reference values in healthy subjects, aged 3-18 years. J Clin Endocrinol Metab. 2005 Apr;90(4):2015–21.
17 Nakamura Y, Fujishima F, Hui XG, Felizola SJ, Shibahara Y, Akahira J et al. 3βHSD and CYB5A double positive adrenocortical cells during adrenal development/aging. Endocr Res. 2015;40(1):8–13.
18 Nakamura Y, Hornsby PJ, Casson P, Morimoto R, Satoh F, Xing Y et al. Type 5 17β-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. J Clin Endocrinol Metab. 2009 Jun;94(6):2192–8.
19 Turcu AF, Nanba AT, Chomic R, Upadhyay SK, Giordano TJ, Shields JJ et al. Adrenal-derived 11-oxygenated 19-carbon steroids are the dominant androgens in classic 21-hydroxylase deficiency. Eur J Endocrinol. 2016 May;174(5):601–9.
20 Rege J, Nakamura Y, Satoh F, Morimoto R, Kennedy MR, Layman LC et al. Liquid chromatography-tandem mass spectrometry analysis of human adrenal vein 19-carbon steroids before and after ACTH stimulation. J Clin Endocrinol Metab. 2013 Mar;98(3): 1182–8.
21 Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I et al. Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. J Clin Endocrinol Metab. 2006 Jul;91(7): 2643–9.
22 Kamrath C, Hochberg Z, Hartmann MF, Remer T, Wudy SA. Increased activation of the alternative "backdoor" pathway in patients with 21-hydroxylase deficiency: evidence from urinary steroid hormone analysis. J Clin Endocrinol Metab. 2012 Mar;97(3):E367–75.
23 O'Reilly MW, Kempegowda P, Jenkinson C, Taylor AE, Quanson JL, Storbeck K-H, et al: 11-oxygenated C19 steroids are the predominant androgens in polycystic ovary syndrome. J Clin Endocrinol Metab 2017Mar 1; 102(3):840–48.
24 Jones CM, Mallappa A, Reisch N, Nikolaou N, Krone N, Hughes BA, et al: Modified release and conventional glucocorticoids and diurnal androgen excretion in congenital adrenal hyperplasia. J Clin Endocrinol Metab 2017 Jun 1;102(6):1797-1806.
25 Baquedano MS, Madjinca S, Aliberti P, Saraco NI, Warman M, Rivarola MA. Expression of Androgen Receptor (AR) and the Alternative "Backdoor" Pathway to Dihydrotestosterone (DHT) in Normal and 21-Hydroxylase Deficient Human Adrenal Cortex. Abstract SAT-0753. Endocr Rev. 2014 Aug;35(4 Suppl):e753.
26 Flück CE, Meyer-Böni M, Pandey AV, Kempná P, Miller WL, Schoenle EJ et al. Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. Am J Hum Genet. 2011 Aug;89(2):201–18.
27 Marti N, Malikova J, Galván JA, Aebischer M, Janner M, Sumnik Z et al. Androgen production in pediatric adrenocortical tumors may occur via both the classic and/or the alternative backdoor pathway. Mol Cell Endocrinol. 2017 Sep;452:64–73.
28 Allis CD, Jenuwein T. The molecular hallmarks of epigenetic control. Nat Rev Genet. 2016 Aug;17(8):487–500.
29 Azzi S, Abi Habib W, Netchine I. Beckwith-Wiedemann and Russell-Silver Syndromes: from new molecular insights to the comprehension of imprinting regulation. Curr Opin Endocrinol Diabetes Obes. 2014 Feb;21(1): 30–8.
30 Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. Nat Genet. 2003 Mar;33(3s Suppl):245–54.
31 Lo R, Weksberg R. Biological and biochemical modulation of DNA methylation. Epigenomics. 2014;6(6):593–602.
32 Yun M, Wu J, Workman JL, Li B. Readers of histone modifications. Cell Res. 2011 Apr; 21(4):564–78.
33 Jenuwein T, Allis CD. Translating the histone code. Science. 2001 Aug;293(5532):1074–80.
34 Kaikkonen MU, Lam MT, Glass CK. Non-coding RNAs as regulators of gene expression and epigenetics. Cardiovasc Res. 2011 Jun; 90(3):430–40.
35 Ha M, Kim VN. Regulation of microRNA biogenesis. Nat Rev Mol Cell Biol. 2014 Aug; 15(8):509–24.
36 Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009 Jan; 136(2):215–33.
37 Kometani M, Yoneda T, Demura M, Koide H, Nishimoto K, Mukai K et al. Cortisol overproduction results from DNA methylation of CYP11B1 in hypercortisolemia. Sci Rep. 2017 Sep;7(1):11205.
38 Howard B, Wang Y, Xekouki P, Faucz FR, Jain M, Zhang L et al. Integrated analysis of genome-wide methylation and gene expression shows epigenetic regulation of CYP11B2 in aldosteronomas. J Clin Endocrinol Metab. 2014 Mar;99(3):E536–43.
39 Demura M, Wang F, Takashi Y, Kometani M, Sawamura T, Cheng Y et al. Differential DNA methylation of human CYP11B1 and CYP11B2 promoters in association with adrenal zonation. Abstract MON-571. Endocr Rev. 2012 Jun;33(3 Suppl):e571.
40 Yoshii Y, Oki K, Gomez-Sanchez CE, Ohno H, Itcho K, Kobuke K et al. Hypomethylation of CYP11B2 in Aldosterone-Producing Adenoma. Hypertension. 2016 Dec;68(6):1432–7.
41 Missaghian E, Kempná P, Dick B, Hirsch A, Alikhani-Koupaei R, Jégou B et al. Role of DNA methylation in the tissue-specific expression of the CYP17A1 gene for steroidogenesis in rodents. J Endocrinol. 2009 Jul; 202(1):99–109.
42 Baquedano MS, Perez Garrido N, Goñi J, Saraco N, Aliberti P, Berensztein E et al. DNA methylation is not involved in specific down-regulation of HSD3B2, NR4A1 and RARB genes in androgen-secreting cells of human adrenal cortex. Mol Cell Endocrinol. 2017 Feb;441:46–54.
43 Sewer MB, Jagarlapudi S. Complex assembly on the human CYP17 promoter. Mol Cell Endocrinol. 2009 Mar;300(1-2):109–14.
44 Flück CE, Miller WL. GATA-4 and GATA-6 modulate tissue-specific transcription of the human gene for P450c17 by direct interaction with Sp1. Mol Endocrinol. 2004 May;18(5): 1144–57.
45 Martinez-Arguelles DB, Papadopoulos V. Epigenetic regulation of the expression of genes involved in steroid hormone biosynthesis and action. Steroids. 2010 Jul;75(7):467–76.
46 Liu J, Li XD, Vaheri A, Voutilainen R. DNA methylation affects cell proliferation, cortisol secretion and steroidogenic gene expression in human adrenocortical NCI-H295R cells. J Mol Endocrinol. 2004 Dec;33(3):651–62.
47 Udhane S, Kempna P, Hofer G, Mullis PE, Flück CE. Differential regulation of human 3β-hydroxysteroid dehydrogenase type 2 for steroid hormone biosynthesis by starvation and cyclic AMP stimulation: studies in the human adrenal NCI-H295R cell model. PLoS One. 2013 Jul;8(7):e68691.
48 Martin LJ, Tremblay JJ. The human 3beta-hydroxysteroid dehydrogenase/Delta5-Delta4 isomerase type 2 promoter is a novel target for the immediate early orphan nuclear receptor Nur77 in steroidogenic cells. Endocrinology. 2005 Feb;146(2):861–9.
49 Udhane SS, Pandey AV, Hofer G, Mullis PE, Flück CE. Retinoic acid receptor beta and angiopoietin-like protein 1 are involved in the regulation of human androgen biosynthesis. Sci Rep. 2015 May;5(1):10132.
50 Romero DG, Plonczynski MW, Carvajal CA, Gomez-Sanchez EP, Gomez-Sanchez CE. Microribonucleic acid-21 increases aldosterone secretion and proliferation in H295R human adrenocortical cells. Endocrinology. 2008 May;149(5):2477–83.

51 Robertson S, MacKenzie SM, Alvarez-Madrazo S, Diver LA, Lin J, Stewart PM et al: MicroRNA-24 is a novel regulator of aldosterone and cortisol production in the human adrenal cortex. Hypertension 2013 Sep;62(3):572–8.
52 Robertson S, Diver LA, Alvarez-Madrazo S, Livie C, Ejaz A, Fraser R et al. Regulation of Corticosteroidogenic Genes by MicroRNAs. Int J Endocrinol. 2017;2017:2021903.
53 Krill KT, Gurdziel K, Heaton JH, Simon DP, Hammer GD. Dicer deficiency reveals microRNAs predicted to control gene expression in the developing adrenal cortex. Mol Endocrinol. 2013 May;27(5):754–68.
54 Huang CC, Yao HH. Inactivation of Dicer1 in Steroidogenic factor 1-positive cells reveals tissue-specific requirement for Dicer1 in adrenal, testis, and ovary. BMC Dev Biol. 2010 Jun;10(1):66.
55 Hu Z, Shen WJ, Cortez Y, Tang X, Liu LF, Kraemer FB et al. Hormonal regulation of microRNA expression in steroid producing cells of the ovary, testis and adrenal gland. PLoS One. 2013 Oct;8(10):e78040.
56 Baquedano MS, Saraco N, Berensztein E, Pepe C, Bianchini M, Levy E et al. Identification and developmental changes of aromatase and estrogen receptor expression in prepubertal and pubertal human adrenal tissues. J Clin Endocrinol Metab. 2007 Jun;92(6):2215–22.
57 Yap OWS, Bhat G, Liu L, Tollefsbol TO: Epigenetic modifications of the Estrogen receptor beta gene in epithelial ovarian cancer cells. Anticancer Res 2009 Jan;29(1):139–44.
58 Klinge CM. miRNAs regulated by estrogens, tamoxifen, and endocrine disruptors and their downstream gene targets. Mol Cell Endocrinol. 2015 Dec;418(Pt 3):273–97.
59 Turcu AF, Auchus RJ. The next 150 years of congenital adrenal hyperplasia. J Steroid Biochem Mol Biol. 2015 Sep;153:63–71.
60 Baquedano MS, Maceiras M, Saraco N. Aliberti Paula, Berensztein E, Rivarola M et al: Dihydrotestosterone (DHT) Stimulates Progesterone Secretion and Decreases Cortisol Production By H295R Human Adrenocortical Cells: Nuclear Receptors, Ligands and Co-Regulators. Abstract SAT-276. Endocr Rev. 2015 Apr;36(2 Suppl):e276.
61 Gillman MW. Developmental origins of health and disease. N Engl J Med. 2005 Oct;353(17):1848–50.
62 Ibáñez L, Dimartino-Nardi J, Potau N, Saenger P. Premature adrenarche – normal variant or forerunner of adult disease? Endocr Rev. 2000 Dec;21(6):671–96.
63 West AN, Neale GA, Pounds S, Figueredo BC, Rodriguez Galindo C, Pianovski MA et al. Gene expression profiling of childhood adrenocortical tumors. Cancer Res. 2007 Jan;67(2):600–8.
64 Michalkiewicz E, Sandrini R, Figueiredo B, Miranda EC, Caran E, Oliveira-Filho AG et al. Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. J Clin Oncol. 2004 Mar;22(5):838–45.
65 Zsippai A, Szabó DR, Szabó PM, Tömböl Z, Bendes MR, Nagy Z et al: mRNA and microRNA expression patterns in adrenocortical cancer. Am J Cancer Res. 2011;1:618–28.
66 Jouinot A, Assie G, Libe R, Fassnacht M, Papathomas T, Barreau O et al: DNA methylation is an independent prognostic marker of survival in adrenocortical cancer. J Clin Endocrinol Metab. 2017 Mar 1;102(3):923–32.
67 Zheng S, Cherniack AD, Dewal N, Moffitt RA, Danilova L, Murray BA et al.; Cancer Genome Atlas Research Network. Comprehensive Pan-Genomic Characterization of Adrenocortical Carcinoma. Cancer Cell. 2016 Aug;30(2):363.
68 Doghman M, El Wakil A, Cardinaud B, Thomas E, Wang J, Zhao W et al. Regulation of insulin-like growth factor-mammalian target of rapamycin signaling by microRNA in childhood adrenocortical tumors. Cancer Res. 2010 Jun;70(11):4666–75.
69 Baquedano MS, Berensztein E, Saraco N, Dorn GV, de Davila MT, Rivarola MA et al. Expression of the IGF system in human adrenal tissues from early infancy to late puberty: implications for the development of adrenarche. Pediatr Res. 2005 Sep;58(3):451–8.