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Adrenal cortex development and related disorders leading to adrenal insufficiency

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ABSTRACT

The adult human adrenal cortex produces steroid hormones that are crucial for life, supporting immune response, glucose homeostasis, salt balance and sexual maturation. It consists of three histologically distinct and functionally specialized zones. The fetal adrenal forms from mesodermal material and produces predominantly adrenal C_{19} steroids from its fetal zone, which involutes after birth. Transition to the adult cortex occurs immediately after birth for the formation of the zona glomerulosa and fasciculata for aldosterone and cortisol production and continues through infancy until the zona reticularis for adrenal androgen production is formed with adrenarche. The development of this indispensable organ is complex and not fully understood. This article gives an overview of recent knowledge gained of adrenal biology from two perspectives: one, from basic science studying adrenal development, zonation and homeostasis; and two, from adrenal disorders identified in persons manifesting with various isolated or syndromic forms of primary adrenal insufficiency.

1. Introduction

Adrenal glands are key endocrine organs that participate in the hormonal regulation of vital aspects of human life, including stress and immune response, sexual maturation and salt balance. They are composed of two tissues with distinct function and developmental origin. The adrenal cortex produces steroids and descends from precursors of the coelomic epithelium (mesoderm); while the inner medulla, surrounded by the cortex, contains chromaffin cells that secrete catecholamines and originate from cells of the fetal neural crest (ectoderm). This article will focus on the adrenal cortex.

The first part of this review article discusses the recent advancements in adrenal investigations that study adrenocortical structure, development and homeostasis. This field has seen notable breakthroughs within the past 15 years and has led to important results on how cell dynamics, tissue structure and steroidogenic function are interconnected. Relevant implications of these studies include the possibility of reprogramming pluripotent cells to tunable steroid-producing cells as a therapeutic approach to compensate for missing steroid function that may lead to opportunities to cure disorders of adrenal insufficiency tomorrow.

The second part of this review article will focus on the steroidogenic function of the adrenal cortex. This aspect of adrenal physiology has been thoroughly investigated since the first description of clinical cases of adrenal insufficiency by Thomas Addison (1856). This report was followed by several efforts in isolating adrenal extract and single cortical steroids (known today as 'corticosteroids') with the therapeutic intention of replacing missing steroids in affected patients. These studies have all contributed towards the current comprehensive knowledge of the biochemical and genetic characteristics of the pathways of adrenal steroidogenesis. By contrast, the therapeutic approach to adrenal insufficiency in patients has seen little improvement over the last years, with life-long steroid replacement therapy remaining the only treatment available so far. The primary role of steroids for the quality of life, as well as the urgent need for better solutions to adrenal insufficiency syndromes are underscored by the severe pathological effects of steroid deficiency, ranging from mild manifestations like low blood pressure and chronic fatigue to life-threatening salt-wasting adrenal crises that may result in sudden death due to Addison's crisis. Therefore, still today, patients with adrenal insufficiency do have a reduced life expectancy (Hummel et al., 2016).

The third part of this article summarizes human adrenal disorders resulting from disruption of steroidogenesis and/or adrenal development, homeostasis and structure. In particular, we discuss selected

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Abbreviations

DZ	'definitive zone' or 'definitive cortex'
FZ	'fetal zone' or 'fetal cortex'
CYP11B1	11β-hydroxylase
HSD17B6	17β hydroxysteroid dehydrogenase 6
CYP21A2	21-hydroxylase
HSD3B2	3β-hydroxysteroid dehydrogenase
SRD5A1	5α-reductase
ATP	adenosine triphosphate
AHC	Adrenal hypoplasia congenita
ACTH	Adrenocorticotropin Hormone
FDXR	Adrenodoxin Reductase
AGP	adrenogonadal primordium
AKR1C2	Aldo-keto reductase family 1 member C2
AKR1C4	Aldo-keto reductase family 1 member C4
AS	Aldosterone Synthase
CREM	AMP-responsive element modulator
ABS	Antley Bixler syndrome
CaMKs	Calcium/calmodulin-dependent protein Kinases
PRKAR1A	cAMP-dependent protein kinase type I-alpha regulatory subunit
CREB	cAMP-responsive element binding protein
CITED2	Cbp/p300-interacting transactivator 2
CAH	congenital adrenal hyperplasia
CRH	corticotropin releasing hormone
cAMP	Cyclic Adenosine Monophosphate
POR	Cytochrome P450 Reductase
dpc	days post conception
DadE	Definitive adrenal-specific enhancer
DAB2	Disabled homolog 2
DMD	Duchenne muscular dystrophy
E	embryonic day
FGD	Familial glucocorticoid deficiency
FAdE	fetal adrenal-specific enhancer
GPX1	glutathione peroxidase 1
GKD	glycerol kinase deficiency
Gaq	Guanine nucleotide-binding protein G(q) subunit alpha
HOX	homeobox proteins
hiSCs	human induced steroidogenic cells
LGR	Leucine-rich repeat-containing G-protein coupled Receptor
MCM4	minichromosome maintenance 4
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NNT	Nicotinamide Nucleotide Transhydrogenase
NPC	Niemann Pick type C
DAX1	nuclear repressor protein dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1
OMIM	Online Mendelian Inheritance in Man
OTC	ornithine transcarbamylase
PTCH1	Patched 1
PRDX3	peroxiredoxin 3
PBX1	pre–B-cell leukemia transcription factor 1
PAI	primary adrenal insufficiency
PPNAD	primary pigmented nodular adrenocortical disease
PCNA	proliferating cell nuclear antigen
PKA	Protein Kinase A
ROS	reactive oxygen species
RAAS	Renin-Angiotensin-Aldosterone system
RSPOs	R-spondins
SHH	Sonic Hedgehog
S1P	sphingosine-1-phosphate
SGPL1	sphingosine-1-phosphate lyase 1
StAR	Steroidogenic Acute Regulatory protein
SF-1	Steroidogenic Factor 1
TXNRD2	thioredoxin reductase 2
HPA	this hypothalamic-pituitary-adrenal
AAAS	triple A syndrome
WT1	Wilms' tumor protein 1
WIF	Wnt inhibitory factor
zF	zona Fasciculata
zG	zona Glomerulosa
zR	zona Reticularis

monogenic disorders of adrenal insufficiency caused by congenital adrenal hypoplasia, hyperplasia and other syndromic and non-syndromic disorders affecting adrenal function.

Finally, in the last part of this update, we report on novel models used for adrenal research and their opportunities and challenges to enhance the knowledge of the biology of the adrenal cortex forward.

Overall, this article highlights critical aspects of adrenal cortex development, homeostasis, function and disease, with a focus on novel aspects that have emerged in the past decade. It does not claim to be comprehensive but will refer to such reviews whenever possible. The reader will notice that substantial amount of data collected so far about adrenocortical structure and development originates from animal studies (mostly mouse models) (Table 1), while data on steroid biochemistry and disorders originate from "human experiments of nature" (Table 2). The authors indicate therefore throughout the text whether described data derive from animal or human studies, to help the reader discriminate between the two. In fact, although animal studies have traditionally informed on key human adrenal mechanisms (e.g. the role of Steroidogenic Factor 1 (SF-1) in development and function of adult steroidogenic organs, including the adrenal), the transferability of most animal findings to human physiology has yet to be experimentally tested. Notable discrepancies between rodent and human physiology are evident. For instance, in adrenal zonal organization of most animals a region resembling the human *zona Reticularis* (zR) is missing and with it the developmental event of adrenarche. Species-specific expression of CYP17A1 coding for 17α-hydroxylase/17–20 lyase activity enables the human, but not the mouse adrenals to produce cortisol and adrenal androgens. Besides, adrenal development in human features the development of a functional transitory 'fetal zone' or 'fetal cortex' (FZ), which is absent in the mouse. These discrepancies then translate into remarkable differences in the clinical outcome of monogenic variants, whereby genes implicated in human adrenal pathologies may not result in a similar phenotype when inactivated in mice, or vice versa. Therefore, readers are invited to use caution in the interpretation of results of adrenal studies, especially if they are interested in a comprehensive, all-including view of the adrenal physiology in human, which still poses many unanswered questions.

2. Adrenocortical structure, development and homeostasis

The adrenal cortex is a highly dynamic, vital steroidogenic hub, implicated in the control of many homeostatic functions mediated by three types of steroid hormones: mineralocorticoids, glucocorticoids and adrenal androgens. Steroid production is compartmentalized in as many epithelial concentric layers, referred to as 'zones', thanks to the segregation of specific enzymes and cofactors. The subdivision of the adrenal cortex in distinct functional zones is conventionally referred to as 'functional zonation'. Teleologically, functional zonation allows discrete regulation of each steroid type, and consequent independent control of each steroid function. The adrenal cortex is surrounded by a

Table 1
Selected genes involved in adrenal development, zonation and homeostasis. Lessons learned from mouse developmental biology and their suggested human correlate according to Online Mendelian Inheritance in Man (OMIM).

Mouse gene name	Phenotype associated with gene inactivation in mouse	Human gene name	OMIM	Human disorder
Axin2	Accelerated ossification leading to premature fusion of cranial structures (Yu, 2005)	*AXIN2*	114500 608615	Oligodontia-colorectal cancer syndrome
Cited2	Embryonic lethality associated with cardiac malformations, adrenal agenesis, abnormal cranial ganglia, exencephaly, liver dysgenesis, XY sex reversal, eye, lung and placental defects. Conditional inactivation show failure in maintenance of adult hematopoietic stem cells (Bamforth et al., 2001; Buaas et al., 2009; Chen et al., 2008; Kranc et al., 2009; Qu et al., 2007; Val et al., 2007; Weninger et al., 2005; Withington et al., 2006; Xu et al., 2008).	*CITED2*	614431 614433	Atrioventricular septal defects
Ezh2	Lethality before gastrulation. Conditional inactivation results in primary glucocorticoids insufficiency and aberrant zonal differentiation (Mathieu et al., 2018; Shen et al., 2008)	*EZH2*	277590	Weaver syndrome
Gata4	Defects of cardiac and pancreatic development, impaired male and female gonadal function (Carrasco et al., 2012; Kyrönlahti et al., 2011a, 2011b; Tevosian et al., 2015)	*GATA4*	615542 607941 614430 187500 614429	Testicular and cardiac anomalies, including atrioventricular septal defects
Gata6	Eary death at implantation, associated with extraembryonic tissue defects. Conditional inactivation results in adrenal hypoplasia (Koutsourakis et al., 1999; Tevosian et al., 2015)	*GATA6*	614475 614474 600001 217095 187500	Cardiac defects included in the Tetralogy of Fallot, pandreatic agenesis
Gli1	No evident phenotype (Park et al., 2000)	*GLI1*	618123 174400	Polydactyly
Hoxb5	Variable anteriorizing homeotic vertrebrae transformations and rostral shift of the shoulder girdle (Rancourt et al., 1995)	*HOXB5*	-	-
Hoxb9	Developmental defects in thoracic skeletal elements (Chen and Capecchi, 1997)	*HOXB9*	-	-
Lef1	Growth retardation and postnatally lethality associated with defective developent of multiple organs, including mammary glands, vasculature and body hair (van Genderen et al., 1994)	*LEF1*	-	Somatic sebaceous tumors.
Nr0b1	Abnormal reproductive development in the hemizygote, ranging from defects in testes development and spermatogenesis to complete male to female sex reversal, progressive adrenal failure (Scheys et al., 2011; Yu et al., 1998)	*NR0B1*	300018 300200	Congenital adrenal hypoplasia.
Nr5a1	Agenesis of adrenal glands and gonads, defects of the ventromedial hypothalamic nucleus and pituitary gonadotrophs, neonatal lethality (Beuschlein et al., 2002)	*NR5A1*	612964 613957612964 612965 617480	Haploinsufficiency leads to andrenocortical insufficiency, ovarian and spermatogenic failure, 46, XX sex reversal.
Pbx1	Late gestational death, hypoplasia or aplasia of multiple organs, impaired hematopoiesis, skin edema, axial and appendicular skeleton defects, absent adrenal glands, impared developent of bone, kidney and pancreas (DiMartino et al., 2001; Kim et al., 2002; Schnabel et al., 2003a, 2003b; Selleri et al., 2001)	*PBX1*	617641	Congenital anomalies of kidney and urinary tract syndrome with or without hearing loss, abnormal ears, or developmental delay.
Prkar1a	Embryonic lethality during organogenesis due to multiple developmental patterning defects (Amieux et al., 2002)	*PRKAR1A*	101800 160980 255960 610489	Carney complex, pigmented nodular adrenocortical disease (PPNAD).
Rnf43	Hyperproliferation of intestinal and stomach epithelium (Neumeyer et al., 2019)	*RNF43*	617108	Sessile serrated polyposis cancer syndrome.
Rspo3	Lethality during organogenesis due to impaired placenta formation. Conditional inactivation results in imparied adrenal development and loss of cell replenishment in the adult adrenal (Aoki et al., 2007; Vidal et al., 2016)	*RSPO3*		
Shh	Mid-gestation death associated with impaired development of limbs, eyes, ears, external genitalia and mouth (Chiang et al., 1996)	*SHH*	142945 611638 269160 147250	Holoprosencephaly, microphthalmia with coloboma, schizencephaly, single median maxillary central incisor.
Tcf21	Lethality around birth due to hypoplastic lungs and kidneys, with abnormal vasculature of these organs and the hemopericardium. Asplenia only in one mouse model (Lu et al., 2000; Quaggin et al., 1999)	*TCF21*	-	-
Wnt4	Perinatal lethality associated with impaired development of the kidney, lung and pituitary gland and female reproductive system. Conditional inactivation results in impaired zG differentiation. (Caprioli et al., 2015; Drelon et al., 2016; Heikkilä et al., 2002; Stark et al., 1994; Treier et al., 1998; Vainio et al., 1999)	*WNT4*	611812 158330	SERKAL syndrome, Müllerian aplasia and hyperandrogenism.
Wt1	Late gestational death, impaired renal, gonadal, adrenal, splenic, pulmonar, cardiac and mesothelial development (Bandiera et al., 2013; Kreidberg et al., 1993)	*WT1*	194080 136680 608978 156240 256370 194070	Denys-Drash syndrome, Frasier syndrome, Meacham syndrome, mesothelioma, nephrotic syndrome and Wilms tumor.
Znrf3	Lethality around birth associated with abnormal lens development. Conditional inactivation results in adrenal hyperplasia (Hao et al., 2012, 2012, 2012)	*ZNRF3*	-	-

mesenchymal capsule, which is composed of a layer of elongated, fibroblast-like cells and fibrous tissue that supports the cortex by providing structural containment and critical signaling information for the development, adult maintenance and regeneration of the gland.

2.1. Distinct histological characteristics and functions of the cortical zones

The first report that describes the subdivision of the adrenocortical zones came in the second half of the nineteenth century, with the observation by Julius Arnold that the human and bovine adrenal cortex

are subdivided into three distinct topological and morphological regions (Dostoiewsky, 1886). Later studies built on this report by indicating that each zone secretes unique types of steroids, and highlighted a strong correlation between morphology and function (Swann, 1940). This correlation is clearly exemplified by syndromic disorders that feature adrenal dysplasia such as X-linked adrenal hypoplasia congenita and the Pallister-Hall syndrome (described more in depth in the third part of this review article and in Table 2), whereby disruption of the stereotypical zonal organization and cellular structure is accompanied by loss of steroidogenic activity.

The cortical zona Glomerulosa (zG) clearly illustrates the tight correlation between structure and function typical of the entire adrenocortical tissue. The zG is the outer zone of the adrenal cortex and is responsible for the synthesis of the mineralocorticoid aldosterone under the control of the Renin-Angiotensin-Aldosterone system (RAAS), high potassium levels and elevated blood pH. Aldosterone controls systemic salt and acid-base homeostasis by acting predominantly on the distal tubules and collecting ducts of the kidney to promote reabsorption of sodium and excretion of potassium and hydrogen ions (Bollag, 2014; Nishimoto et al., 2013). The zG is the smallest among the cortical zones and makes up about 15% of the entire adrenocortical tissue in humans and mice (Nussdorfer, 1980; Pignatti, 2014). Cells within the zG are arranged in round-shaped glomeruli surrounded by a basement membrane, which separates glomeruli and cortical vasculature. While classical histological analysis may lead to the conclusion that zG glomeruli are isolated entities, 3D reconstruction recently revealed that glomeruli are tightly packed globular structures interconnected through openings in the basement membrane (Leng et al., 2020). Finally, each glomerulus contains one or more rosettes, defined as aggregates of five or more cells converging to one single center enriched in cadherin junctions. This structural framework is shaped and maintained in the mouse by the activity of the WNT and FGF signaling pathways, and shows a high level of conservation across species, suggesting it is critical to sustain zG function (Leng et al., 2020). Consistent with this hypothesis, recent studies bring clear evidence that steroid function and morphology within the zG are tightly linked. In this respect it is worth mentioning here a recent study by Guagliardo et al., which shows that calcium bursts, fundamental units of zG layer activity, display phase stability within each rosette. In mice, disruption of cadherin junctions within the rosettes reduces the number of clustered cells, the duration of evoked calcium bursts and, ultimately, leads to reduced aldosterone production (Guagliardo et al., 2020). The strong connection between morphology and function in zG is further substantiated by the expression of zone-specific genes with both structural and steroidogenic functions (extensively reviewed in Seccia et al., 2017). Some of these genes are also commonly used as markers in experimental animal models to determine zG identity. They include Disabled homolog 2 (DAB2) (Romero et al., 2007), Guanine nucleotide-binding protein G(q) subunit alpha (Gaq) (Pignatti et al., 2020; Taylor et al., 2020) and β-catenin (Basham et al., 2019; Berthon et al., 2010; Eberhart and Argani, 2001; Kim et al., 2008; Walczak et al., 2014). While Aldosterone Synthase (AS), encoded by the *CYP11B2* gene, is also restricted to the zG, its expression is often confined to a subset of cells and its use as a zone marker may lead to underestimating the extent of the zG area (Bassett et al., 2004; Pignatti et al., 2017; Walczak et al., 2014). In spite of this, since AS expression levels are directly proportional to the systemic needs of aldosterone reflected by potassium and angiotensin levels in the circulation (Heitzmann et al., 2008; Makhanova et al., 2006), it is reasonable to consider AS an indicator of zG activation.

Concentric to the zG lies the zona Fasciculata (zF), which has strikingly different functional and morphological properties than the zG. The zF occupies the largest fraction of the adrenal cortex and produces the glucocorticoid cortisol in humans (corticosterone in rodents). Cortisol is implicated in mobilization of protein, glucose and lipid depots to produce an adequate response to stress and hypoglycemia, and acts as an immunomodulatory agent inhibiting the production of a wide range of pro-inflammatory cytokines including interferon-gamma and tumor necrosis factor-alpha (Kadmiel and Cidlowski, 2013) while also being involved in immune cell recruitment, differentiation as well as activation (reviewed in Strehl et al., 2019). The enzymatic machinery resulting in cortisol production is activated by the cAMP/PKA signaling, which ultimately controls zF steroidogenic function by direct stimulation of critical enzymes, including Steroidogenic Acute Regulatory protein (StAR) and CYP11A1 (Ruggiero and Lalli, 2016). The Cyclic Adenosine Monophosphate/Protein Kinase A (cAMP/PKA) signaling pathway is initiated by binding of the polypeptide Adrenocorticotropin Hormone (ACTH) to its cognate G-protein coupled receptor MC2R. The accessory protein MRAP is essential for shuttling MC2R from the endoplasmic reticulum to the plasma membrane where it transduces ACTH signal (Metherell et al., 2005; Mountjoy et al., 1992). At a systemic level, ACTH is produced within the anterior pituitary gland under the control of the corticotropin releasing hormone (CRH), which in turn originates in the hypothalamic paraventricular nucleus. In addition to this hypothalamic-pituitary-adrenal (HPA) axis, cortisol secretion is also subject to a circadian rhythmicity entrained to the day-night cycle and to local, intra-adrenal circadian oscillators (Son et al., 2018). Besides, adrenal function is also under the control of the splanchnic nerve, which provides neural input to regulate differential steroid production upon stress or non-stress conditions (Engeland, 1998). The morphology of the zF differs from that of the zG in that cells are larger, less densely packed and much richer in lipid droplets. zF cells are organized in centripetal columns flanked by fenestrated blood vessels that facilitate the exchange of hormones, cholesterol and nutrients between the steroidogenic cells and the circulation (Lever, 1952). The steroid 11β-hydroxylase, encoded by *CYP11B1*, is a commonly used marker for the identification of the zF in human, rats and mice (Gomez-Sanchez et al., 2014; Ogishima et al., 1992; Wotus et al., 1998).

Finally, the zR represents the innermost cortical layer, lies at the interface with the adrenal medulla and is responsible for the production of high levels of androgens and androgen precursors in humans (Nakamura et al., 2012). Contrary to the outer zones, the zR is not present at birth and starts to progressively grow and assume its final shape during childhood, between the age of 6 and 9 in both boys and girls. The development of a functional zR and the consequent rise in circulating androgens is referred to as 'adrenarche' (Auchus, 2011). During adrenarche, increased circulating androgens result in stereotypical systemic effects including growth of pubic and axillary hair and development of adult body odor. How the zR forms and what stimulates its development is still not clear, mainly because of lack of experimental models. Indeed, a functional zR is present in humans and has been thoroughly characterized in rhesus macaques both morphologically and biochemically (Conley et al., 2011), while in Great Apes adrenarche has been inferred by the identification of fluctuating postnatal levels of androgen precursors (Conley et al., 2012; Nguyen and Conley, 2008). Lower species including the ferret and spiny mouse have also been recognized to produce adrenal androgens, although to what extent this activity reflects human adrenarche remains to be established (Lamers et al., 1986; Quinn et al., 2013; Rosenthal and Peterson, 1996). Overall, the existence of a zR in a subset of species suggests that unique molecular events are necessary for this zone to emerge (Conley et al., 2012). In this regard, a recent work from Martinez's group shows that a functional zR-like tissue can develop in a zR-deficient mouse strain following overt activation of the cAMP/PKA signaling pathway (Dumontet et al., 2018). Whether the activation of the cAMP/PKA signaling explains adrenarche in humans and higher primates is still to be established, especially in consideration of the fact that ACTH, the main trigger of cAMP/PKA activity, does not raise in correlation with DHEA during adrenarche (Auchus and Rainey, 2004).

2.2. Aspects of prenatal development of the adrenal glands

The adult adrenal cortex markedly differs in structure and function

Table 2
Reported monogenetic causes of primary adrenal insufficiency and associated phenotype in humans.

	Disorder	Gene	OMIM	Associated clinical features in addition to PAI
Defects of steroid biosynthesis (e.g. congenital adrenal hyperplasia)	Congenital lipoid adrenal hyperplasia (LCAH)	StAR	201710	46,XY DSD, gonadal insufficiency
	P450 side chain cleavage syndrome (CAH)	CYP11A1	118485	46,XY DSD, gonadal insufficiency
	3β-hydroxysteroid dehydrogenase deficiency (CAH)	HSD3B2	201810	46,XY DSD and 46,XX DSD, gonadal insufficiency
	21-hydroxylase deficiency (CAH)	CYP21A2	201910	46,XX DSD, androgen excess syndrome, testicular adrenal rest tumors
	11β-hydroxylase deficiency (CAH)	CYP11B1	202010	46,XX DSD, hypertension, androgen excess syndrome
	17-hydroxylase deficiency (CAH)	CYP17A1	202110	46,XY DSD, hypertension, gonadal insufficiency
	P450 oxidoreductase deficiency (CAH)	POR	613571	46,XY DSD, 46,XX DSD, gonadal insufficiency, Antley-Bixler skeletal malformation syndrome; changes in drug metabolism
	Steroidogenic factor 1 deficiency	NR5A1 (SF1)	184757	46, XY DSD, gonadal insufficiency
	Aldosterone synthase deficiency	CYP11B2	124080	Isolated mineralocorticoid deficiency
Adrenal dysgenesis (e.g. hypoplasia, agenesis)	X-linked adrenal hypoplasia congenita (AHC)	NROB1 (DAX1)	300200	Hypogonadotropic hypogonadism, in some cases gonadotropin independent precocious puberty
	IMAGe syndrome	CDKN1C	614732	IUGR, bone disorders and anomalies, genital anomalies, hypercalcemia,
		POLE1	618336	dysmorphic facial features, immunodeficiency
	MIRAGE syndrome	SAMD9	617053	Myelodysplasia, infections, restriction of growth, genital anomalies, enteropathy
	SERKAL syndrome	WNT4	611812	46,XX DSD, IUGR, cleft lip/palate, dysplastic kidneys and lungs, heart defects, diaphragmatic hernia, intestinal malrotation
	Pallister-Hall syndrome	GLI3	165240	Hypothalamic hamartomas, mesoaxial and postaxial polydactyly, bifid epiglottis, imperforate anus, genitourinary anomalies
	Meckel syndrome	MKS1	249000	Cystic renal disease, CNS malformation - occipital encephalocele, polydactyly, hepatic abnormalities
	Pena-Shokeir syndrome	*DOK7*, RAPSN	208150	Arthrogryposis, facial anomalies, IUGR, camptodactyly, fetal akinesia, polyhydramnion, pulmonary hypoplasia, cardial defects, intestinal malrotation
	Pseudotrisomy 13		264480	Holoprosencephaly, polydactyly, craniofacial anomalies
	Hydrolethalus syndrome	HYLS1	236680	Hydrocephaly, micrognathia, polydactyly abnormal genitalia, congenital heart defects, respiratory organ defects
	Galloway-Mowat syndrome	WDR73	251300	Nephrotic syndrome, microcephaly, encephalopathy, hiatus hernia
ACTH resistance/FGD	Familial glucocorticoid deficiency (FGD)	MC2R	202200	Mostly normal production of mineralocorticoids, tall stature
		MRAP	607398	
	FGD - Deficiency of mitochondrial ROS detoxification	NNT	614736	Only glucocorticoid deficiency
		TXNRD2	606448	Only glucocorticoid deficiency
		GPX1		Only glucocorticoid deficiency
		PRDX3		Only glucocorticoid deficiency
	FGD - DNA repair defect	MCM4	609981	NK cell deficiency, short stature, microcephaly, recurrent viral infections, chromosomal breakage
	AAA syndrome – Triple A (Allgrove syndrome)	AAAS	231550	Alacrimia, achalasia, deafness, mental retardation, hyperkeratosis
Cholesterol synthesis disorders	Wolman disease	LIPA	278000	Xanthomatous changes in the liver, adrenal, spleen, lymph nodes, bone marrow, small intestine and thymus, diffuse punctate adrenal calcification, hepatosplenomegaly, poor weight gain, hypercholesterolemia, steatorrhea
	Smith-Lemli Opitz disease	DHCR7	270400	Multiple congenital malformation and mental retardation syndrome
	Abeta-lipoproteinemia	MTP	200100	Ataxia, retinopathy, acanthocytosis, pathologic fat absorption
	Familial hypercholesterolemia	LDLR	143890	Xanthomas, corneal arcus, and coronary artery disease
	Sitosterolemia (Phytosterolemia)	ABCG5 ABCG8	210250	Short stature, gonadal failure, xanthomas, arthritis, coronary heart disease
Peroxisomal defects	X-linked adrenoleukodystrophy	ABCD1	300100	Progressive neurodegeneration, dementia, progressive behavioral
		ABCD2	300371 601081	disturbances, vision and hearing loss, spasticity and seizures; accumulation of very long chain fatty acids
	Neonatal adrenoleukodystrophy	PEX1	601539	Hypotonia, seizures, diffuse encephalopathy, sensorineural hearing loss, peripheral neuropathy, mild facial dysmorphism; autosomal recessive
	Infantil Refsum disease	PHYH, PEX7	266500	Anosmia, retinitis pigmentosa, neuropathy, deafness, ataxia, ichthyosis
	Zellweger syndrome	PEX1 and other PEX genes	214100	Severe neurologic dysfunction with handicaps, craniofacial abnormalities, severe mental retardation, hepatomegaly, growth failure, stippled epiphysis, genitourinary anomalies
Mitochondrial defects	Kearns-Sayre syndrome	mitDNA del	530000	Progressive external ophthalmoplegia, pigmented retinopathy, cardiac conduction block, cerebellar ataxia; other endocrine pathologies
	(Combined) mitochondrial complex deficiency	MRPS7	617872	Sensorineural deafness, liver and kidney failure, hypogonadism, neurodevelopmental delay. Lactic acidosis.
	(Leigh syndrome)	NDUFAF5	612360	IUGR, brain anomalies and neurodegeneration resulting in neurodevelopmental delay, spasticity, chorea and seizures. Diaphragmatic hernia. Lactic acidosis.
		GFER	613076	Progressive myopathy with congenital cataract and developmental delay
Lysosomal defect	Sphingosine-1-phosphate lyase 1 deficiency	SPGL1	603723	Steroid-resistant nephrotic syndrome, optionally accompanied by ichthyosis, primary hypothyroidism, cryptorchidism, immunodeficiency and neurological defects
Autoimmune disorder	Autoimmune polyglandular syndrome type 1 (APS1, APECED)	AIRE	240300	Hypoparathyroidism, candidiasis, autoimmune hypergonadotropic hypogonadism, autoimmune thyroid diseases alopecia, chronic autoimmune hepatitis, pernicious anemia, vitiligo

from the developing adrenal. Here we provide an overview of the genes and molecular pathways that are critical for the correct formation of the prenatal adrenal cortex. Most of this knowledge has been gained from studies in mice. Table 1 summarizes identified genes and reports on associated phenotypes when these genes are disrupted in mice and humans.

The adrenal cortex originates around 28–30 days post conception (dpc), corresponding to embryonic day 9.5 (E9.5) in the mouse, from bilateral and symmetrical streaks of cells comprised between the coelomic epithelium and the dorsal aorta (Hatano et al., 1996). These cells form the adrenogonadal primordium (AGP) and express the progenitor markers GATA4, Wilms' tumor protein 1 (WT1) and Cbp/p300-interacting transactivator 2 (CITED2) (Bandiera et al., 2013; Val et al., 2007). WT1 is a zinc finger transcription factor, associated with a subset of early onset nephroblastomas, also known as Wilms' tumor (Hohenstein and Hastie, 2006). Inactivation of WT1 in the mouse results in dysgenesis of several organs including the adrenal glands; this is due to a critical role of WT1 for the formation and maturation of the AGP, possibly because of the direct contribution of WT1 to the expression of SF-1 (Hohenstein and Hastie, 2006; Kreidberg et al., 1993, p. 1; Moore et al., 1999; Wilhelm and Englert, 2002). SF-1, encoded by the *NR5A1* gene, is a transcription factor belonging to the nuclear receptor superfamily, essential for the differentiation of several organs, including gonads, spleen and adrenals (Luo et al., 1994). Contrary to GATA4, WT1 and CITED2, SF-1 expression persists in all the steroidogenic cells of the adult adrenal cortex, where it controls the expression of several cytochrome P450 steroid hydroxylases (Parker and Schimmer, 1997). Val and colleagues hypothesized that the specification of adrenal and gonadal lineages in the AGP is decided based on a threshold of SF-1 expression, whereby SF-1 is stronger in the fraction of the AGP destined to become the adrenal cortex (Val et al., 2007). Consistent with this hypothesis, differential expression of SF-1 is achieved in the AGP thanks to the interaction of WT1 with the cofactor CITED2, which positively regulates WT1 transcriptional activity (Val et al., 2007). Other *trans*-acting factors have been involved in the regulation of SF-1, including the complexes of pre–B-cell leukemia transcription factor 1 (PBX1) with the homeobox protein PKNOX1 and the homeobox proteins B9, B5 and C5 (HOXB9, HOXB5 and HOXC5, respectively). These complexes were shown to activate SF-1 in the AGP independent of WT1, by binding the fetal adrenal-specific enhancer (FAdE) located on the 4th intron of the *NR5A1* gene (Zubair et al., 2006).

Between 32 and 40 dpc (E10.5 in the mouse) the medial AGP separates and migrates dorsally to become the adrenal primordium (Fig. 1). Around the same developmental time SF-1 expression becomes self-sustained in a positive feedback loop by *trans*-activation of NR5A1 binding sites also present in the FAdE promoter (Zubair et al., 2006). In addition, genetic manipulation of WT1 expression in the mouse showed that WT1 must be shut down during the formation of the adrenal primordium to allow the progressive differentiation of the adrenal cortex (Bandiera et al., 2013). Indeed, overt activation of WT1 blocks the development and progressive differentiation of the adrenal by promoting the expression of the transcription factors Gli1 and Tcf21, which are known markers of progenitor cells in both the developing and adult adrenal (Huang et al., 2010; King et al., 2009; Wood et al., 2013).

Between 48 and 52 dpc (E12.5 in the mouse), a subset of neural crest cells migrate to form clusters within the adrenal primordium; these clusters later coalesce in the center of the adrenal gland and differentiate to active catecholamine-producing chromaffin cells, which form the adrenal medulla (Lumb and Schwarz, 2015). At the same time, mesenchymal cells surrounding the primordium form the adrenal capsule by creating a cell layer around the adrenal primordium (Mesiano and Jaffe, 1997). After encapsulation, two morphologically different concentric zones can be identified within the adrenal primordium in humans and primates, the inner FZ and the outer and smaller 'definitive zone' or 'definitive cortex' (DZ). The FZ, characterized by large lipid-loaded cells, is responsible for producing DHEA that is subsequently converted to estrogen by the placenta. Instead, the DZ is made of tightly packed, basophilic cells that remain almost inactive until the end of the second trimester of pregnancy, when this zone becomes a main hub of cortisol production (Goto et al., 2006; Hanley et al., 2001; Ishimoto and Jaffe, 2011). Tracing experiments in mice showed that the emergence of the DZ is paralleled by the inactivation of the FAdE promoter but is still characterized by the expression of SF-1. The evidence for a Definitive adrenal-specific enhancer (DadE) that accounts for SF-1 expression in the later stages of development and in the adult adrenal is still missing. The cessation of the FAdE activity in the developing adrenal appears to rely at least on two molecular events, the SUMOylation of SF-1 and the co-repressive activity of the nuclear repressor protein dosage-sensitive

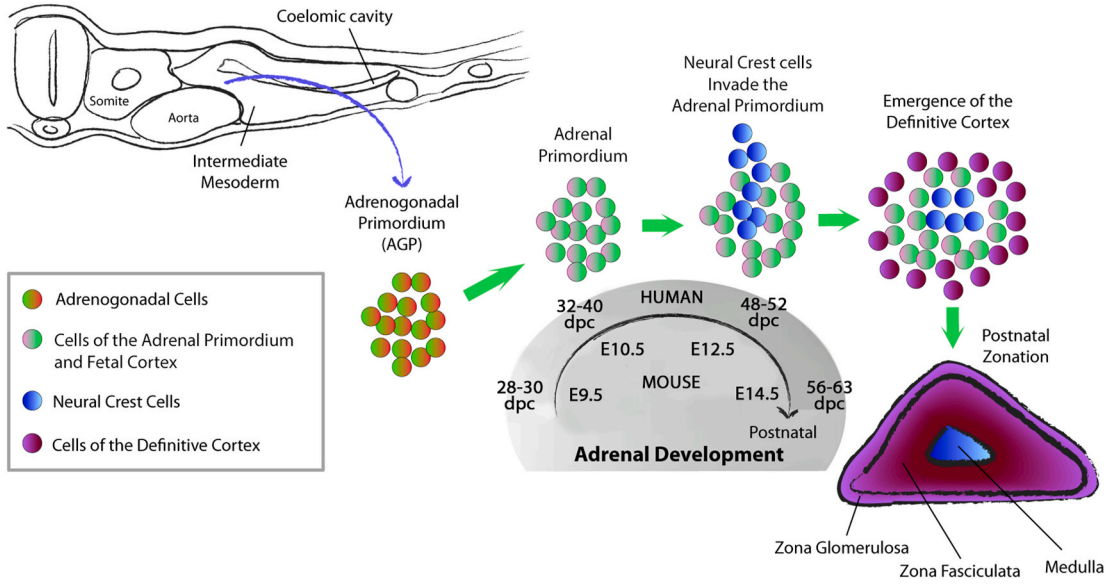

Fig. 1. Embryonic adrenal development. Schematic illustration showing the cellular rearrangements during mouse and human adrenal development. The adrenogonadal primordium (AGP) originates from a streak of cells localized between the coelomic epithelium and the dorsal aorta (red arrow) around 28–30 dpc (E9.5). At 32–40 dpc (E10.5), the adrenal primordium separates from the AGP. Neural crest cells, precursors of the chromaffin cells of the medulla, invade the AGP between 48 and 52 dpc (E12.5). From 56 dpc (E14.5) onward, the fetal cortical cells are slowly replaced by the definitive cortex, which gives rise to the zG and zF around the time of birth.

sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (*DAX1*) (Xing et al., 2017). DAX1 is a member of the nuclear receptor superfamily shown to act as a corepressor for SF-1 transcriptional activity on numerous genes involved in adrenal development and steroidogenesis (Ferraz-de-Souza et al., 2009; Lalli and Sassone-Corsi, 2003; Lehmann et al., 2002; Li et al., 2011; Zhou et al., 2008). The gene encoding DAX1, *NR0B1*, was first cloned as the responsible gene for X-linked cytomegalic adrenal hypoplasia congenita (Mantovani et al., 2006). Inactivation of DAX1 in the mouse results in accelerated differentiation possibly due to premature exhaustion of adrenal progenitor population, suggesting a role for this gene in regulating the balance between adrenal progenitor maintenance and recruitment/differentiation (Scheys et al., 2011). Finally, while the descendants of the DZ will eventually give rise to zG and zF, soon after birth the FZ undergoes a dramatic involution accompanied by a significant decrease in adrenal mass and a rapid fall in circulating DHEA and DHEA-S (Bocian-sobkowska et al., 1998; Bruch, 1936).

2.3. Homeostatic maintenance and remodeling of the adult adrenal cortex

2.3.1. A wide spectrum of progenitor populations

Active endocrine tissues are characterized by a dynamic adaptation in size and function in response to systemic and local stimuli. In the adult adrenal cortex, remodeling depends on two main contributors: one, the recruitment of new steroidogenic cells from undifferentiated progenitors and two, the proliferation of differentiated cells. Historical autologous graft and pulse-chase experiments have validated a model in which adrenal renewal occurs in a centripetal fashion (Salmon and Zwemer, 1941; Wyman and Walker, 1929). According to this model, cells from the capsule and subcapsular regions, including the zG, are progressively displaced in the most internal layers of the cortex up to the corticomedullary region, where they undergo cell death. Lineage tracing experiments demonstrated that adrenocortical cells transdifferentiate during their centripetal migration by changing their morphology and function consistent with the zone they occupy. Therefore, capsular cells can give rise to zG cells, which can transdifferentiate into more internal zF cells. In turn, zF cells were shown to give rise to cells that reminisce the human zR in terms of molecular profile and steroidogenic potential (Dumontet et al., 2018; Freedman et al., 2013; Grabek et al., 2019; King et al., 2009). The origin of most adrenocortical cells from the outer regions of the cortex is consistent with the presence of tissue-resident progenitors in the capsule and zG. This concept has been validated in mice using lineage tracing of discrete cell populations, specifically $Gli1^+$ and $Wt1^+$ cells (located in the adrenal capsule), and Sonic Hedgehog $(Shh)^+$, $Cyp11b2^+$, $Axin2^+$ and $Wnt4^+$ cells (located in the zG) (Bandiera et al., 2013; Freedman et al., 2013; Grabek et al., 2019; King et al., 2009). Short-term fate mapping invariably displays these cells in the capsule or zG, consistent with the expression pattern of each marker, while long-term tracing identifies clusters/stripes of steroidogenic cells that progressively extend from the outer to the inner cortical regions, in agreement with their role as progenitor cells. Despite these common traits, the expression patterns of the distinct progenitor populations overlap only partially, or do not overlap at all, suggesting that a spectrum of different populations exist. In fact, Shh^+ and $Cyp11b2^+$ cells are subpopulations of Axin2-and Wnt4-expressing zG cells, whereas Wt1 and Gli1 markers are expressed in the capsule and overlap only in a minority of cells (Bandiera et al., 2013; Grabek et al., 2019; Walczak et al., 2014). Besides, evidence is accumulating that distinct progenitor populations contribute to the steroidogenic compartment to different extents. This extent depends on multiple factors, including the intrinsic properties of each cell type (i.e. plasticity and quiescence status), the developmental stage of the adrenal, the sex of the animal and the presence of any regenerative stimulus that may lead to accelerated tissue turnover (reviewed in Hammer and Basham, 2021). For instance, progenitor cells localized in the zG lead to a complete turnover of the steroidogenic cortex within 3–6 months. In turn, capsular progenitors appear to act as reservoir stem cells prevalently recruited in response to supraphysiological demands, including regenerative/remodeling stimuli (Bandiera et al., 2013; Finco et al., 2018). However, a sexual dimorphic phenotype exists, since cell turnover in female adrenals still partially relies on the recruitment of capsular progenitors, while male adrenals maintain their homeostasis mainly by proliferation of differentiated cells in the outer cortical regions (Grabek et al., 2019). This scenario indicates a notable heterogeneity among progenitor populations, which ultimately serves the plasticity requirements of adrenal homeostasis and regeneration (Hammer and Basham, 2021; Walczak et al., 2014).

2.3.2. Signaling pathways involved in the regulation of adrenal homeostasis

While the relationship among the diverse progenitor cell populations might still be a matter of interesting debate and further investigation, the regulatory roles of the SHH, WNT and cAMP/PKA pathways in the regulation of adrenal homeostasis, regeneration and disease has been firmly established with the use of animal models of gene inactivation and hormonal treatment. The importance of these pathways in adrenal physiology is further corroborated by the pathological consequences of the disruption of critical signaling components, including GLI3 (Pallister Hall syndrome, linked to adrenal insufficiency; Table 2), β-catenin (adrenal hyperplasia and tumors) and cAMP-dependent protein kinase type I-alpha regulatory subunit (PRKAR1A, associated with primary pigmented nodular adrenocortical disease – PPNAD). Therefore, the SHH, WNT and cAMP/PKA pathways will be described briefly for their role in adrenal physiology. For a detailed coverage of the molecular aspects of these pathways and related disorder, the reader is addressed to more focused review articles (Blassberg and Jacob, 2017; Nusse and Clevers, 2017; Ruggiero and Lalli, 2016).

In the adrenal, the SHH pathway is activated by the zG-secreted SHH ligand that binds its cognate receptor Patched 1 (PTCH1) expressed on the adrenal capsule. In turn, PTCH1 transduces the signal to the intracellular transcription factors GLI, including GLI1, which is also expressed in the capsule (Bitgood and McMahon, 1995; Ching and Vilain, 2009; Huang et al., 2010; King et al., 2009). The peculiar expression pattern of the different components of the SHH pathway underlies a signaling bridge between the cortical zG and the capsule, which is key to the maintenance of adrenal progenitors. Indeed, disruption of the SHH pathway results in reduced adrenal mass caused by thinning of both the cortex and the capsule, which is consistent with loss of progenitor cells in both compartments (Ching and Vilain, 2009; Huang et al., 2010; King et al., 2009).

Similarly, the WNT pathway is active thanks to a molecular crosstalk between capsule and cortex. The canonical form of the WNT pathway relies on the intracellular levels of β-catenin molecules, which act as signal transducers and co-factors in cooperation with the transcription factors of the TCF/LEF family. Levels of β-catenin are negatively regulated by a multimeric destruction complex that targets serine and threonine residues close to the N-terminal of the protein. Phosphorylation is commonly followed by ubiquitination and proteasomal degradation of β-catenin. Instead, when Wnt ligands engage the seven-transmembrane receptor Frizzled, the destruction complex is sequestered to the plasma membrane and β-catenin is free to accumulate in the cytosol and nucleus, where it participates to the transcription of target genes. The canonical Wnt signaling is extensively regulated at the level of ligand-receptor interaction. For instance, Secreted Frizzled-related proteins (Sfrp and Frzb) and Wnt inhibitory factor (WIF) bind and sequester Wnt ligands away from their Frizzled receptor. In addition, Frizzled is constitutively targeted to degradation by the E3 ubiquitin ligase activity of RNF43 and ZNRF3, which are single-pass membrane proteins classified among the suppressors of Wnt signaling. In turn, RNF43 and ZNRF3 are bound and internalized by the complex formed by membrane Leucine-rich repeat-containing G-protein coupled Receptor (LGR) 4/5/6 and their ligands R-spondins (RSPOs), which overall provide an additional level of complexity to the regulation of the WNT signaling.

Despite this complexity, nuclear expression of β-catenin is a simple and validated indicator of active WNT signaling utilized both in diagnostic routine and animal studies (Basham et al., 2019; Eberhart and Argani, 2001; Walczak et al., 2014). In the adrenal, nuclear β-catenin and established WNT-target genes such as LEF1 and AXIN2 are expressed in the outer regions of the cortex in a gradient fashion, whereby WNT activity is stronger in the zG and weaker in the outer regions of the zF (Basham et al., 2019). This gradient of WNT activity is maintained by RSPO3, which is secreted by the adrenal capsule and binds the receptor LGR5 expressed in the outer cortical cells (Vidal et al., 2016). In the mouse adrenal, the WNT signaling pathway is implicated in the preservation of the zG, by driving the expression of critical zG-specific differentiation markers (Berthon et al, 2010, 2014; Drelon et al., 2016; Leng et al., 2020; Pignatti et al., 2020), and in the maintenance of cell turnover in the cortex, by allowing the replenishment of the lost cortical cells (Kim et al., 2008). Consistent with this, disruption of the WNT pathway by inactivation of *Rspo3* results in loss of zG markers within 24 h, and in halving of the adrenal mass within 6 weeks (Vidal et al., 2016). It is reasonable to speculate that these two functions of the WNT pathway are tightly linked, since the zG represents an important physical reservoir of progenitor cells for the entire cortex.

The role of the WNT pathway in promoting and maintaining the zG identity is contrasted by the cAMP/PKA signaling pathway, which is prevalently active in the zF (Ruggiero and Lalli, 2016). As mentioned in the first section of this review article, the cAMP/PKA signaling pathway is initiated by binding of the polypeptide ACTH to the seven-transmembrane receptor MC2R. Signal transduction involves the release of the alpha subunit of the stimulatory G protein and the conversion of adenosine triphosphate (ATP) into cAMP by the enzyme adenylyl cyclase. These events result in the dissociation of the regulatory subunit of PKA from its catalytic subunits, which in turn phosphorylate and activate multiple downstream signaling intermediates including the AMP-responsive element modulator (CREM) and the cAMP-responsive element binding protein (CREB) transcription factors. Among the four different PKA regulatory subunits identified in humans PRKAR1A has a particular clinical relevance, since null mutations in this gene result in overactivation of the pathway and lead to PPNAD, a rare hypersecretion disorder associated to the Carney complex syndrome (Groussin et al., 2002).

Over-activation of the cAMP/PKA signaling pathway in the mouse adrenal cortex results in reduced zG and increased zF differentiation and function (Drelon et al., 2016; Dumontet et al., 2018; Sahut-Barnola et al., 2010). Although the molecular bases are not known, this effect is likely mediated by the suppression of WNT4, the most characterized WNT ligand expressed in the zG. Indeed, inactivation of *Wnt4* phenocopies cAMP/PKA activation, in that it results in decreased *Axin2* and *Lef1* transcripts (zG markers) and expanded zF territory (Drelon et al., 2016).

Phosphodiesterases are selective inhibitors of the cAMP/PKA pathway for their capacity of hydrolyzing cAMP to inactive AMP, and may also play a role in establishing zG/zF zonation by inhibiting zF differentiation. A recent study indicates that the cGMP-dependent 3′,5′-cyclic phosphodiesterase encoded by the *Pde2a* gene is expressed within the mouse zG and is a direct target of the WNT pathway, which opens the possibility that PDE2A suppresses the cAMP/PKA signaling in the zG and inhibits the expression of zF markers (Pignatti et al., 2020). Another evidence that supports the role of phosphodiesterases in the inhibition of the cAMP/PKA adrenal signaling comes from the mouse model of adrenal insufficiency caused by disruption of the *Ezh2* gene, which encodes a histone methyltransferase (Mathieu et al., 2018). Indeed, *Ezh2*-deficient female mice show increased ectopic expression of the phosphodiesterases PDE1B, 3A, and 7A in the zF, and display diminished zF differentiation and expansion of the zG territory. Besides, inactivation of Ezh2 also leads to reactivation and expansion of GATA4 and WT1 fetal markers, which is indicative of ongoing dedifferentiation of adrenal cells to a progenitor-like state (Mathieu et al., 2018). Altogether, these results provide evidence for a role of adrenal phosphodiesterases in adult adrenal zonation and steroidogenesis.

3. Adrenal steroidogenesis

3.1. Steroidogenesis of the human adult adrenal cortex

Suggested by the concept of functional zonation, the structural characteristics of each adrenal zone are instrumental to produce distinct steroid hormones. Fig. 2 provides an overview of the steroidogenic activity within each zone of the human adrenal. It shows genes/enzymes and cofactors involved in the stepwise conversion of cholesterol into the three key steroid pathways leading to mineralocorticoids in the zG, glucocorticoids in the zF and adrenal androgens in the zR.

All cortical steroids are synthetized from cholesterol which is either stored in esterified form or freely available within cells for steroid production (Miller, 2017). Cholesterol recruitment from endosomes and lipid droplets seems to involve proteins encoded by *STARD3*, *STARD3NL*, Niemann Pick type C 1 (*NPC1*) and *NPC2* (reviewed in Gallo-Payet and Battista, 2014). Steroidogenesis is carried out by monooxygenases of the cytochrome P450 family and hydroxysteroid dehydrogenases located in the endoplasmic reticulum and in mitochondria (Miller and Auchus, 2011). Shuttling of cholesterol to the mitochondria, and from there to the inner mitochondrial membrane are the initial and limiting steps for the biosynthesis of all adrenal steroids (Miller, 2017; Miller and Auchus, 2011). Despite the exact mechanisms underlying these processes have not been completely clarified, it is established that the StAR is responsible for shuttling about 90% of the cholesterol present in the inner mitochondrial membrane (Miller, 2017). Here, cholesterol is converted to pregnenolone by the side-chain cleavage enzyme, encoded by the *CYP11A1* gene and supported by cofactors adrenodoxin (FDX1) and the enzyme adrenodoxin reductase (FDXR). While StAR and CYP11A1 are ubiquitously expressed within the adrenal cortex, the zonal segregation of other enzymes accounts for the compartmentalization of steroid production (Nishimoto et al., 2012; Rege et al., 2014).

Within the zG, aldosterone is the product of sequential processing of pregnenolone into progesterone, 11-deoxycorticosterone and corticosterone, with the enzymatic reactions driven by 3β-hydroxysteroid dehydrogenase (HSD3B2), 21-hydroxylase (CYP21A2) and CYP11B1, respectively (Fig. 2). AS, encoded by the *CYP11B2* gene, catalyzes the limiting and final step of aldosterone biosynthesis, and is expressed in patchy pattern selectively within the zG (Pignatti et al., 2017). CYP11B2 is quickly regulated by aldosterone-releasing stimuli (e.g. Angiotensin II, elevated potassium and blood pH), which trigger the expression of CYP11B2 transcripts and proteins through rapid intracellular bursts of calcium and activation of the Calcium/calmodulin-dependent protein Kinases (CaMKs) (Guagliardo et al., 2020). This highly reactive and dynamic system offers a rapid way to control blood pressure and pH by almost instant release of aldosterone and is systemically controlled by the renin-angiotensin feedback loop.

Within the zF, cortisol production depends on the expression of the 17α-hydroxylase enzyme, encoded by the *CYP17A1* gene, which converts pregnenolone into 17α-hydroxypregnenolone. The following downstream enzymatic reactions result in the formation of 17α-hydroxyprogesterone, 11-deoxycortisol and cortisol, and are mediated by HSD3B2, CYP21A2 and CYP11B1, respectively (Fig. 2). Cortisol release is rapidly induced following the activation of the HPA stress response, and its expression largely depends on ACTH via the cAMP/PKA signaling-mediated stimulation of critical enzymes, including CYP11A1 and StAR transcripts (Ruggiero and Lalli, 2016).

Output of the human zR, mainly DHEA, DHEA-S and 11β-hydroxyandrostenedione, is due to the low expression of the HSD3B2 gene, the enhanced activity of the 17,20-lyase function of CYP17A1 and the increased expression of the sulfotransferase SULT2A1. Combined, these molecular events are hallmarks of the zR and herald adrenarche. CYP17A1 has both a hydroxylase and a lyase activity. Phosphorylation

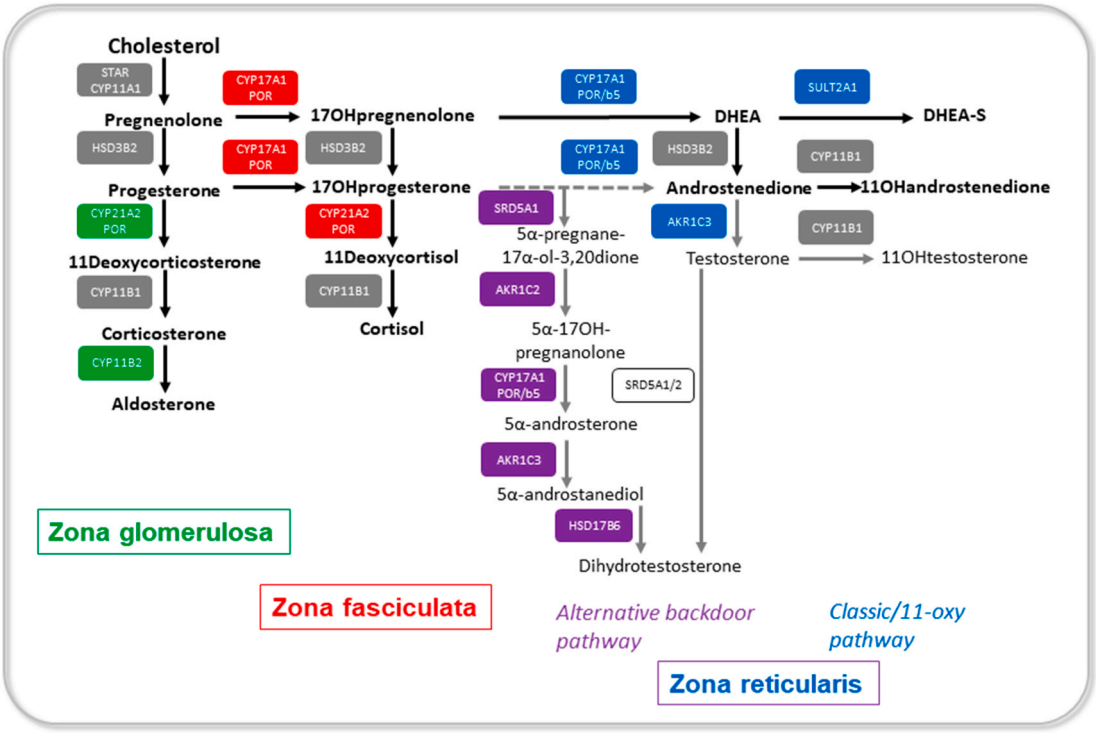

Fig. 2. Adrenal steroid biosynthesis in humans. The adrenal cortex is structured into three concentric zones, which produce specific steroids. Mineralocorticoids (MC) are synthesized in the outermost layer (zona glomerulosa); glucocorticoids (GC) are produced in the middle layer (zona fasciculata); and androgens (AA) are produced in the inner layer (zona reticularis). The scheme provides an overview of the biochemical pathways leading to the three main groups of steroids and shows intermediate products as well as involved enzymes and cofactors catalyzing stepwise conversion of cholesterol into specific products. Enzymes expressed in all zones are colored in grey, while enzymes essential for the production of a specific class of steroids are given in green for the MC path, red for the GC path and blue and purple for the AA path. In the AA path enzymes included in the classic and 11-oxyandrogen path are given in blue, enzymes of the alternative backdoor path in purple. Steroids that are normally produced in the adult adrenal cortex are given in **bold**, while steroids that are not usually produced in relevant quantities are given in normal font.

of CYP17A1 and the concomitant expression of the allosteric regulator cytochrome *b*5 favor the lyase activity, which facilitates the conversion of 17α-hydroxypregnenolone to DHEA (Auchus et al., 1998; Zhang et al., 1995). SULT2A1 drives the conversion of DHEA to DHEA-S with the support of the co-substrate 3′-phosphoadenosine 5′-phosphosulfate (Noordam et al., 2009; Weinshilboum et al., 1997). It is important to note that adrenal steroidogenesis of the human adrenal cortex differs from steroidogenesis of a rodent's adrenal cortex mainly because of species-specific expression of *CYP17A1* (Miller, 2017; Miller and Auchus, 2011). As mouse adrenals do not normally express *Cyp17*, they produce corticosterone, while human adrenals also produce cortisol and C_{19} steroids.

DHEA and its sulfated metabolite are the most abundant adrenal androgens, but they are bioinactive and mainly serve as substrate for peripheral conversion into more potent androgens. But recent evidence shows that bioactive androgens including testosterone and 11-oxy androgens are also produced within the adrenal glands via the catalytic activities of the type 5 17β-hydroxysteroid dehydrogenase encoded by the *AKR1C3* gene and the CYP11B1 hydroxylase (Fig. 2) (Nakamura et al, 2009, 2012). Among the bioactive androgens, 11-hydroxyandrostenedione was found to be the second most abundant and with relevant bioactivity at the androgen receptor either itself or after peripheral conversion to highly bioactive androgens 11-ketotestosterone and 11-ketodihydrotestosterone (Turcu et al., 2020).

In addition, the adrenals during the period of fetal sexual maturation as well as with disorders of adrenal steroidogenesis have been recognized as a hub for production of alternative active androgens through the 'backdoor pathway' (Fig. 2) (Biason-Lauber et al., 2013; Fukami et al., 2013; Kamrath et al., 2013; Reisch et al., 2019). Together with the 'classic' DHEA-mediated pathway, the backdoor pathway is involved in the production of active androgens in disorders of steroidogenesis and sex differentiation of both 46,XY and 46,XX fetuses. More in detail, the backdoor pathway has been first described in tammar wallaby pouch offspring (Auchus, 2004; Wilson et al., 2003). It leads to the production of dihydrotestosterone from 17α-hydroxyprogesterone via the enzymes 5α-reductase (SRD5A1), Aldo-keto reductase family 1 member C2 and C4 (AKR1C2, AKR1C4), CYP17A1, AKR1C3 and 17β hydroxysteroid dehydrogenase 6 (HSD17B6) through a number of alternative intermediate steroid metabolites not comprised in the classic pathway (Flück et al., 2011; Wilson et al., 2003).

Importantly, the function of all monooxygenases of the cytochrome P450 family involved in steroidogenesis critically depends on the reducing capacity of two co-factors, the FDXR, embedded in the inner mitochondrial membrane, and the Cytochrome P450 Reductase (POR), localized to the membrane of the endoplasmic reticulum (Pandey and Sproll, 2014; Sheftel et al., 2010). While POR directly transfers redox equivalents from Nicotinamide Adenine Dinucleotide Phosphate (NADPH) to microsomal cytochrome P450 proteins, FDXR first transfers electrons to two forms of adrenodoxin, encoded in humans by the FDX1 and FDX2 genes, which in turn reduce the target mitochondrial enzymes (Hanukoglu, 2017). The critical role of POR in steroidogenesis is underscored by the consequences of pathogenic genetic variations in the *POR* gene, which are associated with disorders of steroid production and sexual development (Table 2). On the other hand, FDXR insufficiency in human is linked with auditory neuropathy and optic atrophy (Paul et al., 2017), while its pathological role in steroidogenesis still needs to be clearly determined.

3.2. Steroidogenesis of the fetal adrenal

Data on ontogeny of the steroidogenic activity of the human fetal adrenal result from hormonal measurements from cord blood, amniotic fluid and tissues as well as expression profiling of involved genes (e.g. steroid enzymes). These data have been comprehensively reviewed elsewhere (Ishimoto and Jaffe, 2011; Mesiano and Jaffe, 1997).

In contrast to the adult adrenal cortex, steroid biosynthetic function of the fetal adrenal seems less diverse with prominent DHEA and DHEA-S production starting at around 8–10 weeks of gestation and rising thereafter constantly to reach abundant levels of about 200 mg/day at the end of pregnancy (Ishimoto and Jaffe, 2011). This is mainly due to expression of CYP11A1 and CYP17A1 and their redox partners in the relative absence of HSD3B2 throughout most of pregnancy in the adrenal FZ, which comprises most of the steroidogenic activity. However, enabled by spatiotemporal specific expression of HSD3B2, relevant levels of cortisol are also produced in a narrow time window in the first trimester (8–12 weeks gestation). This seems important for safeguarding the female external genitalia from virilization as exemplified by 46,XX girls with congenital adrenal hyperplasia due to pathogenic *CYP21A2* variants causing ambiguous genitalia, and their successful treatment with dexamethasone (Goto et al., 2006). In the second trimester cortisol synthesis occurs only indirectly through the use of placental derived progesterone entering the circulation and then being further metabolized to cortisol in the fetal adrenals (Johnston et al., 2018). This continued cortisol production into the second trimester seems necessary for normal homeostatic feedback regulation and programming of the HPA, stress hormone axis. Only after 24–28 weeks gestation and especially towards late gestation intra-adrenal direct cortisol production resumes to prepare the fetus (e.g. the lungs) for postnatal life. By contrast, fetal adrenals do not produce aldosterone until late gestation due to lack of CYP11B2 expression (Ishimoto and Jaffe, 2011; Johnston et al., 2018). Shortly before term, the DZ of the fetal adrenal is the location where aldosterone production starts. Cells of the DZ will then develop into the zG and zF of the postnatal adrenal cortex due to expression of CYP11A1 and CYP17A1 and their redox partners in the relative absence of HSD3B2.

It is noteworthy that the function of the fetal adrenal needs to be seen in a wider context with the feto-placental unit and other fetal organs (e.g. liver, testis) that all participate in the steroid intermediate metabolism (Ishimoto and Jaffe, 2011). Steroids produced by the fetal adrenal and progesterone produced by the placenta, play essential roles in the maintenance of pregnancy, intrauterine homeostasis, fetal (sex) development and maturation, as well as the initiation of parturition. Adrenal androgens DHEA/DHEAS are precursors for placental estrogen production. However, recent studies show that they also serve as substrate for 5α-androsterone production by the placenta (O'Shaughnessy et al., 2019). Likewise, 5α-17OH-pregnanolone produced in the placenta, fetal testis or liver can be metabolized to 5α-androsterone in the fetal adrenals in a sex dimorphic fashion. Together with testosterone 5α-androsterone has been identified as a key player of normal male sex development. On the other hand progesterone produced by the placenta has also been reported to be a precursor for cortisol production in the second trimester, since fetal adrenals lack HSD3B2 activity (Johnston et al., 2018).

Expression of genes determining fetal adrenal steroidogenesis is regulated by transcription factors. Among them SF-1/NR5A1 and DAX1/NR0B1 play crucial roles for organogenesis of the adrenal cortex from earliest stages on (see also 2.1.). But SF-1 is also a major transcriptional activator of several genes of steroidogenesis, including STAR, CYP11A1, CYP17A1, CYP21A2, HSD3B2, CYP11B, SULT2A1. By contrast, DAX1 is an opponent of SF-1 stimulated transactivation. Transcription factors of the Nur family (e.g. NGFI-B, NURR1) regulate expression of HSD3B2, CYP21A1 and HSD11B2 and thus dictate fetal adrenal steroidogenesis fundamentally. But regulators of NGFI-B expression are unknown. Several other transcription factors such as GATA4, GATA6, CITED2, PBX1 and COUP-TFII have been related to adrenal development, but their role in regulating adrenal steroidogenesis is not entirely clear (Johnston et al., 2018).

4. Disorders of human adrenal development and function

Genetic disorders affecting human adrenal development and/or steroidogenesis lead to primary adrenal insufficiency (PAI), which is clinically defined by cortisol deficiency. Table 2 summarizes currently described monogenetic causes of PAI and their associated phenotype. Disorders that affect not only the adrenals and steroidogenesis but disrupt also other organ functions are called syndromic forms. They include metabolic disorders of cholesterol synthesis, peroxisomal, mitochondrial and lysosomal defects as well as the autoimmune polyglandular syndromes, which will not be discussed in detail as they mostly do not primarily affect adrenal development and typically start off with normal adrenal function. The following sections will focus on genetic disorders (non-syndromic and syndromic) leading to adrenal dysgenesis, hyperplasia and deficient adrenal steroid biosynthesis in the first place (as summarized in the first part of Table 2). Several reviews on PAI have been published in the last 3 years (Buonocore and Achermann, 2020; Fluck, 2017; Guran, 2017; Hannah-Shmouni and Stratakis, 2018; Maharaj et al., 2019; Miller, 2018; Roucher-Boulez et al., 2018).

4.1. Congenital adrenal hyperplasia (CAH)

Genetic disorders of adrenal steroidogenesis leading to cortisol deficiency are better known as CAH because the resulting ACTH overproduction will stimulate adrenal cortex growth through proliferation of adrenocortical cells (Miller et al., 2020; Stewart and Newell-Price, 2016). In case of STAR deficiency, additional intracellular lipid accumulation will lead to lipoid CAH. Structurally, the development of the three zones of the adult adrenal cortex seems unaffected by congenital steroid biosynthetic defects although little evidence exists. To date, genetic disorders related to each step of the classic steroid biosynthetic pathway have been described and are principally characterized by clinical signs and biochemical markers of related deficiencies of downstream steroid hormones and upstream precursor excess (Fig. 2 and Table 2). However, clinical diagnosis of CAH may be more complicated than theoretically thought as some enzymes are able to catalyze multiple reactions (also in extraadrenal tissues), have partial activity and supporting cofactors. They can therefore lead to a disease pattern that may be only recognized by comprehensive phenotyping and steroid profiling using chromatographic mass spectrometric methods. Moreover, a final genetic diagnosis should be attempted in all cases because similar phenotypes may be caused by variants in more than one gene and vice versa. In the following section some aspects of specific CAHs will be discussed. For a more comprehensive description of the specific clinical findings of all CAHs, we refer to medical textbooks, e.g. (Miller et al., 2020; Stewart and Newell-Price, 2016).

The first CAH described in the medical literature and solved to the molecular genetic level was 21-hydroxylase deficiency due to autosomal recessive, pathogenic variants of the *CYP21A2* gene (Miller, 2013). It is also the most frequently occurring form of CAH with a worldwide incidence of its classic form in about 1 in 10′000–15′000 newborns (Miller and Auchus, 2011; Stewart and Newell-Price, 2016). Numerous pathogenic variants have been reported in affected persons with very good genotype-phenotype and structure-function correlation. Loss of 21-hydroxylase activity in its severest form leads to aldosterone and cortisol deficiency and adrenal androgen excess (Fig. 2). Milder forms that retain some enzyme activity might only lead to a relative cortisol deficiency, but a constant androgen excess, and are known as non-classic, late-onset CAH. As the first trimester fetal adrenals are supposed to produce cortisol to lower ACTH and thereby adrenal androgen production for the prevention of masculinization of female fetuses, girls with 21-hydroxylase deficiency will not be able to produce

cortisol and will therefore be born with variable degrees of virilized external genitalia reflecting the degree of CYP21A2 enzyme deficiency and intrauterine androgen excess. Androgen excess of 21-hydroxylase deficiency pre- and postnatally has been shown to result from 17-hydroxyprogesterone excess feeding into the alternative backdoor pathway of androgen biosynthesis as well as the pathway of 11-oxyandrogens (Kamrath et al, 2013, 2018) (Fig. 2).

Similarly, pathogenic variants in the *CYP11B1* gene will result in cortisol deficiency and androgen excess, but because the enzyme block lies one step downstream marker steroids comprise 11-deoxycorticosterone and 11-deoxycortisol. Accumulation of 11-deoxycorticosterone, which is an active mineralocorticoid, will lead to hypertension over time. By contrast, genetic mutations of the CYP11B2 enzyme supporting the last step of aldosterone production in the zG cause isolated mineralocorticoid deficiency without affecting cortisol production, and therefore do not actually belong to the group of CAH.

Genetic disorders of the initial steps of adrenal steroidogenesis include STAR and CYP11A1 deficiencies, which are essential for the production of all adrenal steroid hormones (Fig. 2) (Miller, 2017; Miller et al., 2020). STAR is the cholesterol shuttler to the mitochondria where in the inner membrane the side chain cleavage system consisting of CYP11A1 and cofactors FDX and FDXR will convert cholesterol to pregnenolone. Severe STAR deficiency is responsible for lack of steroid hormone production by the adrenals and gonads, but not the placenta in which mitochondrial STAR import occurs through other mechanisms (Miller, 2017). In dependent organs, about 86% of cholesterol import is STAR-dependent, allowing minimal residual steroid production at first. However, this has been shown to disappear over time due to massive intracellular lipid accumulation and consecutive cell destruction, described in the literature as second hit (Miller, 2017; Miller et al., 2020). Persons with classic lipoid CAH are unable to synthesize corticosteroids and sex hormones. Thus karyotypic 46,XY male fetus will not only suffer from PAI, but will also present with female typical external genitalia at birth as lack of intrauterine (testicular) androgen production will fail to support normal masculinization. Later on, pubertal development and fertility will be affected in both sexes, although the ovary might show some functionality initially as its second hit is delayed to puberty since it is functionally quiescent during fetal life.

Severe enzyme deficiency of CYP11A1 is clinically indistinguishable from STAR with the exception that it does not lead to lipid accumulation. Interestingly, milder non-classic forms of both STAR and CYP11A1 deficiencies exist and lead to PAI without affecting sex hormone production.

Autosomal recessive mutations of HSD3B2 also affect all three pathways of adrenal steroidogenesis and gonadal steroidogenesis. 3β-hydroxysteroid dehydrogenase deficiency leads to an accumulation of C_{19} steroids produced over the delta 5 pathway (pregnenolone, 17-hydroxyprenenolone, DHEA; Fig. 2), which may result in female virilization while not providing sufficient precursors for proper testosterone production and male masculinization (Burckhardt et al., 2015; Miller et al., 2020).

The enzyme CYP17A1 has two catalytic properties, e.g. 17-hydroxylase activity allowing pregnenolone and progesterone to enter the cortisol biosynthetic pathway, and 17,20 lyase activity for the synthesis of adrenal DHEA (Fig. 2). Most pathogenic variants of *CYP17A1* cause deficiency of both enzymatic functions and manifest with low cortisol and sex hormone levels, but elevated mineralocorticoids. Only very few specific variants lead to isolated 17,20 lyase deficiency and thus cause isolated lack of sex hormone production. Cytochrome *b*5 (CYB5) and POR are essential cofactors supporting 17,20 lyase activity. Mutations of *POR* and *CYB5* may therefore copy the phenotype of isolated 17,20 lyase deficiency. Likewise, mutations in backdoor genes *AKR1C2/4* have been reported with similar phenotypes (Biason-Lauber et al., 2013), and similar phenotypes have also been observed with some (heterozygous) variants in the *SF-1/NR5A1* gene (Fabbri-Scallet et al., 2020).

Finally, genetic variants of cofactor POR can cause non-syndromic as well as syndromic PAI (Fluck et al., 2004). POR supports all microsomal type 2 P450 enzymes including steroidogenic CYP17A1, CYP21A2 in the adrenals and CYP19A1 in estrogen producing tissues (e.g. placenta, ovary) (Pandey and Fluck, 2013). Thus, POR mutations will show an adrenal steroid profile resembling combined CYP17 and CYP21 deficiency. In most cases, it will also cause atypical sex development in both sexes through inadequate androgen production involving the classic and backdoor androgen biosynthetic pathways as well as placental androgen to estrogens conversion. Maternal virilization in POR deficiency underscores the importance of the interplay of fetal adrenal steroidogenesis with the feto-placental unit (Fluck et al., 2020). The severest phenotype of genetic variants in *POR* manifest as Antley Bixler syndrome (ABS), which is characterized by craniosynostosis, radiohumeral synostosis, PAI and ambiguous genitalia. The skeletal phenotype of POR deficiency can be explained by its effect on interacting P450s of cholesterol biosynthesis (e.g. *CYP51A1*) as well as retinoic acid metabolism (e.g. *CYP26B1*) that are critical for bone formation (Fluck and Pandey, 2013).

4.2. Adrenal hypoplasia congenita (AHC)

Pathogenic variants or deletion of the transcription factor DAX1 encoded by the *NR0B1* gene and located on chromosome Xp21 cause X-linked AHC. In this most common form of primary adrenal hypoplasia in boys, the DZ of the fetal adrenal does not develop, and the FZ is vacuolated and cytomegalic (Miller et al., 2020; Suntharalingham et al., 2015). Affected boys suffer from salt-loss and cortisol insufficiency either in early infancy or childhood. Mutations of *DAX1/NR0B1* also cause hypogonadotropic hypogonadism with incomplete pubertal development. *NR0B1* gene deletions may be part of a contiguous gene deletion syndrome variably including a mental retardation gene (*IL1RAPL1*) and genes for glycerol kinase deficiency (*GKD*) as well as ornithine transcarbamylase (*OTC*) and Duchenne muscular dystrophy (*DMD*).

Surprisingly, human mutations in *SF-1/NR5A1,* the master transcription factor for adrenal and gonadal development and steroidogenesis, only very rarely lead to PAI (Suntharalingham et al., 2015). In the broadest sense, it might therefore also be classified as a form of adrenal hypoplasia. However, most human *SF-1* mutations affect the gonads and sex development only. By contrast, *Nr5a1* knockout mice reveal adrenal agenesis, gonadal (testicular) dysgenesis with a XY female phenotype and persistent Müllerian structures (uterus). They also have hypogonadotropic hypogonadism and hyposplenism (Suntharalingham et al., 2015).

4.3. Familial glucocorticoid deficiency (FGD)

FGD or ACTH unresponsiveness/resistance syndromes comprise another group of monogenetic disorders causing PAI (Table 2). Grossly elevated ACTH and very low levels of cortisol are characteristic clinical findings. Mutations of the ACTH receptor (*MC2R*) were the first underlying genetic causes identified in non-syndromic FGD (Clark et al., 1993), while mutations in the *MRAP* gene (Metherell et al., 2005) required for proper function and transport of the MC2R protein to the cell membrane location followed (Miller et al., 2020). They manifest early in life with mostly isolated glucocorticoid deficiency and hyperpigmentation (due to massive ACTH stimulation of MC1R receptors in the skin). FGDs are also characterized by absent adrenarche owing to low adrenal androgen production informing on the essential role of ACTH signaling for zR functionality (Novoselova et al., 2019). Adrenal glands are small and the cortex consists of a disorganized zG without zF and zR (Clark and Weber, 1998). Recent studies in *Mrap−/−* KO mice mimicking the human FGD phenotype revealed grossly dysmorphic adrenals with thickened capsule, deranged zonation and deranged WNT4/β-catenin and SHH signaling (Novoselova et al., 2019).

FGD may also be caused by pathogenic variants in genes involved in

the reactive oxygen species (ROS) detoxification and energy transfer system of the respiratory chain in mitochondria (Maharaj et al., 2019). Obviously adrenal steroid biosynthesis relies heavily on this system. The first mutations in a gene of this kind discovered in a relevant number of patients with FGD were found in Nicotinamide Nucleotide Transhydrogenase (*NNT*) (Maharaj et al., 2019). NNT reduces NADP + at the expense of NADH oxidation and H+ movement down the electrochemical potential across the inner mitochondrial membrane. Lack of NNT activity in *Nnt* null mice impairs peroxide metabolism in intact mitochondria, and hence increases oxidative stress, which seems to impede steroidogenesis. Adrenal histology shows mild hyperplasia and disorganization of the zF, but otherwise normal zonation and steroid enzyme expression. Likewise, genetic variants of thioredoxin reductase 2 (*TXNRD2)*, glutathione peroxidase 1 (*GPX1)* and peroxiredoxin 3 (*PRDX3)* comprised in the thioredoxin and glutathione redox systems were found in patients with a profile of FGD/PAI (Table 2). *Txnrd2* null mice exhibit embryonic lethality with severe growth restriction, cardiac and hematopoietic anomalies but no adrenal defects were reported (Maharaj et al., 2019).

4.4. Syndromic forms of PAI affecting adrenal structure and function

Although it would be conceivable that many genetic disorders identified in syndromic PAI might overlap with genes involved in fetal and adult adrenal development, there is in fact only little overlap so far. This might be due to the fact that such genetic defects are embryonic lethal or that they have simply not yet been identified. One such syndrome is the SERKAL syndrome caused by recessive mutations in the *WNT4* gene (Mandel et al., 2008). It has so far only been reported in two aborted fetuses of a consanguineous family. According to WNT4 signaling playing essential roles in organogenesis (see Table 1), fetuses revealed multiple defects including female to male sex reversal and kidney, adrenal, and lung dysgenesis. Discrepancies between genes involved in PAI and adrenal development can also be ascribed to the fact that most developmental genes have been investigated in the mouse, and it is plausible that some genes identified in mouse studies are not involved in human adrenal development and organization.

Two syndromic forms of PAI have been found among persons manifesting at first with an FGD phenotype, namely the triple A syndrome (AAAS) and the minichromosome maintenance 4 (MCM4) syndrome.

Triple A (also known as Allgrove) syndrome combines PAI with Achalasia of the esophagus, Alacrima. However (progressive) neurological symptoms are also found in most triple A patients including intellectual impairment, sensorineural deafness, peripheral and cranial neuropathies, optic atrophy, parkinsonism and autonomic dysfunction (Huebner et al., 2000; Miller et al., 2020). Alacrima and dysphagia are usually the first clinical signs. Cortisol deficiency is found in most patients, but manifests typically not before adulthood, while mineralocorticoid insufficiency is only rarely encountered. Triple A syndrome is due to autosomal recessive variants in the AAAS gene (12q13) coding for the ALADIN nuclear pore scaffolding protein (Handschug et al., 2001). ALADIN interacts with ferritin heavy chain protein FTH1 during nuclear translocation that renders cells susceptible to oxidative damage. Deficiency of ALADIN impairs the redox potential of adrenal cells and inhibits steroidogenesis. ALADIN is also involved in the regulation of formation of the mitotic spindle. Remarkably, lack of ALADIN in mice does not recapitulate the triple A syndrome phenotype with normal structure and function of adrenals (Huebner et al., 2006).

MCM4 mutations were found with FGD in the Irish Traveler population exclusively (Hughes et al., 2012). The syndrome is characterized by isolated cortisol deficiency, growth failure, increased chromosomal breakage, and natural killer cell deficiency. Patients with MCM4 mutations are susceptible to infections and might carry a higher tumor risk. MCM4 plays a role in DNA replication control (i.e. S-phase). *Mcm4* null mice are embryonic lethal, but hypomorphic *Mcm4* mutant mice survive and recapitulate the human phenotype showing abnormal, non-steroidogenic adrenals (Hughes et al., 2012).

Several other syndromes have PAI included in their spectrum either showing adrenal agenesis or dysplasia (Table 2). But the exact mechanism explaining how the disordered adrenal development and function is caused remains unknown for many of them. In the following we will discuss a couple of syndromes that have been genetically solved only recently.

The IMAGe syndrome comprises Intrauterine growth retardation, Metaphyseal dysplasia, Adrenal hypoplasia, and Genitourinary anomalies (Vilain et al., 1999). Linkage analysis in affected patients and their families identified dominant missense mutations in the specific proliferating cell nuclear antigen (PCNA) domain of the *cyclin-dependent kinase inhibitor 1C* (*CDKN1C)* gene (Arboleda et al., 2012). The *CDKN1C* gene encodes p57KIP2, which inhibits several cyclin-dependent kinases. This gene lies in the imprinted region of chromosome 11p15.5. Only the maternal allele is expressed. Interestingly, different variants in this gene cause Beckwith-Wiedemann overgrowth syndrome (Hatada et al., 1996). By contrast to gain-of-function mutations causing IMAGe syndrome, loss-of-function Beckwith mutations inhibit the cell cycle. IMAGe mutations typically cause restricted growth (Arboleda et al., 2012). Thus variants in the same gene cause both the IMAGe adrenal hypoplasia syndrome with IUGR and the Beckwith-Wiedemann adrenal hyperplasia overgrowth syndrome (Eggermann et al., 2014). Interestingly, biallelic mutations in *POLE1* also cause IMAGe syndrome (Logan et al., 2018). POLε is one of the leading polymerases for DNA replication essential for transmission of genetic information. All *CDKN1C* mutations identified in IMAGE syndrome cluster in the PCNA binding domain. At replication initiation PCNA loads with POLε providing a mechanistic link between *CDKN1C* and *POLE1*.

MIRAGE syndrome is characterized by Myelodysplasia, Infections, Restricted growth, Adrenal hypoplasia, Genital anomalies, Enteropathy and early death (Narumi et al., 2016). Affected individuals have heterozygous mutations of the *SAMD9* gene on chromosome 7q. These mutations cause gain-of-function of the growth repressor SAMD9. SAMD9 is involved in endosome fusion and plays a role in growth factor signaling transduction. Heterozygous *SAMD9* mutations enhance its endosome-fusing activity and may thereby lead to abnormal tissue development including dysgenetic and hypoplastic adrenal glands, ovaries and thymus, and result in overall growth restriction and short survival. In some patients progressive loss of mutated *SAMD9* by somatic adaptive changes in the bone marrow leading to monosomy 7 rescued the negative effect on growth and survival, but increased their risk for myelodysplastic syndromes (Buonocore et al., 2017).

Recessive loss-of-function mutations in the gene for sphingosine-1-phosphate (S1P) lyase 1 (*SGPL1*) cause syndromic PAI associated with steroid-resistant nephrotic syndrome, variably accompanied by ichthyosis, primary hypothyroidism, cryptorchidism, immunodeficiency and neurological anomalies (Janecke et al., 2017; Lovric et al., 2017; Prasad et al., 2017). SGPL1 enzyme catalyzes the final breakdown of sphingolipid S1P. S1P regulates cell migration, differentiation, survival as well as angiogenesis and development. However, the pathogenesis of SGPL1 deficiency within a target organ remains unknown (Lovric et al., 2017). The pathogenesis of PAI in SGPL1 deficiency includes both compromised adrenal development as well as disrupted steroidogenesis

(Prasad et al., 2017). Adrenals of *Sgpl1* null mice revealed marked alterations in adrenocortical zonation, while CYP11A1 expression was significantly decreased. They die within a few weeks and show impaired gonadal steroidogenesis and reproduction (Prasad et al., 2017).

5. Adrenal research models and their opportunities and challenges

5.1. Human disorders

Human disorders are experiments of nature that offer unique opportunities to better understand biology. Through comprehensive phenotyping and genotyping many diseases have been solved to the molecular level and hinted pathophysiology amenable for diagnostic and therapeutic opportunities. Recent advances in next generation sequencing (NGS) using an unbiased approach revealed a genetic diagnosis for many disorders in genes that were not suspected previously. This is clearly also the case for disorders of the adrenal cortex. Currently, NGS approaches are able to find a plausible genetic cause in 60–80% of individuals with PAI (Amano et al., 2017; Guran, 2017). However, NGS creates big data with numerous genetic variants of unknown significance for each individual tested. In addition to bioinformatic tools for analyzing such data, novel gene variants should be tested for their disease causing effect by functional studies and validated in other affected persons (Richards et al., 2015). For these studies several models may be employed.

5.2. Mouse models

The use of animal models for the study of adrenal physiology is often limited due to significant species-specific differences in fetal adrenal structure, steroidogenic pathways and endocrinology of pregnancy (Basham et al., 2016; Ratajczak et al., 2010; Ruiz-Babot et al., 2018). Therefore, different human disorders are recapitulated by a specific animal model to variable extent. For instance, inactivation of both alleles of *WNT4* results in the SERKAL syndrome, which presents with a high mortality rate during fetal life with associated lung, kidney and adrenal dysgenesis; likewise, mice homozygous for *Wnt4* exhibit perinatal lethality with a similar phenotype. In contrast, while *Tcf21* deletion in mice leads to hypoplasia of lungs, kidneys and vasculature, in human this gene is not linked to any disease. On the other hand, the *AAAS* gene is linked to the AAA syndrome in human, but there is no adrenal phenotype in the mouse (see Tables 1 and 2). All these results indicate that researchers and caregivers must use caution when interpreting evidence obtained in other species.

Still, gene inactivation and overactivation studies, together with hormonal manipulation in mouse models have been instrumental to understand key mechanisms in adrenal functional zonation and adult homeostasis. In particular, the past 15 years of adrenal research on mouse models have unveiled the identity and stemness potential of adrenal progenitor cells, opening new venues for understanding the mechanisms of cell differentiation and translating these mechanisms for *in vitro* differentiation of adrenocortical cells (Hammer and Basham, 2021). Results from recent studies on mouse models have started to reveal sex dimorphic aspects of adrenal homeostatic maintenance and regeneration, which have the potential to shed light on the sex bias that characterizes many adrenal disorders (Dumontet et al., 2018; El Wakil et al., 2013; Grabek et al., 2019).

5.3. Cells models and organoids

In the past, mechanistic studies of steroidogenesis have predominantly relied on immortalized cell systems (Rainey et al., 2004). Lately, it has been shown that human fetal adrenal cells retain age-, stem- and endocrine characteristics when grown as organoids in culture (Poli et al., 2019). Such organoids bear the potential to better assist in studies of normal adrenal gland development as well as in studies of the pathophysiology of adrenal hyper- and hypoplasia. In addition, human derived (pluripotent stem) cell models help for mechanistic or diagnostic studies and bear the potential to be developed into personalized cell-based therapeutics. Using this approach, patient derived mesenchymal cells from urine were recently developed into human induced steroidogenic cells (hiSCs) by expression of SF-1 and activation of the PKA and LHRH pathways (Ruiz-Babot et al., 2018). Remarkably, hypocortisolism of hiSCs originating from CAH patients could be rescued by overexpression of the wild-type gene of the disease-causing enzyme.

6. Conclusion and perspectives

Failure of adrenal function is not compatible with survival. Treatment of all forms of adrenal insufficiency consists of replacement of steroid hormones, e.g. mineralocorticoids and glucocorticoids as soon as the diagnosis is made. This can prevent life-threatening adrenal crisis. Sex hormone replacement may be added, if gonadal steroidogenesis is affected. Adrenal androgen excess as a consequence of negative feedback overstimulation through the HPA axis in CAH mainly due to CYP21A2 is often difficult to control with corticosteroid replacement only and may therefore require additional anti-androgenic treatments, especially in females. Thus, there is an unmet need for improved therapy. Better treatment or even a cure for some monogenic forms of PAI may be achieved in the near future through gene therapeutic options. For that comprehensive studies for understanding adrenal cortex development and function in health and disease are fundamental.

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Declaration of competing interest

The authors declare no competing interests.

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