

Novel Insight into Etiology, Diagnosis and Management of Primary Adrenal Insufficiency

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Key Words

Primary adrenal insufficiency · Steroid biosynthesis · Familial glucocorticoid deficiency · Hypocortisolism · Addison's disease · ACTH resistance

Abstract

Primary adrenal insufficiency (PAI) is a rare condition in childhood which is either inherited (mostly) or acquired. It is characterized by glucocorticoid and maybe mineralocorticoid deficiency. The most common form in children is 21-hydroxylase deficiency, which belongs to the steroid biosynthetic defects causing PAI. Newer forms of complex defects of steroid biosynthesis are P450 oxidoreductase deficiency and (apparent) cortisone reductase deficiency. Other forms of PAI include metabolic disorders, autoimmune disorders and adrenal dysgenesis, e.g. the IMAGE syndrome, for which the underlying genetic defect has been recently identified. Newer work has also expanded the genetic causes underlying isolated, familial glucocorticoid deficiency (FGD). Mild mutations of *CYP11A1* or *StAR* have been identified in patients with FGD. *MCM4* mutations were found in a variant of FGD in an Irish travelling community manifesting with PAI, short stature, microcephaly and recurrent infections. Finally, mutations in genes involved in the detoxification of reactive ox-

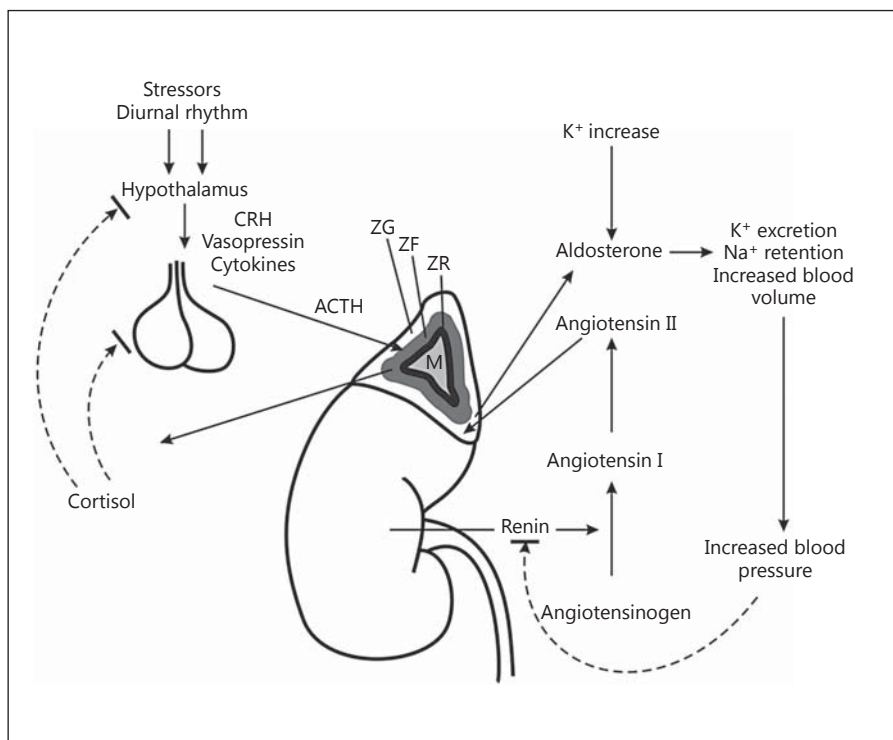
xygen species were identified in patients with unsolved FGD. Most mutations were found in the enzyme nicotinamide nucleotide transhydrogenase, which uses the mitochondrial proton pump gradient to produce NADPH. NADPH is essential in maintaining high levels of reduced forms of antioxidant enzymes for the reduction of hydrogen peroxide. Similarly, mutations in the gene for *TXNRD2* involved in this system were found in FGD patients, suggesting that the adrenal cortex is particularly susceptible to oxidative stress.

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Introduction

Adrenal insufficiency (AI) comprises a fairly large group of disorders characterized by (inappropriately) low production of glucocorticoids (GC) with or without low production of mineralocorticoids (MC). Diagnosis of AI is often delayed due to its unspecific clinical symptoms. However, missed diagnosis of AI or inadequate treatment thereof may be fatal. Recently, new molecular genetic methods have revealed novel genes underlying AI and have enhanced our knowledge of the pathophysiology of these disorders. New formulations of GC which should be able to simulate the circadian rhythm of ste-

Fig. 1. Regulation of GC and MC production in the adrenal cortex. Adrenal cortisol production and secretion is regulated by the HPA axis. Basal diurnal rhythm (regulated by internal ‘clock’ genes) as well as various stress factors prompt the release of CRH which stimulates production and secretion of ACTH from the pituitary gland. ACTH then stimulates cortisol (and androgen) production and release from the zona fasciculata and the zona reticularis. Positive feed-forward regulation pathways are marked with full arrows. Negative feedback control to the hypothalamus and the pituitary gland works directly through cortisol (broken arrows). Aldosterone secretion from the zona glomerulosa is stimulated by 2 major regulators: (1) the renin-angiotensin system and (2) hyperkalemia. Feedback control works through electrolyte and blood pressure changes sensed by osmo- and baroreceptors. ZF = Zona fasciculata; ZR = zona reticularis; ZG = zona glomerulosa; M = medulla of the adrenal glands.



roid secretion are supposed to improve treatment in the near future.

This mini review aims to give an overview and new insights into causes of primary AI (PAI) in children and adolescents as well as new strategies of diagnosis and treatment. For new insights we searched original articles after 2008; to explain relevant pathophysiology we also used published review articles. The PubMed database was searched with the following key terms: ‘adrenal insufficiency, Addison’s disease/crisis, hypocortisolism, (familial) glucocorticoid deficiency, hereditary unresponsiveness to ACTH, ACTH resistance, 11 β -hydroxysteroid dehydrogenase deficiency, (apparent) cortisone reductase deficiency, IMAGE syndrome, and diagnosis and treatment of primary adrenal insufficiency’.

Definition of PAI

PAI is caused by conditions affecting the adrenal cortex, directly resulting in insufficient production of adrenal steroids. In PAI the corticotrophin-releasing hormone (CRH)-ACTH system and the renin-angiotensin system are intact and secrete high amounts of stimulatory hormones. Secondary AI on the other hand is caused

by an impaired effect or secretion of ACTH from the pituitary gland for adrenal stimulation, while tertiary AI results from the disrupted release or effect of CRH from the hypothalamus [1]. For an overview of the hypothalamic-pituitary-adrenal (HPA) axis and its regulatory systems see figure 1.

Clinical Features of PAI

Clinical characteristics of PAI in children may be non-specific. Typical signs include fatigue, vomiting, nausea and abdominal pain. Other symptoms related to GC deficiency are signs associated with hypoglycemia (such as paleness, sweating, disorientation and mood swings), weakness, morning headaches and failure to thrive. MC deficiency may typically manifest as dehydration, collapse, hypotension, tachycardia, dizziness, weight loss and salt craving. In a chronic state of PAI, negative feedback leads to increased ACTH secretion which will overstimulate the melanocortin 1 receptors (MC1R – a family member of the ACTH receptor MC2R) in the skin, leading to typical hyperpigmentation. This hyperpigmentation may be best observed on nail beds, mucous membranes, hand lines, scars and sunlight-unexposed body areas.

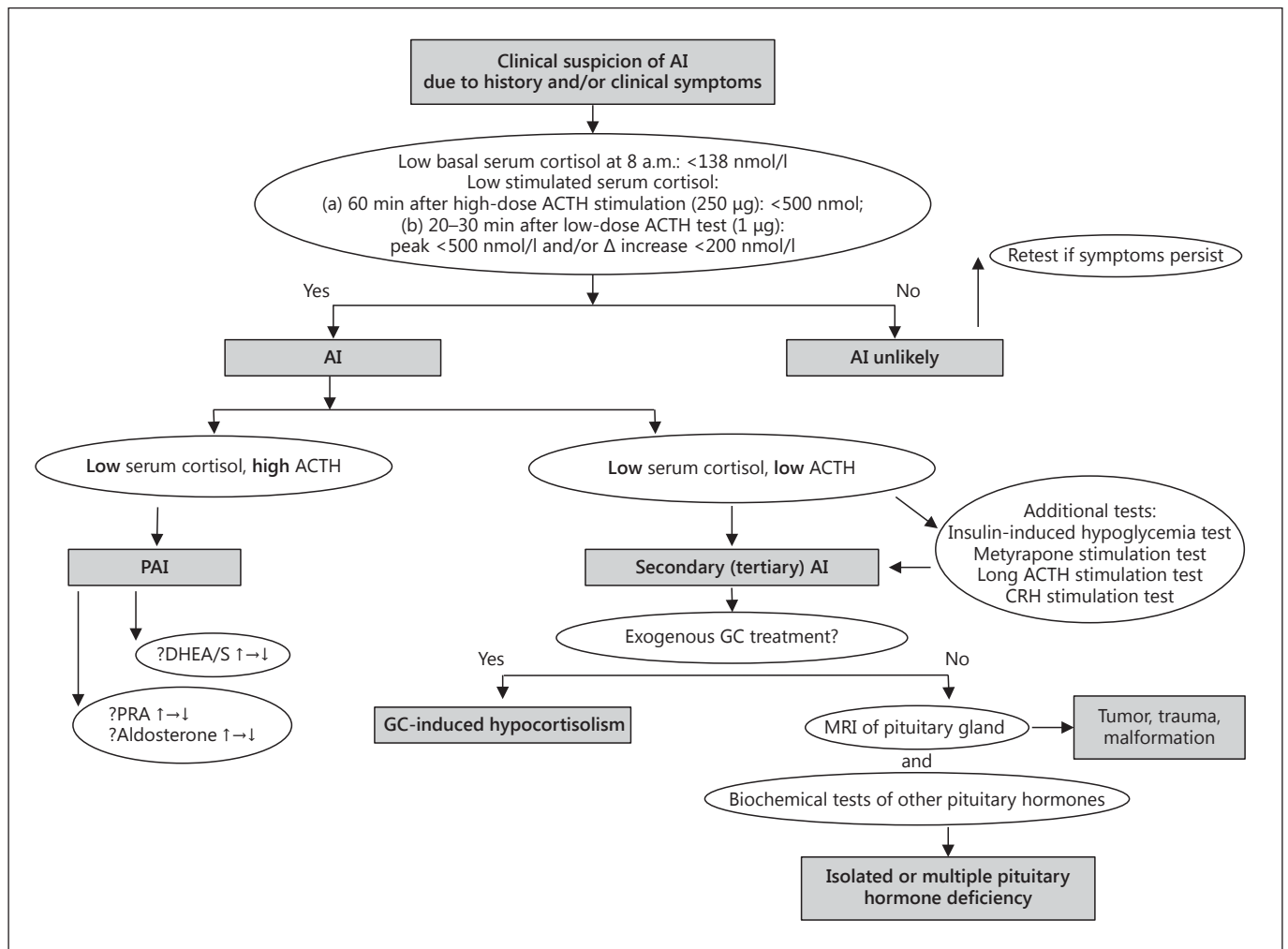


Fig. 2. Flow chart for diagnosing AI. Diagnosis of AI is based on clinical suspicion and low level of basal or stimulated cortisol. Measurement of serum ACTH may distinguish between primary and secondary AI. Drug history, additional endocrine tests, measurements of other pituitary hormones and imaging studies are

used to characterize the exact cause of secondary (or tertiary) AI. In the diagram diagnostic tests are shown in round borders while specific diagnoses of AI are shown in square borders. Normative values are taken from Flück [2].

Biochemical Findings

Typical laboratory findings of AI include hyponatremia with or without hyperkalemia, hypochloremia, metabolic acidosis and fasting hypoglycemia accompanied by low levels of cortisol and aldosterone but high levels of ACTH and plasma renin activity (PRA) [1, 2]. However, often not all biochemical findings are present at clinical presentation; for example hyponatremia is found in 90% of patients, while hyperkalemia is seen in only 50% of patients [3]. Secondary and tertiary AI are not accompanied by MC deficiency and ACTH is typically low or undetectable (fig. 2). Steroid profiling from serum or urine using chro-

matographic mass spectrometric methods (GC/MS or LC/MSMS) presents the best diagnostic tool for unravelling complex defects of adrenal steroidogenesis [4]. Despite the time-consuming preanalytical sample preparation, GC/MS urine steroid profiling is ideal for diagnosing genetic defects in newborn infants, such as 21-hydroxylase (21OHD) or P450 oxidoreductase deficiency (PORD), or in patients with adrenocortical cancers [5]. LC/MSMS is especially suitable for the measurement of circulating steroid hormones in the serum. This method has become more prevalent in many experimental but also reference or clinical laboratories in the past few years. It offers a good alternative for second-tier screening of 21OHD [5].

Diagnosis of PAI

The diagnosis of PAI is established with a low serum cortisol combined with an elevated ACTH (fig. 2) [2]. Evaluation for MC deficiency and work-up for other diseases (including diabetes mellitus, hypothyroidism, etc.) is crucial. For all laboratory evaluations, it is important to interpret the results with an understanding of influencing factors such as age, sex, pubertal stage, timing of blood drawing (e.g. 8 a.m. fasting), and stress level during blood drawing, as well as the type of analytical method applied [3, 6, 7]. GC deficiency is usually determined by measuring a morning (before 9 a.m.) cortisol and ACTH. A low morning cortisol or a cortisol in the lower normal range in stressful situations (such as fever, pain or infection) is a clear marker of hypocortisolism. In addition, morning ACTH values and a short/rapid ACTH test may discriminate between PAI and ACTH deficiency and unmask an inadequate reaction of the adrenal cortex of cortisol secretion upon ACTH stimulation. An extremely elevated serum ACTH in the context of a subnormal morning cortisol confirms the diagnosis of PAI or ACTH resistance. By contrast, low serum ACTH in the context of low morning cortisol is suggestive of secondary or tertiary AI (fig. 2). In complex cases of PAI due to congenital adrenal hyperplasias (CAH) the steroid profile from urine or serum might be more helpful as all steroids and metabolites of interest can be seen in one single analysis [5].

MC deficiency manifests with electrolyte disturbances including hyponatremia, hypochloremia, hyperkalemia and metabolic acidosis. PAI with MC deficiency (e.g. aldosterone) leads to elevated PRA. However, PRA is also influenced by blood pressure, salt intake and renal function (fig. 1). Therefore, besides the assessment of electrolytes, renal function tests and measurements of aldosterone and PRA help to exclude a renal disorder and confirm the diagnosis of PAI.

Adrenal androgens play a less important role in the diagnostics of PAI. Except for some types of CAH (e.g. 21OHD), androgens are generally low in PAI. In addition, adrenal androgens are physiologically low before adrenarche. After adrenarche and puberty, adrenal androgens comprise about half of the circulating androgen pool in women. Therefore, adrenal androgen deficiency might be an issue in adult women and not necessarily of the child.

Because PAI occurs with a wide spectrum of disorders, further diagnostic tests are usually needed to establish the exact diagnosis (fig. 3). These include additional immunological tests (e.g. anti-cortex adrenal antibodies and

anti-21OH antibodies for autoimmune adrenalitis [8]) and metabolic tests (e.g. very long-chain fatty acids) for adrenoleukodystrophy or 7-dehydrocholesterol for Smith-Lemli Opitz disease [9, 10], as well as markers of anticoagulation (APTT, Quick, D-dimers, anti-cardiolipin antibodies for adrenal infarction in the antiphospholipid syndrome [11]). In addition, adrenal imaging (CT or MRI) may be informative. Finally, molecular genetic analysis is essential in confirming the exact diagnosis for congenital forms of PAI [1].

Causes of PAI

PAI is often classified into acute and chronic. However, acute PAI may also suddenly arise from decompensated conditions of chronic AI. The spectrum of PAI differs between children and adults. While more than 80–90% of cases of PAI in adults are caused by autoimmunity [12, 13], CAH is the most frequent cause of PAI in young children [1, 3, 12, 14]. A very common cause of AI in all age groups is abruptly interrupted GC treatment. Table 1 summarizes all reported causes of PAI, both inherited and acquired. In the following text, we will mostly focus on newer forms of inherited PAI.

PAI due to Steroid Biosynthetic Defects

CAH is a group of autosomal recessive disorders characterized by impaired steroidogenesis including hypocortisolism. The most common defect is 21OHD caused by mutations in the *CYP21A2* gene. 21OHD comes in a classic form (either salt-wasting or simple virilizing) and a nonclassic form (late-onset), depending on the remaining enzyme activity. For 21OHD there is a good genotype-phenotype correlation, and numerous studies have described more than 100 mutations (Human Gene Mutations Database, www.hgmd.cf.ac.uk). For review and treatment recommendations, we refer to the newest CAH guidelines published in 2010 [15]. Other types of CAH such as 11 β -hydroxylase deficiency, 3 β -hydroxylase deficiency, 17 α -hydroxylase/17,20-lyase deficiency, congenital lipoid adrenal hyperplasia and P450scc deficiency are less common and more details may be found elsewhere [1, 16]. Generally, these defects can be clinically and biochemically distinguished and are confirmed using genetic tests. Defects in the *StAR* or *CYP11A1* genes on the other hand are more difficult to discriminate from each other as well as from other congenital forms of PAI (e.g. familial GC deficiency, FGD), especially if the protein activity is not completely lost with nonclassic forms. There-

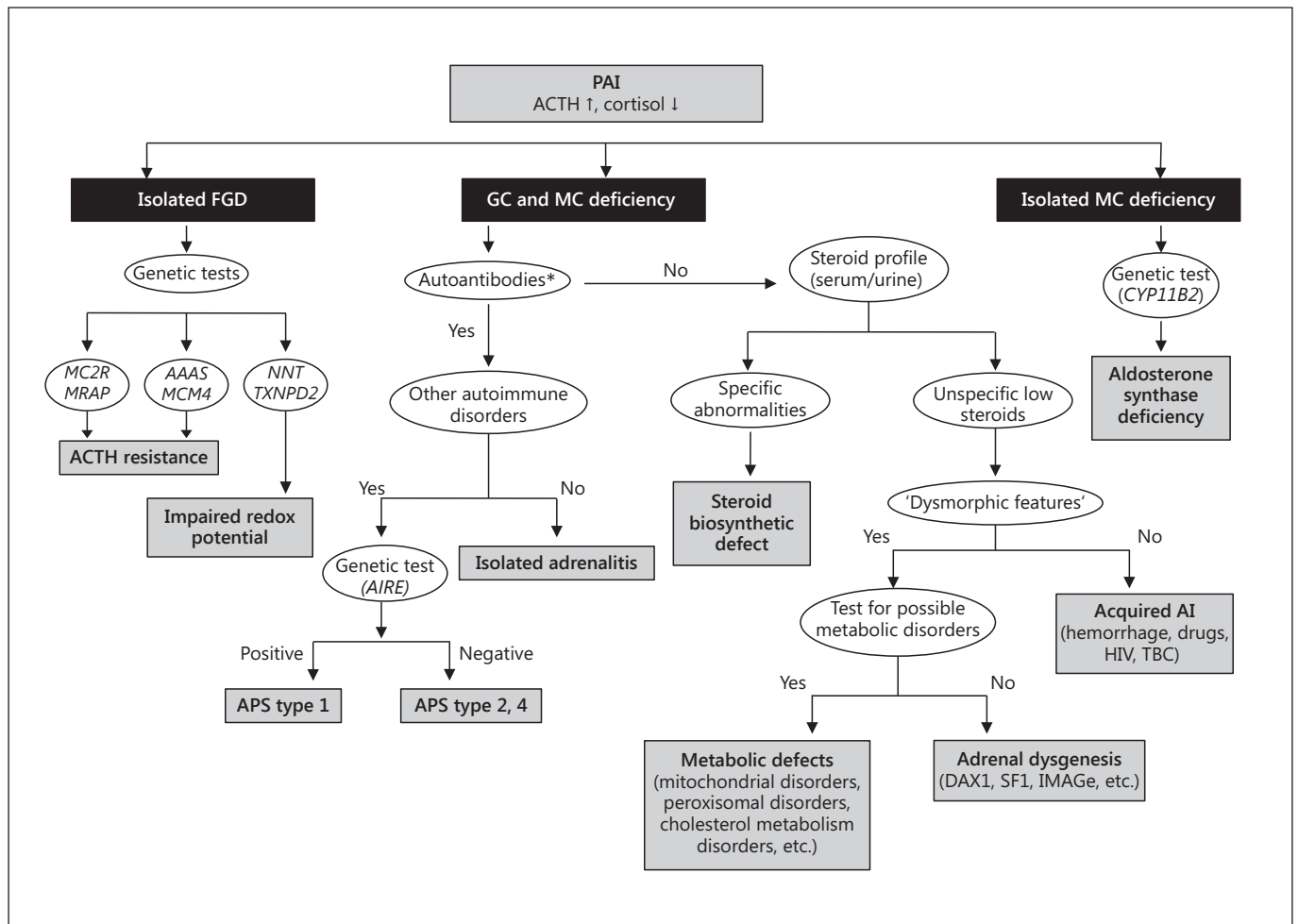


Fig. 3. Flow chart for finding the underlying cause of PAI. PAI may be subdivided into 3 groups according to steroid deficiency: (1) isolated GC deficiency, (2) combined MC and GC deficiency and (3) isolated MC deficiency. Underlying causes of isolated FGD and isolated MC deficiency may be best established by genetic analysis. Combined MC and GC deficiency may be due to autoimmune disorders, defects in steroid biosynthesis (CAH) or adrenal dysgenesis. Metabolic disorders may manifest with PAI as well. Acquired

causes of PAI include drugs, infectious diseases (e.g. HIV), trauma and infarction. Diagnostic tests are given in round boarders. Specific causes of PAI are given in square boarders. Gene names are written in italics. The asterisk indicates antibodies against adrenal cells, adrenal cellular contents or some steroid enzymes such as P450_{sc}, P450_{c17} or P450_{c21}. APS = Autoimmune polyglandular syndrome.

fore, diagnosis of StAR or CYP11A1 deficiency depends largely on genetic analysis [1, 16, 17].

CAH may also be caused by defects in cofactors. P450 oxidoreductase (POR) is a crucial electron donor to all microsomal P450 cytochrome (CYP) enzymes including 17 α -hydroxylase (CYP17A1), 21OHD (CYP21A2) and P450 aromatase (CYP19A1) for steroidogenesis [18, 19]. Clinical presentation of PORD includes AI, 46,XY DSD and 46,XX DSD with incomplete pubertal development and also mild-to-severe skeletal malformations known as

Antley-Bixler craniosynostosis syndrome in its severest form [18, 20]. Clinical and biochemical diagnosis is difficult and requires steroid profiling and genetic testing. Genotype-phenotype correlation has been well described for the most common Caucasian mutation (A287P) and the most common Japanese mutation (R457H) [21, 22]. Prenatal diagnosis of PORD seems possible through steroid profiling of maternal urine and prenatal sonogram focusing on skeletal malformations [23].

Table 1. Causes of PAI in children and adolescents

Disorder	Gene	OMIM	Associated clinical features
<i>Defects of steroid biosynthesis</i>			
Congenital lipoid adrenal hyperplasia	<i>StAR</i>	201710	46,XY DSD, gonadal insufficiency
P450 side chain cleavage syndrome	<i>CYP11A1</i>	118485	46,XY DSD, gonadal insufficiency
3 β -hydroxysteroid dehydrogenase deficiency (CAH)	<i>HSD3B2</i>	201810	46,XY DSD and 46,XX DSD, gonadal insufficiency
21-hydroxylase deficiency (CAH)	<i>CYP21A2</i>	201910	46,XX DSD, androgen excess syndrome, testicular adrenal rest tumors
11 β -hydroxylase deficiency (CAH)	<i>CYP11B1</i>	202010	46,XX DSD, hypertension, androgen excess syndrome
17-hydroxylase deficiency (CAH)	<i>CYP17A1</i>	202110	46,XY DSD, hypertension, gonadal insufficiency
P450 oxidoreductase deficiency (CAH)	<i>POR</i>	613571	46,XY DSD, 46,XX DSD, gonadal insufficiency, Antley-Bixler skeletal malformation syndrome; changes in drug metabolism
Aldosterone synthase deficiency	<i>CYP11B2</i>	124080	Isolated MC deficiency
Cortisone reductase deficiency	<i>HSD11B1</i>	614662	Androgen excess syndrome
Apparent cortisone reductase deficiency	<i>H6PDH</i>	604931	Androgen excess syndrome
<i>Adrenal dysgenesis</i>			
X-linked adrenal hypoplasia congenita	<i>NROB1 (DAX1)</i>	300200	Hypogonadotropic hypogonadism, in some cases gonadotropin-independent precocious puberty
Steroidogenic factor 1 deficiency	<i>NR5A1 (SF1)</i>	184757	46,XY DSD, gonadal insufficiency
IMAGe syndrome	<i>CDKN1C</i>	300290	IUGR, bone disorders and anomalies, genital anomalies, hypercalcemia, dysmorphic facial features
Pallister-Hall syndrome	<i>GLI3</i>	165240	Hypothalamic hamartomas, mesoaxial and postaxial polydactyly, bifid epiglottis, imperforate anus, genitourinary anomalies
Meckel syndrome	<i>MKS1</i>	249000	cystic renal disease, CNS malformation – occipital encephalocele, polydactyly, hepatic abnormalities
Pena-Shokeir syndrome	<i>DOK7, RAPSN</i>	208150	Arthrogryposis, facial anomalies, IUGR, camptodactyly, fetal akinesia, polyhydramnion, pulmonary hypoplasia, cardiac defects, intestinal malrotation
Pseudotrisomy 13		264480	Holoprosencephaly, polydactyly, craniofacial anomalies
Hydrolethalus syndrome	<i>HYLS1</i>	236680	Hydrocephaly, micrognathia, polydactyly, abnormal genitalia, congenital heart defects, respiratory organ defects
Galloway-Mowat syndrome		251300	Nephrotic syndrome, microcephaly, encephalopathy, diaphragmatic hernia
<i>ACTH resistance</i>			
FGD	<i>MC2R</i>	202200	Mostly normal production of MC, tall stature
	<i>MRAP</i>	607398	
DNA repair defect	<i>MCM4</i>	609981	NK cell deficiency, short stature, microcephaly, recurrent viral infections, chromosomal breakage
AAA syndrome (Allgrove syndrome)	<i>AAAS</i>	231550	Alacrimia, achalasia, deafness, mental retardation, hyperkeratosis
Deficiency of mitochondrial radicals detoxification	<i>NNT</i>	614736	Only GC deficiency
	<i>TXNRD2</i>	606448	Only GC deficiency
<i>Cholesterol synthesis disorders</i>			
Wolman disease	<i>LIPA</i>	278000	Xanthomatous changes in the liver, adrenals, spleen, lymph nodes, bone marrow, small intestine and thymus, diffuse punctate adrenal calcification, hepatosplenomegaly, poor weight gain, hypercholesterolemia, steatorrhea
Smith-Lemli Opitz disease	<i>DHCR7</i>	270400	Multiple congenital malformation and mental retardation syndrome
Abeta-lipoproteinemia	<i>MTP</i>	200100	Ataxia, retinopathy, acanthocytosis, pathologic fat absorption
Familial hypercholesterolemia	<i>LDLR</i>	143890	Xanthomas, corneal arcus, and coronary artery disease
<i>Metabolic disorders: peroxisomal defects</i>			
X-linked adrenoleukodystrophy	<i>ABCD1</i>	300100	Progressive neurodegeneration, dementia, progressive behavioral disturbances, vision and hearing loss, spasticity and seizures; accumulation of very long-chain fatty acids
Neonatal adrenoleukodystrophy	<i>PEX1</i>	601539	Hypotonia, seizures, diffuse encephalopathy, sensorineural hearing loss, peripheral neuropathy, mild facial dysmorphism; autosomal recessive

Table 1 (continued)

Disorder	Gene	OMIM	Associated clinical features
Infantile Refsum disease	<i>PHYH, PEX7</i>	266500	Anosmia, retinitis pigmentosa, neuropathy, deafness, ataxia, ichthyosis
Zellweger syndrome	<i>PEX1</i> and other <i>PEX</i> genes	214100	Severe neurologic dysfunction with handicaps, craniofacial abnormalities, severe mental retardation, hepatomegaly, growth failure, stippled epiphysis, genitourinary anomalies
<i>Metabolic disorders: mitochondrial defect</i>			
Kearns-Sayre syndrome	<i>mitDNA del</i>	530000	Progressive external ophthalmoplegia, pigmented retinopathy, cardiac conduction block, cerebellar ataxia; other endocrine pathologies
<i>Autoimmune disorders</i>			
Isolated autoimmune adrenalitis	<i>CLTA-4, HLA-DR3, HLA-DR4, HLA-B8</i>		
Autoimmune polyglandular syndrome, type 1	<i>AIRE</i>	240300	Hypoparathyroidism, candidiasis, autoimmune hypergonadotropic hypogonadism, autoimmune thyroid diseases alopecia, chronic autoimmune hepatitis, pernicious anemia, vitiligo
Autoimmune polyglandular syndrome, type 2	<i>CLTA-4, HLA-DR3, HLA-DR4</i>	269200	Autoimmune thyroid diseases, T1DM, premature ovarian failure, pernicious anemia, celiac disease
Autoimmune polyglandular syndrome, type 4	<i>CLTA-4, HLA-DR3, HLA-DR4</i>		Combination of autoimmune diseases not included in previous groups
<i>Acquired defects</i>			
Hemorrhage			In newborn infants or neonates after delivery, coagulopathy
Infections			Septic shock, Waterhouse-Friderichsen syndrome – meningococcal disease, tuberculosis, fungi, HIV-1, CMV
Surgery, trauma			Bilateral adrenalectomy (McCune-Albright syndrome)
Infiltration			Metastasis, lymphomas, amyloidosis, sarcoidosis, hemochromatosis
Drugs			GC treatment withdrawal syndrome; ketoconazole, rifampicin, phenytoin, phenobarbital, etc.

Cortisone Reductase and Apparent Cortisone Reductase Deficiency

Cortisol is converted to cortisone, a metabolically inactive steroid, by 11 β -hydroxysteroid dehydrogenase type 2 (HSD11B2) which is expressed in MC-responsive tissues (e.g. distal nephron, placenta) – in principal, to protect the MC receptor from cortisol and safeguard it for aldosterone [16]. By contrast, cortisone can be reactivated to cortisol by HSD11B1. Cortisone reductase deficiency (CRD) and apparent CRD are due to inadequate recovery of cortisol from the inactive prohormone cortisone [1, 16]. This conversion is catalyzed by the enzyme HSD11B1 in GC-responsive tissues (liver, brain, adipose tissues, proximal nephron, testis). HSD11B1 is an NADP(H)-dependent oxidoreductase which in vivo prefers the reductive over the oxidative activity if NADPH is abundant [16]. As HSD11B1 is located in the endoplasmic reticulum, its co-factor NADPH to NADP⁺ ratio depends on hexose-6-phosphate dehydrogenase (H6PDH) activity which generates NADPH. Therefore, genetic defects in both *HSD11B1* and *H6PDH* lead to deficient cortisol produc-

tion in affected tissues and prompt a chronic feedback stimulation of ACTH [24–26]. As a consequence of this chronic ACTH stimulation the adrenal cortex will produce more cortisol but also adrenal androgens (C19 steroids). Thus the urinary steroid profile shows characteristically decreased cortisol metabolites and increased cortisone metabolites. CRD is caused by mutations in the *HSD11B1* gene and manifests with hyperandrogenism, premature adrenarche and polycystic ovaries. The first 2 heterozygote mutations in *HSD11B1* (R137C, K187N) were identified in two girls with premature pubarche [26]. Compared to CRD, apparent CRD seems to present with a similar but more severe phenotype. Loss of H6PDH activity leads to reduced NADPH generation, resulting in decreased reductive and enhanced oxidative activity of HSD11B1 – thus overall less cortisone recovery [25]. To date 8 mutations in *H6PDH* have been described [24, 25].

New Insights into FGD

FGD is a rare heterogeneous condition characterized by ACTH resistance with decreased synthesis of GC and

mostly normal production of MC [27]. A high ACTH level can be clinically appreciated with hyperpigmentation of the skin and mucous membranes. In addition, patients suffer from failure to thrive, hypoglycemia, severe infections and fatigue. To date, the underlying genetic disorder is known in approximately 70% of patients with FGD [28]. The most common type is caused by a defect in the ACTH receptor MC2R, which is a 7-membrane G-protein coupled receptor located (almost) exclusively in the adrenocortical cells [29]. To date, 47 mutations have been described in the *MC2R* gene (Human Gene Mutations Database, www.hgmd.cf.ac.uk). The second most common cause of FGD is a defect in the MC2R accessory protein (MRAP, encoded by *MRAP*) which serves as a cofactor of MC2R to promote its trafficking to the plasma membrane [30]. Interestingly, mutations in *StAR* and *CYP11A1* have also been found in patients with FGD [18, 31, 32]. Typically, mutations which do not cause a complete loss of *StAR* or *CYP11A1* activity may be associated with an FGD phenotype, also termed nonclassic lipoid CAH [1, 28, 31].

Recently, Hughes et al. [33] and Gineau et al. [34] found mutations in the mini chromosome maintenance-deficient 4 homolog gene (*MCM4*) in an Irish travelling community manifesting with a special variant of FGD using linkage analysis and targeted next generation sequencing. These patients were found to have short stature, chromosomal breakage, natural killer cell deficiency and progressive PAI characterized by ACTH resistance with GC deficiency and normal MC levels. Typically, patients started with normal adrenal function and developed PAI over time. *MCM4* is part of a heterohexameric helicase complex which is important for DNA replication and genome integrity. *MCM* deficiency leads to cell cycle disturbances and genomic instability and is associated with cancer and developmental defects. The c.71-1insG splice site mutation found in the Irish travelling community was predicted to lead to a frameshift with a prematurely terminated translation product (p.Pro24ArgfsX4). However, in patients an 85-kDa *MCM4* protein was found, most likely due to the use of an alternative translational start site; in contrast, controls expressed 2 – the larger 96-kDa and the smaller 85-kDa *MCM4* protein [33, 34]. *MCM4* knockout is embryonically lethal in mice [35]. Therefore, the pathogenicity of defective *MCM4* has been studied in a heterozygous *MCM4* mouse model (*Mcm4*^{Chaos3/-}/*Mcm3*^{+/-}) [33, 35]. Atypical, nonsteroidogenic cells were found in the adrenal cortex (nonexpressing *CYP11A1* and *CYP11B1*) which displaced steroidogenic cells, presumably decreasing the GC output of the

zona fasciculata. Thus, given the overall important function of *MCM4*, follow-up of those patients is highly recommended, especially for surveillance of tumor development.

Only a couple of years ago, novel genes involved in mitochondrial reactive oxygen species (ROS) detoxification have been implicated in FGD (fig. 4) [28]. The main representative of the group of ROS-containing oxygen atoms is hydrogen peroxide, which is mainly formed through the breakdown of superoxide anions produced by partial oxygen reduction during aerobic respiration [36]. Tight regulation of ROS formation is crucial for signaling pathways controlling cell growth, differentiation, migration and apoptosis [36]. In addition to physiological regulation, ROS are also implicated in the pathophysiology of inflammation, type 2 diabetes mellitus, obesity, arthritis and cardiomyopathy, where in principal an increase in ROS is linked with cytotoxic stress and cell death [37, 38].

Detoxification of hydrogen peroxide is generally provided by catalase, glutathione peroxidase and thioredoxin peroxidase enzymes, which serve as the major antioxidant enzymes in the cell [36]. The catalytic reduction of hydrogen peroxide by thioredoxin peroxidases involves the oxidation of catalytic cysteine residues, while the catalytic mechanism of glutathione peroxidases involves the oxidation of catalytic cysteine or selenocysteine residues (fig. 4) [36]. High concentrations of NADPH are necessary to regenerate reduced glutathione (GSH) from oxidized glutathione (GSSG) and to maintain a high GSH/GSSG ratio. Similarly, the reduction of oxidized thioredoxin depends on NADPH and thioredoxin reductase. To allow these reactions, the required amount of NADPH is produced by nicotinamide nucleotide transhydrogenase (*NNT*, encoded by the *NNT* gene) which is a redox-driven proton pump located in the inner mitochondrial membrane (fig. 4) [38]. The adrenal cortex contains a high amount of P450 steroid enzymes which use NADPH for their catalytic activity. Its function is therefore very sensitive to ROS [39].

In 2012 Meimaridou et al. [40] identified first mutations in the *NNT* gene in 20 patients with pure FGD in whom mutations of *MC2R*, *MRAP* and *StAR* had not been found. Mice carrying a spontaneous *Nnt* mutation showed structural anomalies of their adrenal cortex, higher levels of apoptosis in the zona fasciculata and decreased corticosterone production [40]. Similarly, knock-down of *NNT* in human adrenal NCI-H295R cells revealed increased apoptosis and elevated levels of ROS. The ratio of GSH to GSSG was diminished, indicating

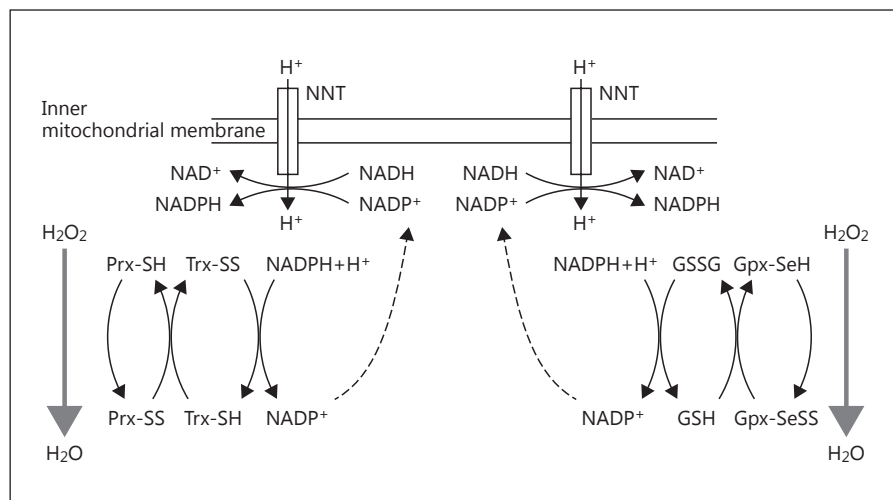


Fig. 4. Overview of the redox cycling reactions involved in the reduction of hydrogen peroxide in the mitochondrion (ROS detoxification). NNT is located in the inner mitochondrial membrane and uses the proton gradient built up by the mitochondrial proton pumps to produce NADPH. High concentrations of NADPH are essential for regenerating GSH and reduced thioredoxin (Trx-SH) from GSSG and oxidized thioredoxin (Trx-SS), respectively. High GSH and Trx-SH levels are required for the detoxification of ROS by glutathione peroxidases (Gpx) and peroxiredoxin (Prx). The catalytic reduction of hydrogen peroxide by Gpx involves the ox-

idation of catalytic thiol groups on selenocysteine (SeH) or cysteine (SH) residues in Gpx. The recycling of oxidized peroxidase enzymes involves oxidation of reduced GSH, while the reduction of oxidized GSSG depends on NADPH and glutathione reductase. By contrast, the catalytic reduction of hydrogen peroxide by thioredoxin peroxidases (Prx) involves the oxidation of SH residues in thioredoxin peroxidases (Trx-SS). The recycling of oxidized peroxidase enzymes (Prx-SH) involves oxidation of Trx-SH. Finally, the reduction of Trx-SS depends on NADPH and thioredoxin reductase [36, 43].

that NNT is important for maintaining the mitochondrial redox potential [40].

Very recently, Metherell et al. [30] in London found a first mutation in the thioredoxin reductases 2 (*TXNRD2*) gene (Y447X) in a consanguineous Kashmiri family presenting with FGD. RT-PCR showed ubiquitous expression of the *TXNRD2* gene in human tissues with high expression in the adrenal cortex [41]. *TXNRD2* knockdown in adrenocortical NCI-H295R cells revealed increased production of ROS and susceptibility to oxidative stress. Two heterozygous mutations in the *TXNRD2* gene have been described previously in 3 patients with dilative cardiomyopathy, but no data are available on their adrenal function [42]. Overall, mammals have three thioredoxin reductases but only *TXNRD2* is synthesized in mitochondria [43]. For further insight we recommend the excellent review of Lu and Holmgren [43].

Adrenal Dysgenesis Caused by IMAGE Syndrome

The IMAGE syndrome was first described in 1999 and is defined by the combination of intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia congenita and genital anomalies [44]. In some cases it

is associated with hypercalciuria and hypercalcemia. The genetic mutations causing IMAGE syndrome have been identified in the PCNA-binding domain of the imprinted cyclin-dependent kinase inhibitor 1C (*CDKN1C*, also known as *P57KIP2*) [45]. *CDKN1C* protein has a conservative structure which serves as a negative regulator of cell cycle progression by inhibiting G1 of CDKs. *CDKN1C* is located in the imprinted region of chromosome 11p15.5. *CDKN1C* mutations were found in familial as well as sporadic cases of IMAGE syndrome. However, familial inheritance is possible through mutations on the maternal allele only.

Interestingly, genetic defects in the same gene may cause Beckwith-Wiedemann syndrome or familial Russell-Silver syndrome (RSS) [46, 47]. Whereas *CDKN1C* mutations found in Beckwith-Wiedemann syndrome result in protein loss of function and thus loss of cell cycle inhibition with overt proliferation [46], mutations found in IMAGE syndrome are localized in a highly conserved region of the PCNA-binding domain of *CDKN1C* and lead to inhibition of growth and differentiation [45, 48]. However, mutations in the PCNA-binding domain of *CDKN1C* are also reported in familial cases of RSS [47].

Remarkably, a mutation of Arg279Leu has been identified in a familial case of RSS manifesting with intrauterine growth retardation but no features of IMAGE syndrome. On the other hand, at the same amino acid location an Arg279Pro mutation was found in patients with IMAGE syndrome. Functional analysis revealed that Arg279Leu (RSS) did not affect the cell cycle, whereas the Arg279Pro mutation (IMAGE) led to a gain of function and thus cell cycle inhibition [47].

Treatment of PAI

AI is potentially life threatening. Adequate supplementation of lacking GC and MC is essential. Patients must be trained in how to adjust therapy during stressful situations and are advised to carry an emergency card [1].

For GC replacement, hydrocortisone is the drug of choice in children as its short half-life allows normal growth (although not mimicking circadian rhythm). A replacement dose of hydrocortisone for PAI is recommended at 8–10 mg/m²/day in children or 15–25 mg/day in adults [6] divided into 3 or 4 doses. Adequate dosing can be monitored by a history of general well-being of the patient and may be achieved without suppressing morning ACTH. However, emerging evidence suggests that doses may be personalized due to a wide variability of individual sensitivity to GC probably caused by polymorphisms in the GC receptor [49]. The challenge of GC treatment is to balance between overtreatment and undertreatment, both predominantly affecting growth during childhood.

MC replacement is accomplished with the drug fludrocortisone, which is given in 1 or 2 doses per day at a total of 50–200 µg/day controlled by blood pressure, normal electrolytes and PRA [1]. Relatively high doses are needed in newborn infants due to a high resistance of MC. In addition, infants with severe MC deficiency may require salt supplementation to maintain their electrolyte balance in the first months of life.

Androgen (e.g. dehydroepiandrosterone, DHEA) replacement is definitively not needed during childhood and controversially discussed in adult women suffering from PAI [50].

Treatment of Patients with 21OHD

In patients with 21OHD (and 11OHD), accompanying hyperandrogenism complicates replacement therapy [1, 15]. Required doses of hydrocortisone are higher (9–15 mg/m²/day) to suppress ACTH and thereby adre-

nal androgen production. However, on the one hand, higher doses may restrict linear growth resulting in lower final height and osteoporosis, while on the other hand, low supplementation with hydrocortisone may lead to increased androgen concentrations and thus potentially to precocious puberty and similarly decreased final height.

Management of Adrenal Crisis

Adrenal crisis is a rare, acute, life-threatening complication of AI. The most important triggering factors include infectious diseases (particularly gastrointestinal infections), perioperative conditions and exhaustive physical activity [51]. Patients with AI and their caregivers must be trained in the management of an imminent adrenal crisis. Emergency therapy consists of immediate administration of hydrocortisone as an intravenous bolus (e.g. 50–100 mg/m² with higher doses recommended in younger children) followed by continuous infusion or repeated boluses every 6–8 h. During less stressful situations (fever, vomiting, minor surgery) the usual doses of oral hydrocortisone are recommended to be doubled or tripled [1].

Novel Modified-Release GC Formulations

In adults suffering from AI, decreased quality of life (especially chronic fatigue) has been linked to insufficient treatment [52]. Current GC replacement therapies cannot mimic the normal circadian rhythm of cortisol. Thus, new strategies and medications for a near physiological GC supplementation are currently being developed. As in type 1 diabetes, timed continuous subcutaneous application of hydrocortisone would be possible but is impractical for daily life [53]. Oral, modified-release hydrocortisone formulations provide a promising novel group of drugs [54–56]. Plenadren[®] (ViroPharma SPRL, Brussels, Belgium) is currently available on the market for the treatment of AI, which provides a rapid increase in cortisol levels after intake in the morning followed by a gradual decrease over the day to almost undetectable levels in the night similar to the normal circadian rhythm [57]. Maybe even more promising for the treatment of AI in 21OHD is Chronocort[®], which is currently being tested in clinical trials [52, 58, 59]. In 2 doses it might be able to provide an optimal cortisol profile to mimic the physiology of GC production and suppress adrenal androgens. Similarly, time-release prednisone (Lodotra[®]; Horizon Pharma GmbH, Mannheim, Germany) has been tested in patients with rheumatoid arthritis [60] and in adult patients with AI [61].

However, it is important to note that none of these newer formulations have been tested in children yet. Modified-release hydrocortisone formulations may also be beneficial to children with AI but clinical trials looking at the outcome of growth particularly are mandatory.

Conclusion and Perspective

Novel genetic approaches have elucidated new causes of PAI and broadened our understanding of its pathomechanisms. In the last decade, we have learned that the same clinical presentation (e.g. FGD) may be due to different genetic defects. We have also learned that one gene may cause a broad range of phenotypes (e.g. StAR, PORD). Cofactor disorders lead to apparent steroid enzyme deficiencies. Recently, genetic mutations disrupting the mitochondrial redox homeostasis were shown to

cause disordered steroidogenesis. Next generation sequencing of genetically unsolved patients with PAI will reveal further genes of known and unknown function and challenge our current knowledge of human adrenal steroidogenesis.

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Disclosure Statement

The authors declare no conflict of interest and declare that neither the manuscript nor parts of it are submitted elsewhere.

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