MECHANISMS IN ENDOCRINOLOGY

Update on pathogenesis of primary adrenal insufficiency: beyond steroid enzyme deficiency and autoimmune adrenal destruction

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Abstract

Primary adrenal insufficiency (PAI) is potentially life threatening, but rare. In children, genetic defects prevail whereas adults suffer more often from acquired forms of PAI. The spectrum of genetic defects has increased in recent years with the use of next-generation sequencing methods and now has reached far beyond genetic defects in all known enzymes of adrenal steroidogenesis. Cofactor disorders such as P450 oxidoreductase (*POR*) deficiency manifesting as a complex form of congenital adrenal hyperplasia with a broad clinical phenotype have come to the fore. In patients with isolated familial glucocorticoid deficiency (FGD), in which no mutations in the genes for the ACTH receptor (*MC2R*) or its accessory protein MRAP have been found, non-classic steroidogenic acute regulatory protein (*StAR*) and *CYP11A1* mutations have been described; and more recently novel mutations in genes such as nicotinamide nucleotide transhydrogenase (*NNT*) and thioredoxin reductase 2 (*TRXR2*) involved in the maintenance of the mitochondrial redox potential and generation of NADPH important for steroidogenesis and ROS detoxication have been discovered. In addition, whole exome sequencing approach also solved the genetics of some syndromic forms of PAI including IMAGe syndrome (*CDKN1C*), Irish traveler syndrome (*MCM4*), MIRAGE syndrome (*SAMD9*); and most recently a syndrome combining FGD with steroid-resistant nephrotic syndrome and ichthyosis caused by mutations in the gene for sphingosine-1-phosphate lyase 1 (*SGPL1*). This review intends do give an update on novel genetic forms of PAI and their suggested mechanism of disease. It also advocates for advanced genetic work-up of PAI (especially in children) to reach a specific diagnosis for better counseling and treatment.

European Journal of Endocrinology
(2017) **177**, R99–R111

European Journal of Endocrinology

Invited Author's profile

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www.eje-online.org
DOI: 10.1530/EJE-17-0128

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Introduction

Primary adrenal insufficiency (PAI) is rare in children and adults, but can have fatal consequences (1, 2, 3). PAI is mostly due to genetic defects in children, whereas acquired forms are more prevalent in adults. PAI is defined as inability to produce sufficient glucocorticoids (GC) and/or mineralocorticoids (MC) in the adrenals. In contrast to PAI where the problem occurs at the level of the adrenals and leads to feedback stimulation of the regulatory hypothalamus–pituitary-axis (HPA) and the renin–angiotensin–aldosterone loop (RAA), secondary AI is caused by central defects leading to insufficient ACTH production that will not affect MC production regulated by RAA. Thus, diagnosis of PAI typically includes elevated production of ACTH and other pro-opiomelanocortin peptides, which lead to hyperpigmentation of the skin and the mucous membranes; besides that salt craving and other non-specific symptoms such as fatigue, failure to thrive and depression are characteristic. Delayed diagnosis of PAI is associated with lower health-related quality of life and increases the risk for life-threatening adrenal crisis. Guidelines for the diagnosis and treatment of PAI have just been published by the Endocrine Society (3), and an expert review on diagnosis and management of secondary AI is also available (4).

Both PAI and secondary AI may be caused by sporadic or inherited genetic defects (2). They both occur in isolated form or as part of a syndromic spectrum and may manifest at birth or later in life. PAI was first described by Addison more than 150 years ago (5). However, only 30 years ago first gene mutations underlying the disorder have been reported in patients with congenital adrenal hyperplasia (CAH) suffering from 21-hydroxylase (*CYP21A2*) deficiency, which is to date the most common inherited form of PAI (about 1:10 500 in Switzerland). This steroid enzyme and thus its gene is essential for both GC and MC production. According to OMIM 613815, two groups of investigators identified the gene and pseudogene of *CYP21* within the MHC class III gene complex on chromosome 6 in 1985 (6, 7). Meanwhile numerous disease causing sequence variations in the *CYP21A2* gene have been reported, which cause classic salt-wasting or simple virilizing CAH with loss or severe inhibition of the 21-hydroxylase enzyme activity, or non-classic, late-onset CAH with less severe enzyme inhibition (2, 8, 9, 10).

As the biochemistry of adrenal steroidogenesis was solved before the involved genes became known (Fig. 1), patients harboring steroid disorders have been excellent experiments of nature to find and characterize new disease causing genes using a gene targeted approach. In fact, over the years and with the genome project completed in 2000 human mutations in almost all genes encoding enzymes of the steroid biosynthetic pathways of the adrenal cortex were described (Table 1) (2, 10).

However, genetic work-up of patient with PAI did not only reveal defects in genes involved in adrenal steroidogenesis, but also revealed genetic mutations in genes involved in adrenal development (1). Here the knockout mouse model of *ftz-f1/sf1* pioneered the finding in humans. Disruption of *ftz-f1/sf1* resulted in agenesis of adrenal glands and gonads manifesting as complete sex reversal in male and PAI in both male and female animals (11). Soon afterwards the first human being harboring *NR5A1/SF1* gene mutations was reported. A phenotypically female baby was diagnosed with PAI soon after the birth and was also found to have 46,XY

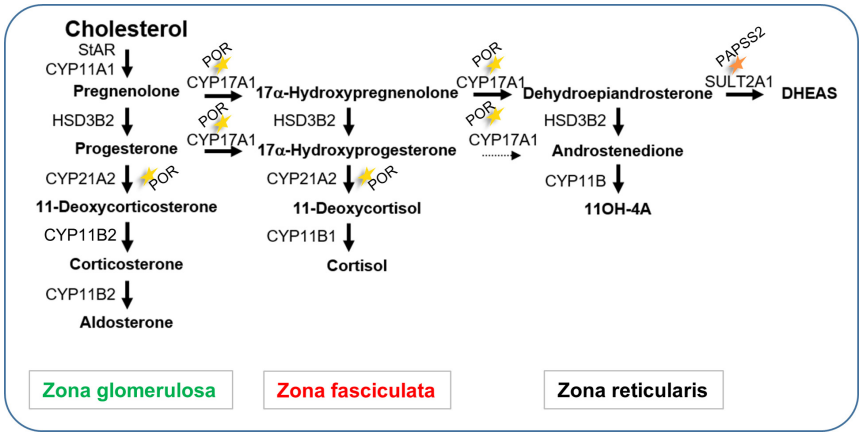

Figure 1

Pathway of steroidogenesis of the human adult adrenal cortex. Step-wise conversion of cholesterol into mineralocorticoids, glucocorticoids and adrenal androgens is shown. Metabolites and involved steroid converting enzymes are depicted. Enzymatic reactions supported by redox partner P450 oxidoreductase (POR) are labeled with a yellow star; the orange star shows cofactor support of sulfonyl transferase SULT2A1 by 3′-phospho-adenosine-5′-phosphosulfate synthase 2 (PAPSS2).

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sex reversal due to a heterozygote *NR5A1/SF1* mutation (12). Meanwhile numerous *SF1* mutations have been reported in many individuals presenting with a broad range of phenotypes with respect to sexual development and reproduction that still remains unexplained (13). However, interestingly PAI has been found to be a rare finding in patients with *SF1* mutations. By contrast, individuals diagnosed with adrenal hypoplasia congenita (AHC) in childhood have been found to harbor mostly mutations in *NR0B1/DAX1*, a coregulator of transcription factor SF1 (14). DAX1 is located on the X chromosome and may cause dosage-sensitive 46,XY sex reversal when duplicated, or may be part of a contiguous gene syndrome together with the Duchenne muscular dystrophy gene (*DMD*; OMIM 300679) and the gene for glycerol kinase deficiency (*GKD*; OMIM 307030). However, the typical phenotype of isolated *DAX1* gene mutations is PAI and secondary hypogonadotropic as well as primary hypogonadism (14). Adrenal dysgenesis has also been described with mutations in several other genes as part of a syndrome complex, e.g. in the Pallister–Hall syndrome due to *GLI3* mutations or in the Pena–Shokeir syndrome due to *DOK7* and/or *RAPSN* mutations (Table 1).

Similarly, genetic work-up of patients with familial isolated glucocorticoid deficiency (FGD)/ACTH resistance revealed mutations in the ACTH receptor gene *MC2R* (15, 16), an obvious candidate for causing PAI. MC2R is a G protein-coupled receptor which is almost exclusively expressed in the adrenal cortex that transmits ACTH stimulation to adrenal GC and androgen production as well as tissue growth and maintenance. Further studies of FGD patients not harboring MC2R mutations revealed mutations in the gene for MRAP, an accessory protein, which enables MC2R targeting and function and thus mimics the phenotype of loss of MC2R function (17). Less obvious and thus only revealed by fine-mapping based on linkage disequilibrium analysis of two cohorts of families with PAI in the context of the Allgrove syndrome led to the discovery of the *aladin* gene/*AAAS* gene (18, 19, 20). Aladin seems to impair redox homeostasis and thus steroidogenesis (21) and is involved in the formation of mitotic spindles and chromosome alignment (22). Allgrove or the triple A syndrome combines PAI of the FGD type with alacrima, achalasia of the esophageal cardia and neurologic deficits that are similar to the neurological deficits seen with adrenoleukodystrophy, an X-linked peroxisomal defect caused by *ABCD1* mutations (23). Additional mutations in genes of peroxisomal and mitochondrial metabolism and of cholesterol synthesis have been identified in several, mostly multiorgan disorders/syndromes including the adrenals (e.g. Zellweger syndrome (*PEX1*) or Kearns–Sayre syndrome (mitDNA del)).

Finally, genetic defects causing autoimmune-mediated PAI either as isolated adrenalitis or in combination with different types of autoimmune polyglandular syndromes (APS) have been described especially in adults (24) (Table 1). Mutations in the autoimmune regulator gene *AIRE* are responsible for APS-1, which typically combines PAI with hypoparathyroidism and chronic mucocutaneous candidiasis (25, 26). By contrast, isolated PAI and APS-2 share the same pattern of complex inheritance (24).

Although so far numerous mutations in many genes had been found to cause PAI, recent advances in genetics and molecular medicine have still revealed several new forms of inborn errors of adrenal GC production due to mutations in genes thus far unexpected to play a role. Disorders of the adrenal cortex have lately been reviewed in journal articles (for e.g. in 2, 3, 27, 28, 29), and book chapters, e.g. (2, 30), and are briefly summarized in Table 1. This article intends to give an update on recently discovered, at first glance unsuspected genetic defects and their pathomechanism and characteristics of disease for PAI illustrating that the spectrum has become even more complex in the post-genomic era. The selection does not claim to be complete and sure enough additional genes will appear on the scene before long.

From enzyme to cofactor deficiencies

In principal, adrenal steroid hormones are produced by step-wise conversion of cholesterol to intermediates that serve again as substrates for specific enzymes to finally produce aldosterone, cortisol and androgens (Fig. 1); e.g. intermediate 17-hydroxyprogesterone (17OHProg) is converted to 11-deoxycortisol by CYP21A2 to finally yield cortisol, but it typically elevated with 21-hydroxylase deficiency CAH. After having found underlying genetic defects in all enzymes involved in steroidogenesis of the adrenal cortex (Table 1), some patients manifesting with a complex profile of disturbed steroidogenesis remained still unsolved. Some of these patients presented with a biochemical profile of combined 21-hydroxylase (CYP21A2) and 17-hydroxylase (CYP17A1) deficiency (31). It was therefore rightly hypothesized that the defect may lie in a cofactor (32). Years later genetic mutations in P450 oxidoreductase (*POR*) were found in these patients (33, 34).

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Table 1 Genetic causes of primary adrenal insufficiency.

Disorder	Gene	OMIM	Associated clinical features in addition to PAI
Defects of steroid biosynthesis			
Congenital lipoid adrenal hyperplasia (CLAH)	*StAR*	201710	46,XY DSD, gonadal insufficiency
P450 side chain cleavage syndrome	*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
Aldosterone synthase deficiency	*CYP11B2*	124080	Isolated mineralocorticoid deficiency
Cortisone reductase deficiency	*HSD11B1*	614662	Androgen excess syndrome
Apparent cortisone reductase deficiency	*H6PDH*	604931	Androgen excess syndrome
Adrenal dysgenesis			
X-linked adrenal hypoplasia congenita (AHC)	*NROB1 (DAX1)*	300200	Hypogonadotropic hypogonadism, in some cases gonadotropin independent precocious puberty
Steroidogenic factor 1 deficiency	*NR5A1 (SF1)*	184757	46,XY DSD, gonadal insufficiency
IMAGe syndrome	*CDKN1C*	300290	IUGR, bone disorders and anomalies, genital anomalies, hypercalcemia, dysmorphic facial features
MIRAGE syndrome	*SAMD9*	617053	Myelodysplasia, infections, restriction of growth, genital anomalies, enteropathy
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 – 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
FGC – deficiency of mitochondrial ROS detoxification	*NNT*	614736	Only glucocorticoid deficiency
	TXNRD2	606448	Only glucocorticoid deficiency
	GPX1		Only glucocorticoid deficiency
	PRDX3		Only glucocorticoid deficiency

(Continued)

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Table 1 Continued.

Disorder	Gene	OMIM	Associated clinical features in addition to PAI
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*	210250	Short stature, gonadal failure, xanthomas, arthritis, coronary heart disease
	ABCG8		
Metabolic disorders: peroxisomal defects			
X-linked adrenoleukodystrophy	*ABCD1*	300100	Progressive neurodegeneration, dementia, progressive behavioral disturbances, vision and hearing loss, spasticity and seizures; accumulation of very long-chain fatty acids
	ABCD2	300371 601081	
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
Metabolic disorders: mitochondrial defect			
Kearns–Sayre syndrome	*mitDNA del*	530000	Progressive external ophthalmoplegia, pigmented retinopathy, cardiac conduction block, cerebellar ataxia; other endocrine pathologies
Metabolic disorders: lysosomal			
Sphingosine-1-phosphate lyase 1 deficiency	*SPGL1*	603723	Steroid-resistant nephrotic syndrome, optionally accompanied by ichthyosis, primary hypothyroidism, cryptorchidism, immunodeficiency and neurological defects
Autoimmune disorders			
Isolated autoimmune adrenalitis	*CLTA-4*, *HLA-DR3*, *HLA-DR4*, *HLA-B8*		
Autoimmune polyglandular syndrome (APS), type 1 (APECED)	*AIRE*	240300	Hypoparathyroidism, candidiasis, autoimmune hypergonadotropic hypogonadism, autoimmune thyroid diseases alopecia, chronic autoimmune hepatitis, pernicious anemia, vitiligo
Autoimmune polyglandular syndrome (APS), type 2	*CLTA-4*, *HLA-DR3*, *HLA-DR4*	269200	Autoimmune thyroid diseases, T1DM, premature ovarian failure, pernicious anemia, celiac disease
Autoimmune polyglandular syndrome (APS), type 4	*CLTA-4*, *HLA-DR3*, *HLA-DR4*		Combination of autoimmune diseases not included in previous groups

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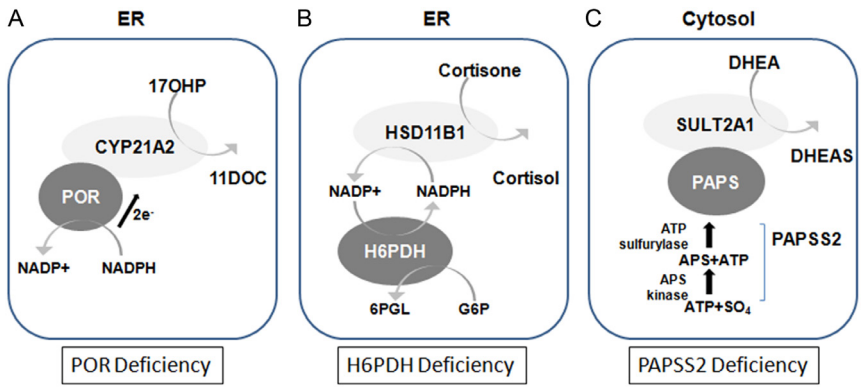

Figure 2

Schemes of cofactor disorders affecting human steroid biosynthesis and metabolism. (A) P450 oxidoreductase (POR) deficiency (PORD). In PORD electron transfer from NADPH to depending P450 redox partners such as CYP17A1 and CYP21A2 of adrenal steroidogenesis through POR is inhibited or disrupted. This may affect adrenal glucocorticoid and androgen production. (B) Hexose-6-phosphate-dehydrogenase (H6PDH) deficiency. In H6PDHD regeneration of NADPH by H6PDH to support 11β-hydroxysteroid dehydrogenase type 1 (HSD11B1) activity is disrupted. This leads to reduced regeneration of cortisol from cortisone in multiple tissues. (C) 3′-Phospho-adenosine-5′-phosphosulfate (PAPS) synthase 2 (PAPSS2) deficiency. PAPSS2 synthesizes PAPS through its ATP sulfurylase and APS kinase activities for sulfation of dehydroepiandrosterone (DHEA) to its sulfate DHEAS. Lack of PAPSS2 activity results in increased DHEA and downstream androstendione levels, while DHEAS is very low. 17OHP, 17-hydroxyprogesterone; 11DOC, 11-deoxycortisol; SULT2A1, sulfotransferase family 2A member 1.

POR is the obligate electron transfer partner from NADPH to all microsomal type II P450 enzymes including CYP21A2, CYP17A1 and CYP19A1 of adrenal and gonadal steroidogenesis (Fig. 2A). It manifests with a very broad phenotype ranging from severely being affected and presenting with an Antley–Bixler syndrome with genital anomalies and PAI to almost being normal and just presenting with a mild PCOS-like phenotype (34, 35). This broad phenotype makes the clinical diagnosis very difficult, but can be explained by several reasons. As POR is the essential electron donor to more than 50 human P450s, its impact may be vast and includes steroid and cholesterol biosynthesis and metabolism as well as drug and xenobiotics metabolism (35). In theory, POR also supplies electrons to heme oxygenase, fatty acid desaturase and elongase, squalene-monooxygenase, cytochrome b5, sterol reductase and many others. The POR protein seems to interact with all its redox partners specifically. Therefore, POR mutations that do not disrupt the electron transfer chain from FMN to FAD within POR directly, but rather disturb the protein–protein interaction will affect different enzyme reactions to variable degrees. In addition, different POR mutations as such affect POR function to variable degrees. Generally, mutations that disrupt the electron transfer from FAD to FMN will affect all reactions severely. By contrast, some POR mutations have been found to inhibit some enzyme activities, whereas they do not affect or stimulate others (35). So far, human *POR* mutations have been shown to affect adrenal and gonadal steroidogenesis and drug metabolism as well as bone formation, whereas effects on other redox partners' function remain to be elucidated. Adrenal insufficiency is often not very severe with POR deficiency as 21-hydroxylase activity is mostly not completely lost.

Similarly, a cofactor disorder has been described as the underlying cause for deficient enzyme activity of 11β-hydroxysteroid dehydrogenase type 1 (*HSD11B1*), which regenerates cortisol from cortisone in multiple peripheral tissues (Fig. 2B) (36). Lack of HSD11B1 leads to relative cortisol deficiency, also known as cortisone reductase deficiency, and results in excess adrenal androgen production due to negative feedback control via the HPA axis. This disorder has thus a similar phenotype as non-classic, late-onset CAH, although it is not a primary adrenal disorder *per se*. So far, heterozygote mutations of HSD11B1 have been described in two unrelated boys presenting with hyperandrogenism and premature pseudopuberty (37). However, because HSD11B1 activity depends on a high NADPH/NADP+ ratio, which is generated *in vivo* through the activity of microsomal hexose-6-phosphate dehydrogenase (*H6PD*), mutations of *H6PD* mimic the same clinical picture as *HSD11B1* mutations. This cofactor disorder is therefore also known as apparent cortisone reductase deficiency (Fig. 2B) (36).

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H6PD mutations illustrate the importance of the redox homeostasis for cortisol metabolism.

Androgen excess is also a possible phenotype for human mutations in the cofactor 3′-phospho-adenosine-5′-phosphosulfate (PAPS) synthase 2 (*PAPSS2*, OMIM 612847) (38). PAPS is the essential sulfate donor to all human sulfotransferases including SULT2A1, which converts adrenal DHEA to its sulfate ester DHEAS that is the most abundant steroid in circulation. Generation of PAPS requires PAPS synthase activities, both ATP sulfurylase and APS kinase (Fig. 2C). As sulfation is a key to proteoglycan and thus extracellular matrix formation and thereby bone development and growth, human mutations of *PAPSS2* have been observed first in individuals with disproportionate short stature due to spondyloepimetaphyseal dysplasia (39). The hormonal phenotype of PAPSS2 deficiency (androgen excess due to elevated DHEA and very low DHEAS) may only be found when searched for. Premature adrenarche, pubarche, axillarchy in children and a profile for hyperandrogenic PCOS can hint for *PAPSS2* mutations. However, cortisol production is normal with PAPSS2 deficiency. Therefore, it may not qualify for a disorder of PAI, but it is definitively to consider in the differential diagnosis of adrenal androgen excess.

A phenotype of androgen excess, but apparent GC deficiency is also seen with familial glucocorticoid resistance (OMIM 615962) due to mutations in the glucocorticoid receptor gene (*NR3C1*, OMIM 138040), in which the glucocorticoid receptor does not respond to cortisol stimulation (40, 41). These patients have inappropriately high serum ACTH and cortisol levels, but no stigmata of Cushing's, and they present with chronic fatigue, androgen excess and arterial hypertension. Also, this disorder is not a primary adrenal problem, but manifests similarly.

PAI caused by disrupting the oxidative stress balance of the cell

That a well-balanced redox potential is important for the regeneration of cortisol in peripheral tissues has been illustrated by H6PDH deficiency. Evidence for the important role of the cellular redox homeostasis for adrenal steroidogenesis came more recently by advanced genetic work-up of individuals presenting with a phenotype of familial glucocorticoid deficiency (FGD), in which no mutations in *MC2R*, *MRAP* or the *AAAS* genes were found (27). Next-generation sequencing (NGS) revealed mutations in genes so far not suspected to cause a phenotype of PAI and FGD (Fig. 3).

FGD due to human mutations in the gene for nicotinamide nucleotide transhydrogenase (*NNT*) was first described in 2012 (42) (Table 1). It meanwhile accounts for approximately 10% of FGD. NNT is located in the inner mitochondrial membrane and is responsible for the generation of NADPH using the energy from the mitochondrial proton gradient (Fig. 3). In the mitochondria, steroidogenic enzymes CYP11A1 and CYP11B2/1 depend on NADPH for the conversion of cholesterol to pregnenolone, 11-deoxycorticosterone to aldosterone and 11-deoxycortisol to cortisol respectively, supported by the cofactor system ferrodoxin reductase (*FDXR*)/ferrodoxin (*FDX1*) (10, 43). Electrons from NADPH are accepted by the flavoprotein ferrodoxin reductase located at the inner mitochondrial membrane, which then transfers them to the iron/sulfur protein ferrodoxin forming a 1:1 complex. Thenc ferrodoxin dissociates with the electron load to form a next complex with the mitochondrial P450 (e.g. CYP11A1, CYP11B1/2). Overall, this electron shuttle seems rather inefficient. To date, no human mutations in *FDXR* or *FDX* have been described. However, NNT and NADPH do not only play a direct role in steroidogenesis, but are also very important in maintaining the right cellular balance of reactive oxygen species (ROS). ROS levels play a critical role in many cellular functions including stress response, immune reactions as well as cell proliferation, differentiation and apoptosis (44, 45). ROS are mainly produced at complex I and III of the respiratory electron transport chain in the mitochondria by electron leakage. However, produced superoxides may be detoxified by two antioxidant systems, namely the glutathione peroxidase or the peroxiredoxin systems and their associated proteins, which both require NADPH. In the adrenal cortex, steroidogenesis requires high mitochondrial metabolic activity that feeds ROS production, whereas ROS may inhibit steroidogenesis through negative feedback to the steroidogenic acute regulatory protein (StAR) (46). ROS has been shown to suppress StAR protein synthesis that is essential for transporting cholesterol into the mitochondria for the initiation of steroid hormone biosynthesis. Overall, it is therefore no longer astonishing that genetic defects such as NNT, which affects NADPH production and the cellular homeostasis of ROS lead to defective steroidogenesis. Thus, it was only a matter of short time that genetic mutations in other components of this system were found (Fig. 3). Recently, human mutations in the *TXNRD2* gene of the thioredoxin system have been described in three

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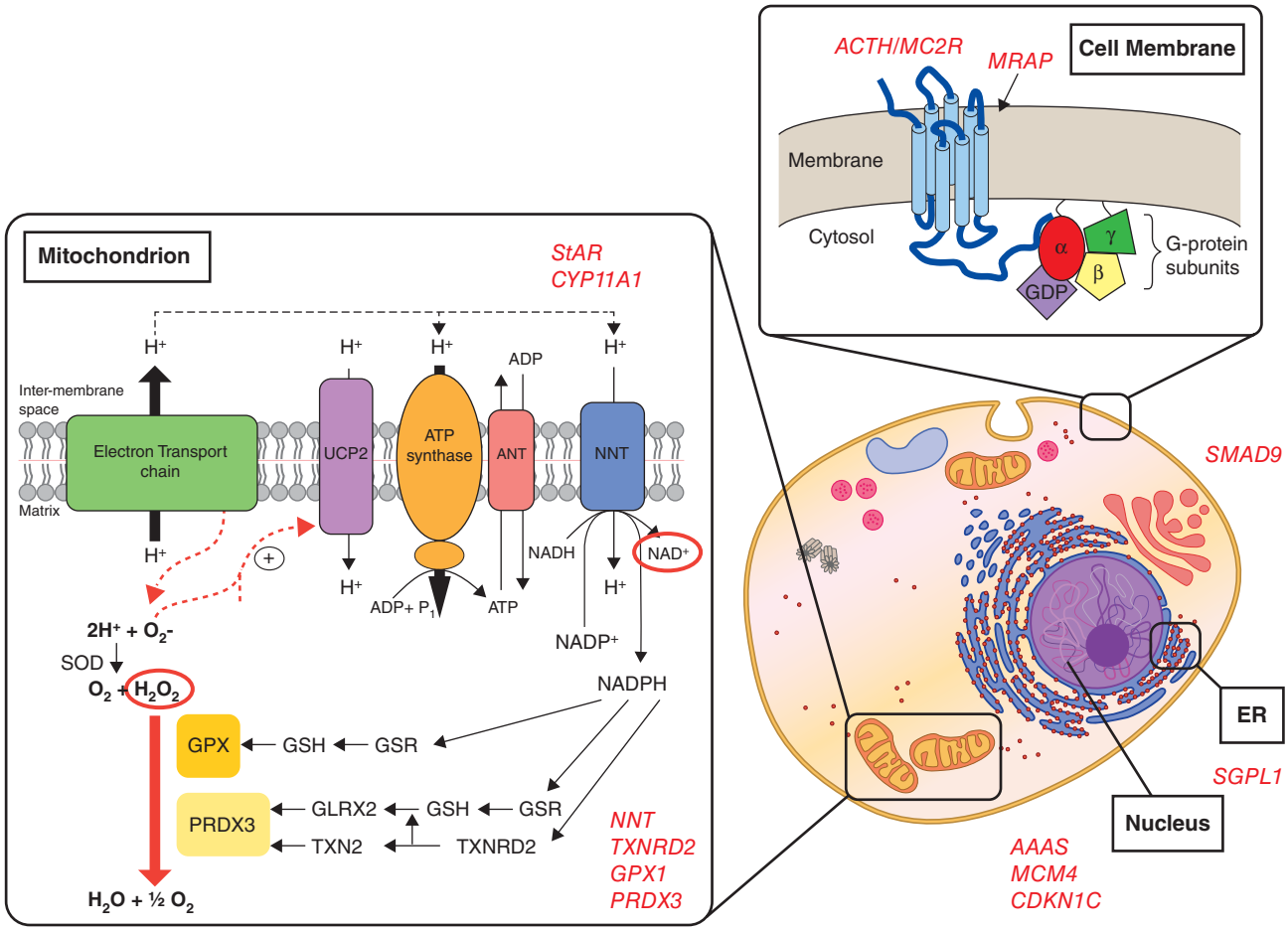

Figure 3

Spectrum of genetic disorders associated with familial glucocorticoid deficiency (FGD). The figure summarizes the known genetic defects (abbreviated gene names given in red and italic) underlying isolated and syndromic forms of FGD and puts them into perspective of their cellular localization. To illustrate the importance of the redox balance for the cell and steroidogenesis in particular, the mitochondrial system for NADPH and ROS production and detoxification is shown in more details. NADPH supports both the thioredoxin (PRDX3) and the glutathione (GPX) systems by electrons. Nicotinamide nucleotide transhydrogenase (NNT) serves as proton pump for generating NADPH. SOD, superoxide dismutase. ACTH/MC2R, melanocortin receptor 2; MRAP, melanocortin receptor accessory protein; StAR, steroidogenic acute regulatory protein; SMAD9, sterile alpha motif domain-containing protein 9 (MIRAGE syndrome); SGPL1, sphingosine-1-phosphate lyase 1; AAAS, achalasia-addisonianism-alacrimia (triple A) syndrome gene; MCM4, minichromosome maintenance complex component 4 (Irish traveler disease); CDKN1C, cyclin-dependent kinase inhibitor 1C (IMAGe syndrome).

related patients manifesting at different ages with FGD (47) (Table 1). Genetic mutations in further components of the mitochondrial antioxidant system will follow without any doubt. Combined mutations in *GPX1* and *PRDX3* have already been identified in the cohort of FGD patients (48).

Of note, PAI in syndromic disorders such as triple A syndrome and X-linked adrenoleukodystrophy (ALD) have both been linked to oxidative stress. However, the exact pathophysiology linking the defective nuclear pore protein complex in AAAS and the abnormal accumulation of VLCFAs in ALD to enhanced ROS generation remains unclear. An extensive review on oxidative stress and adrenocortical insufficiency has been recently published in this journal by the group of Lou Metherell (45).

Novel syndromic forms of PAI

IMAGe syndrome was first defined in 1999 by the spectrum of intrauterine growth restriction, metaphyseal dysplasia,

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congenital adrenal hypoplasia and genital anomalies (49). Patients may also feature hypercalciuria and/or hypocalcemia, craniosynostosis, cleft palate and scoliosis (50). Using a NGS approach, the underlying defect was identified in a rare autosomal-dominant single gene defect, *CDKN1C* (Table 1) (51). CDKN1C is part of an imprinted gene cluster on chromosome 11p15.5, which regulates prenatal and postnatal growth and development. It seems to play a major role in inhibiting cell-cycle progression. Normally, the paternal allele of *CDKN1C* is repressed by imprinting and only the maternal allele is expressed. Thus, specific mutations in the PCNA-binding domain of the maternally inherited allele of *CDKN1C* were found to cause IMAGe syndrome likely through a gain-of-function mechanism. By contrast, loss-of-function mutations in the CDK-binding domain and truncating mutations of the very same gene are known to cause the Beckwith–Wiedemann syndrome (OMIM 130650) manifesting with overgrowth and a predisposition to embryonal malignancies (e.g. Wilms tumor and hepatoblastoma).

Similarly, advanced genetic work-up of an Irish cohort suffering from FGD and growth failure with frequent consanguinity revealed mutations in the *MCM4* gene (Table 1) (52). Affected individuals also showed increased chromosomal breakage and immunological anomalies (e.g. natural killer cell deficiency), which may make them more susceptible for neoplastic lesions. MCM4 is part of a protein complex for DNA synthesis in the S phase and therefore causes disordered DNA repair and replication. Why this leads to a rather specific and narrow phenotype remains unanswered.

Lately, MIRAGE syndrome due to genetic variants in the *SAMD9* gene was reported in 11 patients presenting with myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital anomalies and enteropathy (53) (Table 1). SAMD9 is involved in endosome fusion and is reported to play a role in growth factor signaling transduction. Thus, heterozygote *SAMD9* mutations seem to enhance its intrinsic endosome-fusing activity and may thereby lead to abnormal tissue development including dysgenetic and hypoplastic adrenal glands, ovaries and thymus.

Most recently, novel genetic mutations were identified in patients with a syndrome comprising of steroid-resistant nephrotic syndrome (SRNS) and PAI, optionally accompanied by ichthyosis, primary hypothyroidism, cryptorchidism, immunodeficiency and neurological anomalies. Using NGS on patient cohorts with FGD or SRNS respectively, two groups found concurrently the underlying genetic defect in the gene for sphingosine-1-phosphate (S1P) lyase 1 (*SGPL1*, OMIM 603723) (54, 55). SGPL1 is the intracellular enzyme responsible for the final breakdown of sphingolipid (S1P). S1P regulates cell migration, differentiation, survival as well as angiogenesis and development. S1P may function as an activator of an extracellular signaling pathway mediated by G protein-coupled receptors, or as a direct intracellular second messenger (56). The pathogenesis of SGPL1 deficiency within a target organ may result from (a) an excess of intracellular S1P; (b) an accumulation of other sphingoid bases; and (c) from S1P signaling through the S1P receptor (54). Identified human *SGPL1* mutations were shown to behave as recessive loss-of-function mutations affecting protein expression and localization, enzyme activity and thus degradation of long-chain sphingoids (54). SGPL1 mutations were also shown to alter ceramide composition of cultured fibroblast of patients compared to that in controls. Also, reconstituted human missense mutations led to reduced viability and nephrocyte anomalies in Drosophila reminiscent to the podocyte phenotype seen in humans (54). The pathomechanism of PAI in SGPL1 deficiency includes both compromised adrenal development as well as disrupted steroidogenesis (55). Adrenals of Sgpl1$^{-/-}$ mice revealed marked alterations in adrenocortical zonation, whereas CYP11A1 expression was significantly decreased. Thus, the Sgpl1$^{-/-}$ mouse model was found to reflect the adrenal and renal phenotype found in humans (54, 55). Sgpl1$^{-/-}$ mice die within few weeks and are also reported to have impaired gonadal steroidogenesis; they are infertile. Of note, mutations in upstream components of the sphingolipid metabolism lead to disorders known as sphingolipidoses such as Niemann-Pick, Gaucher or Fabry diseases (57). They are mostly progressive, multisystemic disorders and for some of them a renal phenotype has been reported. By contrast, an adrenal phenotype has not been described for sphingolipidoses so far.

Phenotype–genotype conundrum in PAI and FGD

Prediction of genotype–phenotype is not (always) easy with PAI and FGD. Biochemical steroid profiling can help in defining specific enzyme deficiencies. However, even simple genetic mutations in steroid enzymes such as 21-hydroxylase deficiency can manifest with variable phenotypes due to variable degrees of loss of enzyme activity. This has resulted in the clinical definition of the classic (salt-vasting and simple virilizing) and the

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non-classic (late-onset) form of CAH. With PORD, the phenotypic variability is even broader as this cofactor serves multiple enzymes, and specific mutations may not affect all redox partners similarly as described earlier in this review. Thus, mutations in a single specific gene may manifest with variable phenotypes. By contrast, the non-syndromic FGD phenotype may be caused by mutations in several genes (Table 1) and has already led to controversial discussions about the definition of this group of PAI. For instance, milder mutations of the *StAR* gene or the *CYP11A1* gene, which cause a FGD-like phenotype, have been suggested to be named non-classical (lipoid) CAH (58, 59, 60, 61) instead of being included in the group of FGDs (27, 62). In addition, some genetic defects may not manifest early in life as expected for inborn errors, but appear far beyond childhood only, when genetic disorders are generally not considered first.

Unsolved questions

Although many questions regarding PAI have been solved in the last two decades, there is still a lot more to discover and understand about adrenal steroidogenesis. To date approximately two-third of patients manifesting with PAI are genetically solved, but the pathogenesis of some forms is still not fully understood. The discovery of novel gene variants associated with human diseases will always ask for disease causing mechanisms for understanding gene functions. This is even more of a concern with NGS approaches where mostly too many genetic variations are found and an effective filtering strategy must be applied to find the 'needle in the haystack' to follow-up for eventually finding the disease causing genetic variant(s) (48). In contrast, detailed studies of patients with genetic mutations have always provided unexcelled insight into human biology.

In the following, few examples of unsolved specific questions concerning PAI, which we and others are currently addressing, are provided: (a) Why do most mutations of the *NR5A1/SF1* gene not affect adrenal steroidogenesis in most affected individuals, and how can we explain that heterozygote mutations cause a very broad range of disorders of sex development? (b) Why did we not find genetic mutations in the cofactors ferrodoxin/-reductase (*FDX1/FDXR*, OMIM 103260 and 103270) supporting steroid enzymes such as CYP11A1 in FGD patients so far? (c) How come that some novel genes found in FGD patients that are known to have broad functions in cell growth, differentiation and metabolism manifest with an almost exclusive PAI phenotype? (d) Finally, what else causes FGD that still remains unsolved in almost 40% of cases?

Conclusion: Why bother with genetics of PAI?

In recent years the spectrum of genetic defects causing PAI has increased, but several defects manifest phenotypically indistinguishable (1, 2, 14, 28, 48). In addition, NGS approaches have revealed genetic variations in novel genes and have finally solved the diagnosis in several patients with PAI (27, 48). It is therefore recommended to aim at a genetic diagnosis in patients with PAI, especially in familial cases, syndromic forms and young patients. Providing a rapid genetic diagnosis to patients suffering from PAI bears several advantages. First, a precise diagnosis can be offered and allows to provide clear information about disease spectrum and prognosis according to published literature and genetic databases. Necessary treatments may be started and unnecessary treatments stopped earlier, and screenings for accompanying disorders may be installed. Second, genetic counseling of affected individuals and their families is possible at a more advanced level and prenatal genetic testing and treatment can be offered. Third, genetic work-up of genetically unsolved patients and families harboring rare congenital disorders followed by in-depth studies of the biological implications has provided invaluable insight into normal biology and pathomechanisms of endocrine (and many other) disorders including PAI.

In the past, genetic work-up has been performed by the candidate gene approach, in which a single gene was studied exon by exon. This approach might still be valid for the genetic work-up of specific steroid enzyme defects characterized in details by steroid profiling. The candidate analysis has been followed by gene panels, in which a group of genes that are implicated with a specific phenotype such as PAI are investigated at once. With sequencing becoming easier (available) and cheaper, today's first approach for studying non-syndromic genetic conditions is often whole exome sequencing (WES), at least in the screening and diagnostic application. However, WES does not cover intronic sequences and is not the first choice for the detection of copy number variations, gene conversions or fusions, translocations or transversions, which are often associated with syndromic conditions. For that, other genetic analyses such as whole genome sequencing or array CGH may be applied. Most importantly, analysis of big data obtained by WES requires

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an educated filtering strategy, which is able to screen for disease causing variants in the biological context, for which the genetic analysis has been initiated. Applying this method of genetic analysis to genetically unsolved patients and families with FGD, most of the novel underlying genetic disorders described in this review have been discovered recently (27, 48, 54, 55). In addition, more will follow certainly given the fact that still many patients with FGD remain without genetic diagnosis.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work is supported by grants of the Swiss National Science Foundation (current grant ID 320030–146127).

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Received 13 February 2017
Revised version received 19 April 2017
Accepted 27 April 2017

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