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An Overview of Inborn Errors of Metabolism manifesting with Primary Adrenal Insufficiency

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Abstract

Primary adrenal insufficiency (PAI) results from an inability to produce adequate amounts of steroid hormones from the adrenal cortex. The most common causes of PAI are autoimmune adrenalitis (Addison disease), infectious diseases, adrenalectomy, neoplasia, medications, and various rare genetic syndromes and inborn errors of metabolism that typically present in childhood although late-onset presentations are becoming increasingly recognized. The prevalence of PAI in Western countries is approximately 140 cases per million, with an incidence of 4 per 1,000,000 per year. Several pitfalls in the genetic diagnosis of patients with PAI exist. In this review, we provide an in-depth discussion and overview on the inborn errors of metabolism manifesting with PAI, including genetic diagnosis, genotype-phenotype relationships and counseling of patients and their families with a focus on various enzymatic deficiencies of steroidogenesis.

Keywords

Adrenal insufficiency; Congenital Adrenal Hyperplasia; 21-hydroxylase deficiency; X-ALD; Niemann-Pick Diseases; Genetics; Mitochondrial disease

Introduction

Primary adrenal insufficiency (PAI) is defined by an inability to produce sufficient amounts of glucocorticoid hormones from the adrenal cortex, with or without mineralocorticoid deficiency. Adrenal androgens may also be deficient. The prevalence of PAI in Western countries is approximately 140 cases per million, with an incidence of 4 per 1,000,000 per year (1–3). It took approximately three centuries from the initial description of the adrenal glands to the recognition of PAI: in 1552, Batholemeus Eustachius (1520–1574) of *Collegio della Sapienza* in Rome provided the first detailed description of the adrenal glands; in 1849,

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Thomas Addison, described adrenal insufficiency, a disease that bears his name to date (Addison's disease).

Adrenal insufficiency is divided into three subtypes: primary (PAI; defect in adrenal glands), secondary (defect in pituitary gland with insufficient adrenocorticotrophic hormone (ACTH) production) and tertiary (defect in the hypothalamus with insufficient corticotropin-releasing hormone production). These conditions can occur as isolated or part of a syndrome and may manifest at birth or later in life. The most common causes of PAI are autoimmune adrenalitis (Addison's disease), infectious diseases, adrenalectomy, neoplasia, medications, and various rare genetic syndromes that typically present in childhood although late-onset forms are becoming widely recognized. Examples of genetic causes of PAI include adrenal dysgenesis or hypoplasia syndromes (e.g.: mutations in *ACTHR*, *SFI*, *DAX1* and others) and inborn errors of metabolism (IEM). Acquired forms of PAI are more prevalent in adults, although genetic causes can present as late-onset.

IEM are a rare group of genetic diseases that are most commonly transmitted in an autosomal recessive manner and generally result from deficient enzymatic activities in various biochemical pathways (Table 1). Their collective frequency is 1 in 800 to 1 in 2500 births (4, 5). A few of these disorders affect adrenocortical function across the lifespan, with most presenting in childhood but some in adulthood. IEM typically manifest with non-specific symptoms including nausea and fatigue, whereby signs or symptoms of PAI might be easily overlooked. Since PAI may present with a ketoacidotic attack with hypoglycemia, it should be considered on the differential diagnosis of rule out IEM given the overlap in these features. However, fasting hypoglycemia with ketosis occurring mainly in the morning and in the absence of metabolic acidosis suggests recurrent functional ketotic hypoglycemia, rather than hypoglycemia secondary to PAI. Gluconeogenesis defects and glycogen storage diseases are rare IEM that do not present with PAI. PAI can be a complication of a previously diagnosed IEM, although rarely the first presenting organ dysfunction, except in a few exceptions as detailed in this review.

PAI remains an underrecognized condition likely because of its nonspecific presenting symptoms, which results in a delayed diagnosis (6). Patients often complain of anorexia, abdominal pain, salt craving, weight loss, orthostatic hypotension, sparse axillary and pubic hair (in females due to loss of adrenal androgens) and the characteristic hyperpigmentation of the skin and mucous membranes from excess ACTH and other pro-opiomelanocortin peptides. Over the past several decades, our understanding of the molecular pathophysiology of PAI has led to the recognition of various enzymatic deficiencies of steroidogenesis, with their genetic defects being well characterized, and mutation analysis widely available. Like other diseases caused by enzyme deficiencies, IEM that manifest with PAI present with a wide phenotypic spectrum. In this review, we highlight disorders of steroidogenesis, including cholesterol biosynthesis, cellular uptake, intracellular cholesterol trafficking and mitochondrial cholesterol uptake. We provide a comprehensive discussion on the genetic diagnosis, genotype-phenotype relationships and counseling of patients and their families with various IEM that manifest with PAI. We divide the disease categories into broad disorders rather than their original classification, which is not accurate as these conditions present as part of a spectrum of disease, ranging from attenuated to severe forms. For

example, Niemann-Pick Disease Type A and B are now collectively referred to as acid sphingomyelinase deficiency and Wolman disease lies on the severe spectrum of cholesteryl ester storage diseases. Additionally, this review will not cover non-IEM related PAI syndromes, such as the various types of adrenal hypoplasia congenita, ACTH deficiency, ACTH insensitivity syndrome and autoimmune adrenal insufficiency, as detailed elsewhere (7).

Section 1. Diagnosis of PAI

PAI is a life-threatening condition if unrecognized. The genetic etiology of PAI should be determined in all patients with confirmed disease. As detailed in this review, IEM manifesting with PAI present with non-specific symptoms and should be considered in individuals with unexplained PAI. Avoidance of the term “idiopathic” PAI is encouraged until an exhaustive investigation for PAI is performed in all individuals irrespective of ethnicity, age or sex. Once PAI due to an IEM is suspected clinically, diagnosis can be confirmed by the combination of biochemical and molecular genetic studies, which are outlined in Figure 1.

A random cortisol measurement at the time of illness is a useful screening test for evaluating PAI. However, random cortisol results could be misleading and may cause a delayed diagnosis of PAI or inappropriate discontinuation of glucocorticoid therapy. Assay interference from cross-reactivity with other steroids is possible and could be avoided using tandem mass spectrometry methods, which are expensive and not readily available in testing laboratories.

PAI is a biochemical diagnosis that is established using the standard dose of intravenous cosyntropin stimulation. Peak cortisol levels below 500 nmol/L (18 µg/dL; assay dependent) at 30 and/or 60 minutes indicate PAI. Additionally, plasma ACTH concentrations that are above 2-fold the upper limit of the reference range helps to establish PAI. Elevated plasma renin and/or low or undetectable aldosterone concentration may be the first markers of PAI and help determine the presence of mineralocorticoid deficiency (8). Another early marker of PAI is a low dehydroepiandrosterone sulfate (DHEAS, except in patients with congenital adrenal hyperplasia), defined as concentrations below the lower limit of normal for age. None of these tests (except for the cosyntropin stimulation test) should be used in isolation to make the diagnosis of PAI. Elevated very long chain fatty acids (VLCFA) is almost universal in X-linked adrenoleukodystrophy (X-ALD) cases, which helps narrow the differential diagnosis of PAI (Figure 1) (9). VLCFA is also elevated in Zellweger spectrum disorders, non fasting or hemolyzed samples and in an individual on a ketogenic diet. Future studies addressing PAI in IEM should follow established guidelines for the workup and management of PAI, and include assessment of all adrenocortical layers, including mineralocorticoid (renin and aldosterone), glucocorticoid (cortisol and/or its precursors), and androgen production (DHEAS and less so DHEA) (3).

Section 2. Inborn errors of metabolism manifesting with PAI

1. Cholesterol synthesis disorders—Cholesterol synthesis disorders comprise a group of rare inherited conditions that affect in utero steroidogenesis due to the reliance of the

fetus on its endogenous cholesterol synthesis (10). They typically present with intrauterine growth retardation, gastrointestinal and brain malformations (11). These disorders affect the complex upstream steroidogenic pathway before the conversion of cholesterol to pregnenolone, and include defects in cholesterol biosynthesis, cellular uptake, intracellular cholesterol trafficking and mitochondrial cholesterol uptake (10), as demonstrated in Figure 2. In brief, circulating low-density lipoproteins enters steroidogenic cells through receptor-mediated endocytosis. In the endosomes, cholesterol esters are bound by Niemann-Pick disease, type C2 (NPC2) protein, and through the action of lysosomal acid lipase (LAL), yields free cholesterol. Free cholesterol is then transferred to Niemann-Pick disease, type C1 (NPC1) protein in the endosome, and exported to reach the outer and inner mitochondrial membranes where it can enter the process of steroidogenesis (10).

A. Smith-Lemli-Opitz syndrome and related disorders: Smith-Lemli-Opitz syndrome (SLOS or 7-dehydrocholesterol reductase deficiency; OMIM 270400) is the first human syndrome discovered to be due to an inborn error of sterol synthesis with an estimated incidence of 1 in 10,000 to 1 in 70,000 per year (12, 13). SLOS is an autosomal recessive condition due to mutations in *DHCR7* (11q12-13) that encodes for 7-dehydrocholesterol reductase and manifests with developmental delay, microcephaly, organ anomalies, dysmorphic features (such as syndactyly of the second and third toes) and male undervirilization and/or hypogonadism. PAI and adrenal crisis is a rare manifestation of SLOS, as most patients have compensated adrenocortical function (14). SLOS is biochemically characterized by elevated plasma concentrations of 7-dehydrocholesterol (7-DHC) and 8-dehydrocholesterol (8-DHC).

Several rare autosomal recessive phenocopy disorders of SLOS exist but have not been well characterized with PAI. Lathosterolosis (OMIM 607330) is due to mutations in *SC5D* (11q23.3) leading to a deficiency in 3-beta-hydroxysteroid-delta-5-desaturase (converts lathosterol into 7-dehydrocholesterol) and desmosterolosis (OMIM 602398) due to mutations in *DHCR24* (1p32.3) encoding for 3-beta-hydroxysterol delta-24-reductase. CHILD syndrome (OMIM 308050) is an X-linked disorder due to mutations in *NSDHL* (Xq28) that is involved in the demethylation of C4-methyl groups from the sterol intermediate lanosterol. It is characterized by skin and skeletal abnormalities that typically demonstrate a striking unilateral predominance or distribution. Conradi-Hunermann syndrome (CDPX2; OMIM 302960), also known as chondrodysplasia punctata 2, is an X-linked sterol isomerase deficiency disorder due to a mutation in *EBP* (Xp11.23) that manifests with skin, skeletal, and ophthalmologic anomalies (15).

B. Cholesteryl ester storage disease: Cholesteryl ester storage disease represent a spectrum of autosomal recessive LAL deficiency due to recessive mutations in *LIPA* (10q23.31) (16). In these disorders, insufficient free cholesterol is available for steroidogenesis, leading to PAI. The severe form (previously referred to as Wolman disease or primary xanthomatosis; OMIM 278000) presents with a rapidly progressive metabolic disorder in the newborn period with bloating, steatorrhea, vomiting, failure to thrive, growth failure, jaundice, and hypotonia (16). Attenuated phenotypes can present later in life. Xanthomatous changes lead to hepatosplenomegaly, occasional adrenal calcification, lipid deposition in the intestinal

walls, and/or xanthelasma (17). The diagnosis of the severe form can be suggested by pre- or postnatal ultrasonography of bilateral subcapsular adrenal punctuate calcifications, a sign of extensive fetal adrenal necrosis (18). Due to the rarity and underrecognition of LAL deficiency, precise prevalence rates are not known at this time. One study found that ~3% of pediatric patients with PAI had the severe form of cholesteryl ester storage disease (19), with a prevalence of 1 in 350,000 (20). A founder mutation in *LIPA* (c.260G>T) exist in individuals of Iranian-Jewish ancestry with a prevalence of 1 in 4,200 (21).

C. Niemann-Pick disease: In steroidogenic cells, the endosomal processing of cholesteryl esters requires the action of two important proteins; NPC1 and NPC2, encoded by *NPC1* (18q11-12) and *NPC2* (14q24.3), respectively. NPC2 assists in the transfer of unesterified cholesterol to the cholesterol binding domain of NPC1. Niemann-Pick type C (NPC) is a rare autosomal recessive neurodegenerative disorder with an prevalence of approximately 1 in 89,229 to 1 in 150,000 (22, 23), caused by mutations in *NPC1* or *NPC2*. NPC is characterized by accumulation of cholesterol and glycosphingolipids in endosomes, leading to progressive neurodegeneration and death (24). Niemann-Pick Type D, now referred to as a subtype of NPC, is due to a founder mutation in *NPC1* (p.G992W) in the Canadian Nova Scotian Acadian ancestry (25). Adrenal cells in NPC likely escape PAI because of the presence of alternate pathways for intracellular cholesterol trafficking (10). However, the mechanism by which this escape occurs remains to be elucidated. The first line laboratory testing for evaluating patients with suspected NPC includes measurement of several plasma metabolites (cholestane-3 β , 5 α , 6 β -triol, lyso-sphingomyelin isoforms and bile acid metabolites), which are sensitive and specific diagnostic biomarkers for NPC (26). Genetic analysis should be performed if laboratory testing is suggestive of NPC. The filipin test is no longer considered as first line for evaluating patients with NPC (26).

Acid sphingomyelinase deficiency (ASMD) leads to a group of disorders previously known as Niemann-Pick Disease Type A (NP-A) and Niemann-Pick Disease Type B (NP-B). Acid sphingomyelinase is an enzyme that is widely expressed in human tissues, including in the adrenal glands. A single report from 1987 identified a girl with NP-B and polyglandular involvement, including partial PAI (27). However, in the largest case series on ASMD, death related to adrenal involvement was not ascertained (28). Cortical adrenal storage in ASMD (29), but not NPC, is evident on adrenal imaging (or autopsy) as bilateral adrenocortical enlargement. Additionally, organ transplantation is common in this patient population whereby exogenous glucocorticoid therapy is used for immunosuppression, further complicating the workup of PAI. Since no studies have systematically evaluated adrenocortical function in a series of patients with ASMD, it is still unclear as to whether the adrenocortical enlargement in ASMD from storage predisposes to PAI. Assessment of adrenocortical function in ASMD should follow existing guidelines when clinically indicated (3).

2. Congenital adrenal hyperplasia—Adrenal steroidogenesis in the fetus is directed toward dehydroepiandrosterone (DHEA) and DHEA-S given the low enzymatic levels of 3 β -hydroxysteroid dehydrogenase (Figure 2). Small quantities of adrenal steroids are converted to aldosterone, cortisol and 17-hydroxyprogesterone (17-OHP) to

androstenedione. Absent or reduced 21-hydroxylase enzymatic activity leads to increased production of DHEA, testosterone, dihydrotestosterone, 17-OHP and androstenedione. This is referred to as congenital adrenal hyperplasia (CAH), the most frequent etiology (71.8%) of PAI, while non-CAH etiologies in pediatrics account for 28.2% of PAI, of which 55% are non-autoimmune in etiology (30).

CAH represents a heterogeneous group of autosomal recessive disorders due to single gene defects of adrenal steroidogenesis (31). This results in decreased cortisol and/or mineralocorticoid synthesis, shunting of the accumulated steroid precursors across alternate intra-adrenal pathways, and adrenocortical hyperplasia. Clinical presentation range in severity, and typically result from PAI. Genital ambiguity, disordered sex development, infertility, short stature, hypertension, psychiatric disorders and an increased risk of metabolic syndrome during adolescence and adulthood are common comorbidities. Table 1 lists the various subtypes of CAH. 11 β -hydroxylase and 17-hydroxylase deficiency do not manifest with glucocorticoid deficiency due to the glucocorticoid effects of excess corticosterone and will not be discussed in this review.

A. 21-hydroxylase deficiency: 21-hydroxylase deficiency (21-OHD; OMIM 201910) causes >95% of CAH cases, and affects approximately 1 in 16,000 live births (32). 21-OHD is divided into classic salt-wasting (SW), classic simple virilizing (SV), and nonclassic (NCCAH, mild or late-onset) (33). Certain ethnic groups have a predilection to certain genotypes (34–43), which may have resulted from an ancient founder effect, unequal crossing over during meiosis or gene conversion of point mutations in the pseudogene (44). It is well accepted that 21-OHD is the most common inherited metabolic disorder of PAI.

21-OHD typically manifests with virilization of the external genitalia in newborn females, PAI and precocious pseudopuberty due to androgen overproduction in both sexes. Approximately 75% of patients with classic CAH have SW, which presents with severe life-threatening SW crisis in the neonatal period. Patients with SV escape life-threatening crisis in the neonatal period due to the production of small amounts of aldosterone, and if not detected through newborn screening, may present as toddlers with signs and symptoms of hyperandrogenism. NCCAH is common, affects approximately 1 in 200 (45, 46). Patients with NCCAH are usually asymptomatic but may manifest in childhood or early adulthood with precocious pubarche or a clinical picture resembling polycystic ovary syndrome (45). Approximately a third of patients with NCCAH show a suboptimal glucocorticoid response to the ACTH stimulation test (47). However, patients with NCCAH do not suffer from clinically significant glucocorticoid deficiency; stress dosing during illness is indicated in those who fail the cosyntropin stimulation test.

The 21-hydroxylase enzyme is encoded by *CYP21A2* (6p21.3), a cytochrome P450 type II enzyme of 495 amino acids (48, 49). This gene is located 30kb apart from its duplicated yet inactive pseudogene (*CYP21A1P*) in the human leukocyte antigen (HLA) class III region in the major histocompatibility (MHC) locus (Figure 3). These two genes share approximately 98% sequence homology (Figure 3) (35), and are arranged in tandem repeat with the *C4* (*C4A* and *C4B*) genes. This arrangement is subject to high frequency of genomic recombination, with the transfer of deleterious pseudogene mutations to the active

CYP21A2 gene, which causes the majority of CAH mutations (50). Approximately 25–30% are chimeric genes due to large deletions (51, 52).

The genetic diagnosis of CAH is complex due to the high variability of its genomic region (31). Most patients with CAH are compound heterozygotes carrying two different disease-causing mutations, with the phenotype defined by the mutation retaining the most enzyme activity (53). Four different groups of *CYP21A2* mutations exist (Null, A, B, C) (Table 2) (35). Generally, the disease severity in childhood can be accurately predicted by genotypes that result in the SW and NCCAH forms, while the most phenotypic variability is observed with SV (36, 53–56). Deletions or nonsense mutations that affect critical enzyme functions or alter enzyme stability resulting in a complete loss of function and SW. Missense mutations with 1–2% of normal enzyme activity are found in SV (57–59). Molecular analysis of *CYP21A2* should be extensive and performed in a certified laboratory with adequate quality controls and experience in the analysis of CAH genotypes. Care should be taken to prevent genotyping the pseudogene because genetic results can be complicated due to the duplication, deletion, and recombination of *CYP21A2* in the chromosome 6q21.3 region.

Mutations p.V281L, p.P453S and p.P30L retain 20–60% of normal activity and cause NCCAH (60–62). In general, there is good genotype-phenotype correlation for the NCCAH p.V281L and p.P453S mutations, but phenotypic variability has been described for p.P30L (37, 53). The exon 7 p.V281L (c.1685G>T) mutation associated with NCCAH is very frequent in the Ashkenazi Jewish (AJ) population of New York (estimated in one study to be 1 in 27, with 1 in 3 being heterozygous), which has not been observed in the Asian and Native American populations (53). However, this mutation also occurs in other populations, and recent estimates have demonstrated that the proportion of Ashkenazi Jewish NNCAH carriers and disease affected was not as high as previously reported (46).

B. CAH-X Syndrome: CAH-X refers to a contiguous gene deletion syndrome that involves *CYP21A2* and *TNXB* genes, encoding *tenascin-X*, an extracellular matrix protein (63, 64). Individuals present with signs and symptoms related to CAH and a hypermobility type Ehlers-Danlos syndrome phenotype, manifesting with joint hypermobility, pain and multiple dislocations (63). The prevalence of CAH-X amongst CAH is approximately 9% (65). CAH-X should be suspected in every patient with CAH and hypermobility (31).

C. 3 β -hydroxysteroid dehydrogenase type 2 deficiency: 3 β -hydroxysteroid dehydrogenase type 2 deficiency (HSD3B2; OMIM 201810) is encoded by *HSD3B2* and represents a rare form of CAH, which exists in two isoforms (66). HSD3B2 converts pregnenolone, 17-hydroxypregnenolone and DHEA to progesterone, 17-OHP and androstenedione, respectively. Thus, HSD3B2 deficiency results in gonadal and adrenal dysfunction, with PAI, SW and genital ambiguity.

D. P450 oxidoreductase deficiency: P450 oxidoreductase (POR) deficiency (OMIM 613571) is encoded by *POR* gene (7q11.2) and represents a rare form of CAH that presents as apparent combined *CYP17A1* and *CYP21A2* deficiency (67). Both sexes present with severe sexual ambiguity, PAI, severe SW and possible maternal virilization during pregnancy

(68). Post-natal virilization does not occur. Patients may also present with craniofacial-malformations, which may overlap with a rare craniosynostosis syndrome called Antley-Bixler syndrome (OMIM 201750) due to a homozygous or compound heterozygous mutation in *POR* (67).

E. Lipoid Congenital Adrenal Hyperplasia: The most severe disorder of adrenal steroidogenesis is lipoid CAH (OMIM 201710), which arises due to a homozygous or compound heterozygous mutation in the gene encoding StAR on chromosome 8p11 (69). This leads to a defect in a transport protein, steroidogenic acute regulatory protein (StAR) that regulates cholesterol transfer within the mitochondria, the rate-limiting step in the production of steroid hormones (Figure 2) (70).

Two forms of lipoid CAH exist. The classic form results from complete or near complete absence of all steroid hormones; patients present as phenotypic females (46,XX or 46,XY sex reversal) with PAI and severe SW. The milder or “nonclassic” form present as phenotypic females (46,XY) with variable degrees of genital ambiguity, and PAI. The mild form results in 20–30% of normal StAR activity (71) due to mutation p.R188C, which has been found across various ethnicities.

F. Cholesterol side-chain cleavage (SCC) enzyme deficiency: Cholesterol side-chain cleavage (SCC) enzyme deficiency (OMIM 613743) is an extremely rare form of CAH that results from *CYP11A1* mutations (72, 73). Most of the reported cases are from Eastern Turkey due to the founder homozygous missense mutation p.R451W (74). Two forms exists; the classic form presents in a phenotypic female (46,XX or 46,XY sex reversal) with PAI and SW. The nonclassic form presents as 46,XY with variable degrees of genital ambiguity and PAI (73).

3. X-linked adrenoleukodystrophy—X-linked adrenoleukodystrophy (X-ALD; OMIM #300100) is an X-linked neurodegenerative disorder due to mutations in *ABCD1* (Xq28) (75, 76), which encodes for a peroxisomal trans-membrane protein. Deficiency of this protein leads to peroxisomal dysfunction in the central nervous system and adrenal glands through accumulation of unbranched saturated very long chain fatty acids (VLCFA) (Figure 2) (77). Siemerling and Creutzfeldt published the first case of X-ALD in 1923, of a 7-year-old boy with rapidly progressive spasticity of the lower limbs, behavioural changes, bronzed skin, and dysphagia; on autopsy, there was evidence of adrenal gland atrophy and diffuse cerebral sclerosis (78). The term adrenoleukodystrophy was later coined in the 1970’s (79). Today, X-ALD is accepted as the most common peroxisomal disorder and the second most frequent inherited metabolic disorder of PAI with a hemizygote frequency of 1 in 21,000 in the United States (80).

The clinical manifestations of X-ALD varies widely with an unpredictable disease course (76). Patients may present with chronic myelopathy that manifests with lower limb spasticity and/or peripheral neuropathy (referred to as adrenomyeloneuropathy), debilitating cerebral demyelination, fecal or urinary incontinence and PAI (Table 1) (9). A considerable number of patients have only one or the other X-ALD manifestations, and up to 20% of male cases with idiopathic PAI have X-ALD (81). Affected individuals are asymptomatic/

presymptomatic at birth, and PAI in male patients often develop in childhood, with biochemical evidence of PAI often preceding the development of relevant clinical signs by several years (82). Exceptions to the rule may exist; one rare report described a 78-year-old man with elevated VLCFA and PAI (with no genetic confirmation of disease) who had an unremarkable neurological examination and cerebral MRI (83). Conversely, PAI in females may be the only presenting manifestation of disease in adulthood likely owing to the skewing of X chromosome inactivation. However, the majority of females will go on to develop adrenomyeloneuropathy without PAI, which appears early in adulthood and reaches complete penetrance >60 years of age (9). A rapidly progressive cerebral form of the disease (cerebral adrenoleukodystrophy) may occur in a small number of patients, characterized by severe disability and death during the first decade of life. A contiguous *ABCD1 DXS1357E* deletion syndrome (CADDs) presents with a severe phenotype and death by the first year of life (84).

Elevated VLCFA, ACTH levels and impaired glucocorticoid (and/or aldosterone) responses to the cosyntropin stimulation test are the most frequent biochemical findings. However, a small proportion of females with X-ALD do not have elevations in VLCFA (9). Thus, demonstration of a pathogenic mutation in *ABCD1* is critical in confirming the diagnosis in all affected patients (85). Hair loss or abnormal hair growth, commonly referred to as scanty scalp hair, with PAI should raise the suspicion for X-ALD (86). Females with chronic myelopathy, peripheral neuropathy, urinary or fecal incontinence, and PAI should be carefully investigated for X-ALD (9). With the recent implementation of a comprehensive X-ALD newborn screening in the United States (87), the risks of unnecessary treatment and surveillance among other significant challenges, including cost, resource allocation, care coordination, and extended family testing after case identification (87), will pose new challenges for the fields of genetics and endocrinology.

4. Mitochondrial diseases—Mitochondrial diseases represent a heterogeneous group of conditions that typically manifest with multisystemic involvement and can affect individuals at any age with a prevalence of 1 in 4,300 (88). Hundreds of mutations in several nuclear or mitochondrial genes that encode for mitochondrial proteins have been described. The association between mitochondrial diseases and PAI were initially described in syndromic forms of mitochondrial DNA deletion syndromes that commonly present in childhood, such as Kearns-Sayre syndrome (OMIM 530000) (89) and Pearson syndrome (OMIM 557000) (90). One study from a patient with PAI and Kearns-Sayre syndrome showed trace amount of scar tissue replacing the adrenal glands with 65% of mutant mitochondrial DNA in the adrenal glands (compared with 95% in the liver) (91). Subsequently, reports of other mitochondrial syndromes with PAI have been observed, including MELAS (*MT-TL1* on mitochondrial DNA; OMIM 540000) (92), mitochondrial complex 1 deficiency (*NDUFA5*, 20p12.1; OMIM 612360) (93), and combined respiratory complex deficiencies resulting from a homozygous *QRSL1* mutation (6q21; OMIM 617209) (94). The clinical signs and symptoms of PAI in this patient population may present at any age, and overlap with the non-specific clinical presentation of mitochondrial diseases. Thus, a high index of suspicion for PAI should be suspected in patients with a known mitochondrial disease who present with salt craving, hyponatremia, hypotension, asthenia, or skin hyperpigmentation. PAI has

not been reported as the initial manifestation of a mitochondrial disease, and has been listed as a very rare contributor of PAI by the Endocrine Society Clinical Practice Guideline on the diagnosis of PAI (3).

5. Complex glycerol kinase deficiency—Glycerol kinase deficiency (GKD) is an X-linked disorder that is categorized as isolated (infantile, juvenile, and adult-onset forms) and manifests with postnatal hypoglycaemia and acidosis or as part of a contiguous gene deletion syndrome, referred to as complex GKD. Over 100 male patients and a few symptomatic female carriers have been described (95). Complex GKD presents in infants due to microdeletions in the Xp21.2–p21.3 region, which involves three genes; *GK*, *NROB1* and/or part of *DMD* (Duchenne muscular dystrophy) (96). The first and most severe comorbidity in complex GKD is due to adrenal hypoplasia congenita (*NROB1*), which usually manifests with SW crisis and skin hyperpigmentation in the first few weeks of life (97). The gene *NROB1* (Xp21.2) encodes for DAX1, an orphan member of the nuclear receptor superfamily that acts as a coregulatory protein in fetal adrenal formation (98). Isolated deletions or mutations in *NROB1* causes X-linked congenital adrenal hypoplasia with hypogonadotropic hypogonadism (AHC; OMIM 300200) (97). On biochemistry, complex GKD presents with metabolic acidosis, hyponatremia, PAI, elevated elevated glycerol levels on urine organic acids analysis and in blood that can falsely elevate triglyceride levels (pseudohypertriglyceridemia). Complex GKD should be considered on the differential diagnosis of all male patients presenting with SW.

6. Zellweger spectrum disorders—The Zellweger spectrum disorders (ZSD) are autosomal recessive peroxisome biogenesis conditions with multiple metabolic abnormalities commonly due to mutations in the *PEX* genes (99). ZSD causes hypotonia, distinctive facial features, growth failure, and skeletal abnormalities. There is a wide spectrum of disease severity, ranging from the most severe condition associated with death in the first year of life (previously referred to as Zellweger syndrome) to attenuated phenotypes (previously referred to as neonatal adrenoleukodystrophy and infantile Refsum disease) that present with developmental delays, hearing loss, visual impairment, liver dysfunction, coagulopathy and intracranial bleeding (99). Adult-onset forms that manifest with sensory deficits with normal neurologic development are extremely rare (99, 100). The biochemical evaluation of ZSD includes demonstration of increased VLCFA, phytanic, pristanic, bile and pipecolic acids, and reduced plasmalogens levels. The diagnosis is established with confirmation of biallelic mutations in one of the *PEX* genes.

The first association between ZSD and PAI was first reported in 1984 in 6 asymptomatic patients (101). Subsequent studies showed that up to 29% of patients have PAI (102). PAI typically affects older infants and children with ZSD, and has not been reported in the neonatal period. Therefore, adrenocortical function should be assessed at around one year of age, or earlier if clinically suggestive, and regularly thereafter, in all affected patients with ZSD (99). The mechanism of PAI in ZSD is likely similar to that in X-ALD, and involves the accumulation of VLCFA in the adrenal glands that ultimately impair steroidogenesis (Figure 2).

7. Hereditary cystatin C amyloid angiopathy—Hereditary cystatin C amyloid angiopathy (HCCAA; OMIM 105150) is a rare autosomal dominant multi-organ amyloid deposition disease that is caused by a mutation in cystatin C (*CST3*; 20p11.21) leading to inhibition of several cysteine proteinases (e.g.: cathepsins S, B, and K) (103). A founder mutation in *CST3* occurs frequently in Iceland. Other mutations have been reported in the *APP* (21q21.3) and *ITM2B* (13q14.2) genes (104). Mutated cystatin C forms amyloid in blood vessels, predominantly in the central nervous system, but also in the adrenal glands leading to hemorrhage (104). This predisposes to adrenocortical dysfunction, suboptimal response to the cosyntropin stimulation test and rarely adrenal crisis (105, 106).

8. Sphingosine-1-phosphate lyase 1 deficiency—Sphingosine-1-phosphate (S1P) lyase 1 deficiency is a recently identified autosomal recessive condition due to loss of function mutations in *SGPL1* (10q22.1). The clinical manifestations are broad and include bilateral adrenal calcifications leading to PAI, steroid-resistant nephrotic syndrome, ichthyosis, immunodeficiency and organ anomalies. S1P lyase catalyzes the irreversible degradation of endogenous and dietary S1P, an intracellular and extracellular signaling molecule involved in angiogenesis, vascular maturation, and immunity, which represents the final step of sphingolipid catabolism and downstream from the defect observed in NPC (Figure 2) (107). Increased levels of S1P and sphingosine in the probands blood and fibroblasts have been reported (107–109), with marked alterations in adrenocortical zonation and decreased *CYP11A1* expression in *Sgpl1*^{−/−} mice (108, 109).

9. Fabry disease—Fabry disease (OMIM 300644) is an X-linked condition due to mutations in the *GLA* gene (Xq22.1) with multi-organ involvement due to α -galactosidase-A deficiency and consequent systemic accumulation of globotriaosylceramide (Gb3), possibly including the adrenal glands. A small number of case reports and series showed lower basal cortisol levels (110) and suboptimal cortisol response at the cosyntropin stimulation test (111). However, more recent clinical data from various centers conducting enzyme therapy clinical trials have not documented adrenal insufficiency as either a major or a minor manifestation of Fabry disease. Thus, future well designed studies are required to ascertain the association between PAI and other IEM using standardized diagnostic approaches, and preferably with tandem-mass spectrometry based methods for increased specificity and sensitivity.

Section 3. Genetic counseling of patients with IEM manifesting with PAI

The majority of IEM are transmitted as autosomal recessive and the determination of disease risk is dependent on the carrier status of the patient's parents. Thus, genetic counseling and testing should focus on the future risks of having an affected child, incorporating the families values and attitudes. Additionally, underscoring the risks and benefits of genetic screening and counseling, psychosocial interventions and service delivery are other important aspects of a successful genetic counseling strategy that should be carried out by a certified genetic counselor. Genetic testing should be carried out in an accredited laboratory and interpreted by an experienced team. When one or both disease-causing mutations have been identified in the proband, genetic testing should be offered to other family members, which will also assist in the identification of presymptomatic carriers.

A carrier state is defined as having one normal gene and one gene carrying a mutation. Carriers of an autosomal recessive condition do not show symptoms. The risk of having an affected child, a child who is a carrier, and a child with normal genes if both parents are carriers is 25%, 50%, and 25%, respectively. If one parent is affected with an autosomal recessive IEM and the other parent is a heterozygous carrier, then each child has a 50% chance of inheriting one mutation and a 50% chance of being affected, irrespective of the sex. In the case of CAH due to 21-OHD, the incidence of classic CAH is approximately 1 in 10,000 to 1 in 20,000 per year, with a carrier rate in the general population of approximately 1 in 50 to 1 in 71 (median 1 in 60) (45, 112, 113). The chance that a patient with classic CAH will have a child with classic CAH is 1 in 120 (chance of partner being a carrier ($1/60 \times 1/2$) if the carrier status of the partner is unknown.

X-linked inheritance is observed in some IEM, such as X-ALD, complex GKD and Fabry disease. Thus, the possibility of a son of a female carrier developing X-ALD is 50%; 50% of female off-springs will also be heterozygous carriers. All female off-springs of an affected male will be carriers but none of their male off-springs will be affected. Significant intra-familial phenotype variability has been observed as different clinical phenotypes can occur even among monozygotic twins (114). The notion that females rarely show signs of X-linked recessive conditions (“a carrier”) given the presence of a second unaltered copy of the X chromosome to compensate for an altered gene is inaccurate and should be avoided in clinical practice when managing females with various X-linked disorders. It is becoming increasingly recognized, particularly in IEM, that female heterozygous carriers for an X-linked disease can present with significant manifestations, and at any age, as shown in studies of females with X-ALD and Fabry disease (9, 115). Whether these manifestations are owing to the skewing of X-inactivation in various organs and/or the misclassification of such diseases as X-linked recessive rather than dominant (a mutation in one X chromosome in each cell is sufficient to cause the condition, as indicated by excess of affected female heterozygotes in a family pedigree) remains to be debated and elucidated. Additionally, if a clinician encounters discrepant results when dealing with a patient with an X-linked condition, the following possibilities should be considered; complete gene deletion, contiguous gene syndrome, occurrence of the disorder in a female affected with Turner syndrome (one normal X chromosome, the other is either missing or structurally altered; XO), homozygosity for an X-linked condition, translocations involving the X chromosome, genes located in the pseudoautosomal regions, non-paternity, or gonadal mosaicism. Thus, clinicians are encouraged to clinically evaluate females at risk for X-linked diseases as they would in males with the same condition, addressing all potential disease manifestations and abandoning the classification of X-linked disorders as either recessive or dominant.

Section 4. Conclusions

The routine use of genetic testing and development of comprehensive methodologic approaches for the diagnosis of PAI has improved our ability to phenotype and genotype patients with various IEM. As shown in this review, IEM manifesting with PAI represent a heterogeneous group of disorders that are life-threatening. Life-saving neonatal screening programs exist in the United States to detect some conditions and future screening programs aimed at genetic diagnosis of neonates, such as with X-ALD, will likely result in early

diagnosis and avoidance of adrenal crisis as a presenting sign in this patient population although the risks of unnecessary treatment and surveillance among other significant challenges are yet to be determined. Molecular analysis of these conditions is useful in confirming the diagnosis, and provides a powerful tool in genetic counseling. Clinicians are encouraged to consider these IEM on the differential diagnosis of every patient with PAI, irrespective of ethnicity, age or sex. Future research is needed to assist in the molecular pathogenesis of other IEM that will assist in the management of affected patients and their family members across all age groups.

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Figure 1.

Diagnostic algorithm for primary adrenal insufficiency with an emphasis on inborn errors of metabolism. The cosyntropin stimulation test is a necessary first step in excluding PAI. Once PAI is biochemically established, then rule out of common causes in adults such as autoimmune destruction of the adrenal cortex with measurement of 21-hydroxylase antibodies is necessary. Imaging of the adrenal glands with a computed tomography or magnetic resonance imaging is necessary in excluding adrenal gland atrophy, hemorrhage, infiltration or neoplasia. Baseline serum 17-hydroxyprogesterone level (17-OHP) is used for evaluating congenital adrenal hyperplasia (CAH), particularly the nonclassic type, which presents as late-onset. All patients with negative 21-hydroxylase antibodies should be tested for adrenoleukodystrophy with plasma VLCFAs, irrespective of age or sex. The individual's clinical picture and/or family history may help prioritize testing. Idiopathic PAI is defined as adrenal failure from an unexplained etiology. Revisiting the diagnostic algorithm, including broader genetic testing (e.g. whole exome or genome sequencing), is encouraged in such cases. *Abbreviations:* 17-OHP, 17-hydroxyprogesterone; 21-OHD, 21-hydroxylase antibody; CAH, congenital adrenal hyperplasia; VLCFA, very long chain fatty acid; PAI, primary adrenal insufficiency; X-ALD, X-linked adrenoleukodystrophy. Courtesy of Dr. Fady Hannah-Shmouni, NICHD, NIH.

Figure 2.

Key disorders of adrenal intracellular cholesterol trafficking and steroidogenesis. Key enzymatic deficiencies are highlighted in red. Circulating low-density lipoproteins (LDL) enters steroidogenic cells through receptor-mediated endocytosis into the endosomes, where cholesterol esters are bound by Niemann-Pick disease, type C2 (NPC2) protein. Lysosomal acid lipase (LAL) yields free cholesterol, which is then transferred to Niemann-Pick disease, type C1 (NPC1) protein and exported to reach the outer and inner mitochondrial membranes where it can enter the process of steroidogenesis. Congenital adrenal hyperplasia is caused by deficiency of mitochondrial enzymes that are responsible for steroidogenesis. The orange triangles represent accumulation of very long chain fatty acids (VLCFA) in adrenocortical cells that lead to primary adrenal insufficiency as seen in X-linked adrenoleukodystrophy and Zellweger spectrum disorders. Courtesy of Dr. Fady Hannah-Shmouni, NICHD, NIH.

Figure 3.

The *CYP21A2* gene and its duplicated pseudogene (*CYP21A1P*). A) Both genes are located 30kb apart in the human leukocyte antigen (HLA) class III region in the major histocompatibility (MHC) locus on 6p21.3, where they share approximately 98% sequence homology. They are arranged in tandem repeat with the *C4* (*C4A* and *C4B*) genes. *C4*/*CYP21A2* is flanked by telomeric *RP* (*RP1* and *RP2*) and centromeric *tenascin* (*TNXA* and *TNXB*) genes. With the exception of *C4*, each of the other functional genes (*RP1*, *CYP21A2* and *TNXB*) has a corresponding highly homologous pseudogene (*RP2*, *CYP21A1P* and *TNXA*). Enlarged area represents the 10 exons of *CYP21A2*. B) The high degree of sequence homology between *CYP21A2* and its pseudogene *CYP21A1P* allows for recombination events, whereby unequal crossing-over through intergenic recombination results in large deletions, and the transfer of deleterious pseudogene sequence to the active gene. Adapted from reference 25 (Hannah-Shmouni et al.); Courtesy of Dr. Fady Hannah-Shmouni, NICHD, NIH.

Table 1

List of inborn errors of metabolism known to cause primary adrenal insufficiency.

Inborn errors of metabolism	Causative Gene	Clinical Manifestation
Smith-Lemli-Opitz syndrome	<i>DHCR7</i>	<ul style="list-style-type: none"> Developmental delay, microcephaly, organ anomalies, dysmorphic features (eg: syndactyly of the second and third toes), PAI, male undervirilization and/or hypogonadism.
Cholesteryl ester storage disease (e.g.: Wolman disease)	<i>LIPA</i>	<ul style="list-style-type: none"> Bloating, steatorrhea, vomiting, xanthelasma, failure to thrive, growth failure, jaundice, hepatosplenomegaly, adrenal calcification, PAI, and hypotonia.
21-hydroxylase deficiency	<i>CYP21A2</i>	<ul style="list-style-type: none"> Classic: 46,XX ambiguous genitalia, PAI, salt-wasting, postnatal virilization. Nonclassic: hyperandrogenism during childhood or early adulthood may be asymptomatic.
	<i>CYP21A2</i> and <i>TNXB</i>	<ul style="list-style-type: none"> CAH-X: In addition to the above, joint hypermobility, joint pain, multiple joint dislocations, midline defects including possible cardiac structural abnormalities.
3 β -hydroxysteroid dehydrogenase type 2 deficiency	<i>HSD3B2</i>	<ul style="list-style-type: none"> Classic: 46,XX and 46,XY ambiguous genitalia, PAI, salt-wasting.
P450 oxidoreductase (POR) deficiency	POR	<ul style="list-style-type: none"> 46,XX and 46,XY ambiguous genitalia, PAI, severe salt-wasting, possible maternal virilization during pregnancy. Possible skeletal malformations (Antley-Bixler syndrome). Post-natal virilization does not occur.
Lipoid CAH	StAR	<ul style="list-style-type: none"> Classic: phenotypic female (46,XX or 46,XY sex reversal), PAI, severe salt-wasting. Nonclassic: 46,XY variable degrees of genital ambiguity, PAI.
Cholesterol side-chain cleavage enzyme deficiency	<i>CYP11A1</i>	<ul style="list-style-type: none"> Classic: phenotypic female (46,XX or 46,XY sex reversal), PAI, salt-wasting. Nonclassic: 46,XY variable degrees of genital ambiguity, PAI.
X-linked adrenoleukodystrophy	<i>ABCD1</i>	<p>Affected males:</p> <ul style="list-style-type: none"> Childhood cerebral form: hyperactivity, PAI and progressive impairment of cognition, behavior, vision, hearing, and motor function. Adrenomyeloneuropathy: progressive stiffness and weakness of the legs, sphincter disturbances, sexual dysfunction, PAI in adulthood. PAI without obvious neurologic abnormality; adrenomyeloneuropathy usually develops by middle age. <p>Affected females:</p> <ul style="list-style-type: none"> The majority will develop mild-to- moderate spastic paraparesis later in adulthood, typically without PAI. PAI may be the sole presentation in adulthood.
Mitochondrial diseases	Associated genes are either nuclear or mitochondrial	<p>Syndrome specific</p> <ul style="list-style-type: none"> KSS: progressive external ophthalmoplegia, retinitis pigmentosa, cardiomyopathy, heart block, PAI (rare). Pearson syndrome: exocrine pancreatic dysfunction, pancytopenia, sideroblastic anemia, PAI (rare).

Inborn errors of metabolism	Causative Gene	Clinical Manifestation
		<ul style="list-style-type: none"> MELAS: early-onset stroke-like episodes that do not confine to vascular territories, migraines, lactic acidosis, myopathy, multiple endocrinopathies such as diabetes mellitus or PAI (rare).
Complex Glycerol Kinase Deficiency	<i>GK, DAX1</i> and/or part of <i>DMD</i>	<ul style="list-style-type: none"> Adrenal hypoplasia congenital (PAI), dysmorphic features, pseudohypertriglyceredmia, elevated creatine phosphokinase.
Zellweger spectrum disorders	<i>PEX</i>	<ul style="list-style-type: none"> Severe: Hypotonia, difficulty feeding, distinctive facial features, growth failure, skeletal abnormalities, neonatal-onset seizures, renal cysts, chondrodysplasia punctata, liver disease, progressive apnea, PAI. Mild-moderate: developmental delays, hearing loss, visual impairment, liver dysfunction, coagulopathy, intracranial bleeding. Adult-onset: sensory deficits with normal neurologic development.
Sphingosine-1-phosphate lyase 1 deficiency	<i>SGPL1</i>	<ul style="list-style-type: none"> Bilateral adrenal calcifications leading to PAI, steroid-resistant nephrotic syndrome, ichthyosis, immunodeficiency, organ anomalies.

Abbreviations: CAH, congenital adrenal hyperplasia; CAH-X, congenital adrenal hyperplasia with tenascin-X impairment; KSS, Kearns-Sayre syndrome; MELAS, mitochondrial encephalopathy with lactic acidosis and stroke-like episodes; PAI, primary adrenal insufficiency; StAR, steroidogenic acute regulatory protein

Table 2

Classification of common *CYP21A2* mutations in CAH based on *in vitro* data.

21-hydroxylase deficiency				
Group	Null	A	B	C
Phenotype	SW	SW	SV	NC
<i>In vitro</i> activity of <i>CYP21A2</i>	0%	<1%	1–2%	20–60%
Mutation ^a	30kb deletion ^b 8bp deletion exon 6 cluster p.Q318X p.R356W p.Leu307fs	IVS2-13A/C>G	p.I172N p.I77T	p.P30L ^b p.V281L p.R339H p.P453S

Abbreviations: SW, salt-wasting; SV, simple-virilizing; NC, nonclassic.

^a Nomenclature at the protein level is based on conventional codon numbering. Exon 6 cluster denotes 3 clustered mutations in exon 6.

^b About 4 percent being Group C.

Adapted from reference 25 (Hannah-Shmouni et al.)