Determining the Etiology of Primary Adrenal Insufficiency

PAI may be a symptom of a potentially fatal genetic disease

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Primary Adrenal Insufficiency (PAI)

- A rare disorder with a prevalence of 110–144 cases per million population in the developed countries[1]
  - Congenital adrenal hyperplasia (CAH) is the most common form in children and accounts for 72% of cases, with autoimmune PAI seen in ~10–15%[1]
  - The incidence is 4.4 to 6 new cases per million population a year and rising[1]
- Irreversible damage to the adrenal cortex leads to insufficient production of glucocorticoids, mineralocorticoids, and adrenal androgens[2]
- If left untreated, PAI can be potentially fatal, and prompt diagnosis can avoid unnecessary hospital admissions due to adrenal crisis[3]
- PAI leads to reduced life expectancy, with younger patients (<40 yrs at diagnosis) at elevated risk of premature death[4]
  - In a 2009 Norwegian study,[4] mean life expectancy in patients with PAI was reduced by 3.2 years in female and 11.2 years in male patients

1. Betterle C, Morlin L. *Endocr Dev.* 2011;20:161-172. **2.** Quinkler M et al. *Dtsch Arztebl Int.* 2013;110(51-52):882-888. **3.** Puttanna A et al. *BMJ Case Rep.* 2013;2013. pii: bcr2013010473. **4.** Erichsen MM et al. *Eur J Endocrinol.* 2009;160(2):233-237.

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Symptoms and laboratory changes in PAI[1,2]

Hormone	Symptoms
ACTH (POMC) stimulation	Hyperpigmentation
Glucocorticoid deficiency	Fatigue and decreased performance
	Diminished appetite & weight loss
	Nausea, vomiting, and abdominal pain
	Myalgias and joint pain
	Orthostatic hypotension
	Anemia, lymphocytosis, eosinophilia
	Hypoglycemia
	Hyponatremia
	Hypercalcemia
Mineralocorticoid deficiency	Hypotonia, hypovolemia, creatinine increase, orthostatic dysregulation
	Hyponatremia
	Hyperkalemia
	Salt craving

POMC, proopiomelanocortin.

1. Quinkler M et al. *Dtsch Arztebl Int.* 2013;110(51-52):882-888.

Causes of PAI

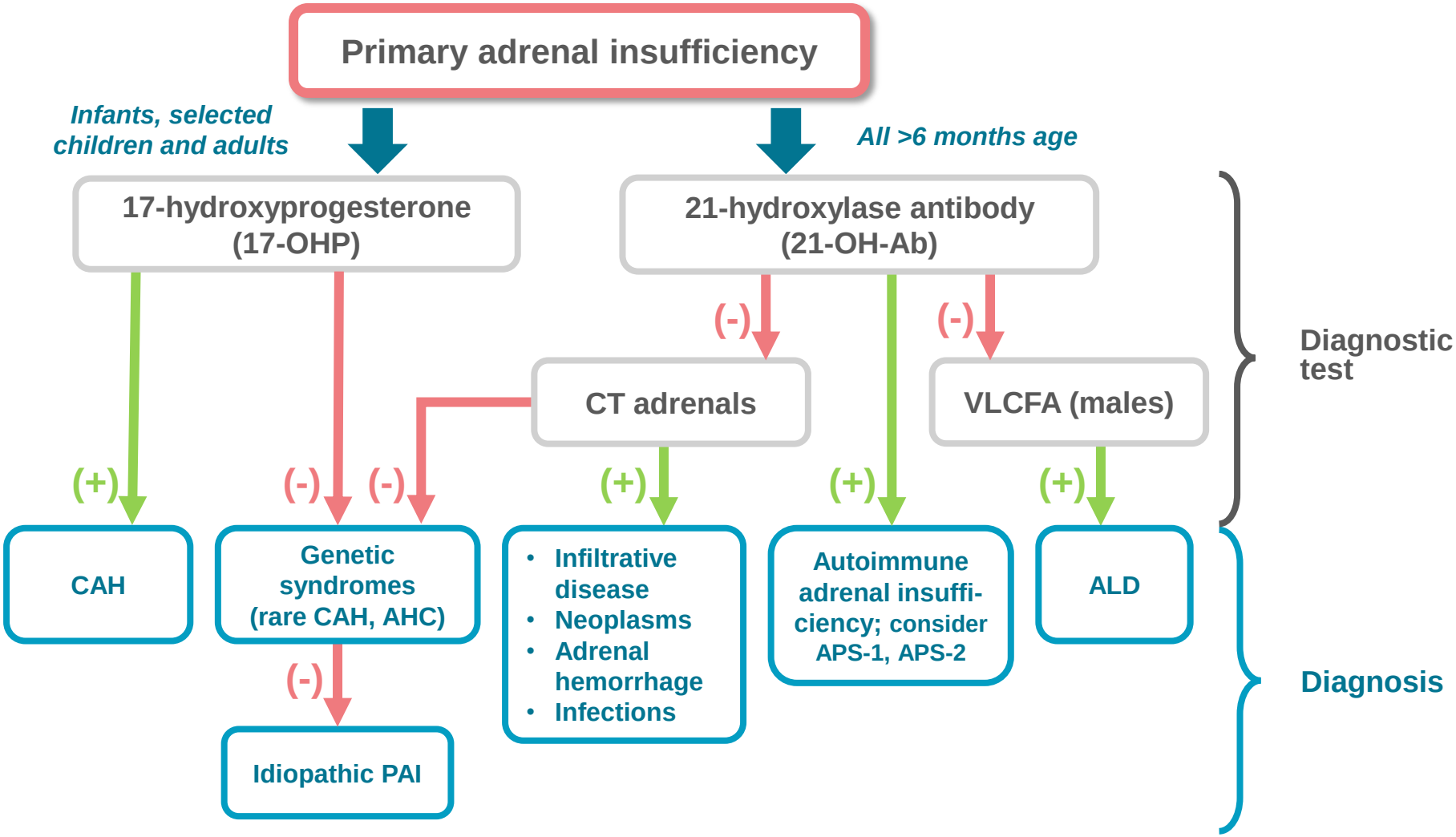
Classification and causes of PAI

Etiology	Pathogenesis	Diagnosis
Autoimmune	T- and B-cell autoimmunity against adrenocortical cells	21-OH-Ab
Genetic	• CAH • Adrenoleukodystrophy (ALD) • Adrenal hypoplasia congenital (AHC) • Hypogonadotropic hypogonadism • Familiar glucocorticoid deficiency (ACTH resistance syndrome), Smith–Lemli–Opitz syndrome, mitochondrial forms (Kearns–Sayre syndrome)	• Serum steroid profile, Sequencing of steroidogenic genes (eg, *CYP21B*) • Serum VLCFA • Sequencing of *NR0B1* (*DAX1*)
Infection	• Mycobacteria • Bacteria (eg, meningococcus and *H. influenzae*) • Fungi (eg, *Pneumocystis carinii*) • Viruses (eg, HIV, herpes simplex, and cytomegalovirus)	Culture, QuantiFERON test, PCR, adrenal CT
Bleeding	• Antiphospholipid syndrome • Anticoagulant therapy • Disseminated intravascular coagulation	Evidence of bleeding on adrenal CT
Surgery	Tumor surgery, Cushing's syndrome, radical nephrectomy	
Infiltrative	Amyloidosis, hemochromatosis, bilateral adrenal metastasis or lymphoma, xanthogranulomatosis	
Medication	Ketoconazole, etomidate, mitotane, metyrapone	

21-OH-Ab, 21-hydroxylase antibody; ACTH, adrenocorticotropic hormone; CT, computed tomography; PCR, polymerase chain reaction; VLCFA, very long chain fatty acids.

Adapted from: Husebye ES et al. *J Intern Med.* 2014;275(2):104-115.

Uncovering the etiology of PAI[1]



AHC, adrenal hypoplasia congenita; APS-1, autoimmune polyendocrine syndrome type 1; APS-2, autoimmune polyendocrine syndrome type 2.

1. PAI Draft Guideline.

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Diagnostic approach to genetic causes of PAI

- During the past 2 decades, genetic mutations associated with several familial causes of adrenal insufficiency (AI) have been identified[1]
  - Both etiologies can be screened for using 21-OH-Ab and baseline serum 17-OHP level[4]
- Children presenting with PAI and normal 17-OHP levels should be further worked up for other potential genetic etiologies[2]
  - Male infants and children should be screened for ALD by measuring VLCFA in a serum sample
  - CT of the adrenal glands can rule out infiltrative disease, tumors, hemorrhages, and infection and further strengthen the case for a genetic etiology
- Early identification of genetic causes of adrenal disease has important prognostic and therapeutic implications for patients and their families[1]

1. Brett EM, Auchus RJ. *Endocr Pract.* 2015;21(4):395-399. **2.** PAI Draft Guideline.

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Genetic basis of familial PAI

Disease	Gene(s)	Inheritance pattern	Associated	Laboratory
AAA	*AAAS*	Autosomal recessive	Alacrima, achalasia	
ALD	*ABCD1*	X-linked recessive	Neurologic, hypogonadism	Males: high VLCFA
AHC	*NR0B1*	X-linked recessive	Hypogonadotropic hypogonadism, delayed puberty	Low gonadotropins and testosterone
AI with gonadal dysgenesis	*NR5A1*	Autosomal dominant More common 46,XY	Gonadal dysgenesis, undervirilization	High gonadotropins, low testosterone
APS-1	*AIRE*	Autosomal recessive	Mucocutaneous candidiasis, hypoparathyroidism	Positive 21-OH-Ab
CAH: 21-OH	*CYP21A2*	Autosomal recessive	Variable—salt wasting, simple virilizing, females with ambiguous genitalia	Elevated 17-OHP, basal or stimulated
CAH: 3β-hydroxysteroid dehydrogenase	*HSD3B2*	Autosomal recessive	Males: undervirilization Females: mild virilization	Elevated 17-OH-pregnenolone, elevated DHEA
Lipoid CAH	*STAR*	Autosomal recessive	XY sex reversal	All steroids low
FGD	*MRAP, MC2R, NNT, MCM4, TXNRD2*	Autosomal recessive	Glucocorticoid only, hyperpigmentation	Elevated ACTH, normal renin and aldosterone

AAA, alacrima-achalasia-adrenal insufficiency (triple A syndrome; Allgrove syndrome); DHEA, dehydroepiandrosterone; FGD, familial glucocorticoid deficiency.

Brett EM, Auchus RJ. *Endocr Pract.* 2015;21(4):395-399.

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Adrenoleukodystrophy (ALD)

- Screening antibody–negative PAI can be the first presentation of another genetic etiology: ALD[1,2]
  - ALD can be suspected in antibody-negative male children with PAI in whom CAH has been ruled out[2]
- ALD is an X chromosome–linked recessive disorder[3-5]
  - Frequency is 1 in 42,000 for hemizygous males (1 in 16,800 for hemizygotes plus heterozygotes)
  - Gene mutation leads to accumulation of VLCFA in brain white matter, adrenal gland, fibroblasts, and plasma
- ALD is characterized by elevated plasma and tissue levels of VLCFA in male patients and in most female carriers[5]
  - VLCFA accumulate secondary to defects in peroxisome β-oxidation associated with mutations in the *ABCD1* gene
- The earliest symptoms of ALD are nonspecific or overlap with those of other medical conditions[6]
- Phenotypic expression of ALD varies widely, even among affected members of the same family[3,4]

1. Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399. **2.** PAI Draft Guideline. **3.** Bezman L et al. *Ann Neurol.* 2001;49(4):512-517.
4. Lombard-Platet G et al. *Proc Natl Acad Sci USA.* 1996;93(3):1265-1269. **5.** Moser HW et al. *Nat Clin Pract Neurol.* 2007;3(3):140-151.
6. Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.

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ALD phenotypes and diagnosis

- Cerebral ALD (CALD) is the most common and severe form of the disease[1,2]
  - Characterized by extensive demyelination of the white matter of the brain
  - Typical age of onset between 5 and 12 years
- Adrenal insufficiency (AI) may be the only early phenotypic expression of the disease before progressing to neurologic symptoms[1,2,3]
- Once neurologic symptoms become evident, progression is rapid and can be fatal if left untreated[3]
  - Timely diagnosis during work-up of AI can save the patient's life
- Diagnosis of ALD is achieved by the demonstration of elevated serum VLCFA[3]
  - In **males**, the VLCFA panel is highly sensitive for detecting ALD and is the appropriate first step in the diagnosis. If VLCFA levels are elevated or VLCFA ratios are abnormal, a mutation analysis should be performed to confirm the diagnosis
  - In **females**, the VLCFA panel is potentially less sensitive for detecting ALD (up to 15% of carriers will have normal results). Therefore, a more definitive test for suspected female carriers is a mutation analysis
- Genetic testing is available through a DNA-based blood test[3]

1. Moser HW et al. The Metabolic Basis of Disease, eds. Scriver CR et al. (McGraw-Hill, New York), pp. 1511-1532. **2**. Moser HW Advances in Human Genetics, eds. Harris H and Hirschhorn K (Plenum, New York), pp 1-1061**. 3.** Moser HW et al. *Nat Clin Pract Neurol.* 2007;3(3):140-151.

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ALD expresses multiple phenotypes—CALD is the most severe[1-3]

Neurological manifestations	Phenotype	Description	Frequency
Inflammatory cerebral demyelination	**Childhood and adolescent CALD**	In children, onset at 3–10 years of age; progressive behavioral, cognitive, and neurologic deficits, leading to total disability and death within 2–5 years. In adolescents, onset at 11–21 years of age; pathology and clinical presentation similar to childhood form, but somewhat slower progression. Treated by bone marrow transplant if diagnosed early.	Childhood, 31–35% Adolescent, 4–7%
	Adult CALD	Dementia, behavioral disturbances; neurologic deficits. Progression parallels that of CCALD, leading to total disability and death. Treated by bone marrow transplant if diagnosed early.	45–60%
Dying-back axonopathy (non-inflammatory)	**Adrenomyelo-neuropathy (AMN)—men**	In men, onset at 20–30 years of age; in women, onset at 40–50 years of age; progressive over decades. Involves mainly spinal cord; distal axonopathy. Progressive stiffness and weakness of legs, impaired vibration sense in the legs, urinary sphincter disturbances, scarce scalp hair, impotence in men.	100%
	Heterozygote—women		65%
Presymptomatic	**Adrenal insufficiency**	PAI without apparent neurologic involvement. Most patients eventually develop inflammatory cerebral phenotype or axonopathy. Regular monitoring required for early detection of inflammatory cerebral phenotypes.	Varies with age; up to 50% in childhood
	Asymptomatic	Biochemical and gene abnormality without demonstrable adrenal or neurologic deficit. Detailed studies often show adrenal hypofunction or subtle signs of AMN. Regular monitoring required for early detection of inflammatory cerebral phenotypes and adrenal insufficiency.	Diminishes with age; common <4 years, very rare >40 years

Adapted from: **1.** Moser HW et al. *Nat Clin Pract Neurol.* 2007;3(3):140-151. **2.** Berger J et al. *Biochimie.* 2014;98:135-142. **3.** de Beer M et al. *Neurology.* 2014;83(24):2227-2231.

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Performing a VLCFA test

- Phenotype cannot be predicted by VLCFA plasma concentration

VLCFA	Normal	Males with ALD	Obligate Female Carriers
C26:0, µg/mL*	0.23+0.09	1.30+0.45	0.68+0.29
C24:0/C22:0	0.84+0.10	1.71+0.23	1.30+0.19
C26:0/C22:0	0.01+0.004	0.07+0.03	0.04+0.02

Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.

Performing a VLCFA test

VLCFA test: codes and logistics	
CPT code(s)	• 82726
Includes	• VLCFA
Preferred specimen(s)	• 1 mL plasma collected in an EDTA (lavender-top) tube
Minimum volume	• 1 mL
Collection instructions	• Fasting sample is required
Specimen container	• Plastic screw-cap vial
Transport temperature	• Frozen
Specimen stability	• Room temperature: unacceptable • Refrigerated: unacceptable • Frozen: 14 days
Methodology	• Gas chromatography–mass spectrometry (GC-MS)

Codes and information accurate for Quest Diagnostics screening laboratory.

Improving Outcomes for Patients With Inflammatory Cerebral Demyelinating Phenotypes (CCALD, ACALD)

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Early identification of CALD improves outcomes

- CALD is characterized by an inflammatory demyelinating process[1]
  - Progressive central demyelination occurs with neurologic deterioration[1]
  - Cerebral lesions resemble those of multiple sclerosis[1]
  - Impairment of cognition, behavior, vision, hearing, and motor function[3]
  - Disability within 2 years, death within 4 years[1]
- If ALD is suspected in males with PAI, testing for elevated plasma VLCFA confirms the diagnosis[1,2]
  - If a diagnosis of ALD is established, extended family screening and consultation with a geneticist is recommended[1]
- Hematopoietic stem cell transplantation (HSCT) done in the early stages of CALD has been shown to be beneficial[4]
  - Clinical trial data support the recommendation that transplant be offered to patients in the early stages of CALD

1. Engelen M et al. *Orphanet J Rare Dis.* 2012;7:51. **2.** Moser HW et al. *Nat Clin Pract Neurol.* 2007;3(3):140-151
3. Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.
4. Mahmood A et al. *Lancet Neurol.* 2007;6(8):687-692.

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Management of ALD

- Multidisciplinary care team may be required:
  - Endocrinologist
  - Neurologist/pediatric neurologist/metabolic disease expert
  - Psychologist/psychiatrist
  - Transplant physician (inflammatory cerebral phenotype)
- Treatment of manifestations[1]:
  - Corticosteroid replacement therapy is essential for those with AI
  - Affected boys with ALD also benefit from the general supportive care of parents as well as from psychological and educational support
- Surveillance: periodic reevaluation of adrenocortical function and twice-yearly MRIs for detection of early cerebral disease[1]
- Follow-up in boys with ALD is important[2]
  - Early detection of AI
  - Early detection of CALD to propose HSCT

1. Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.
2. Engelen M et al. *Orphanet J Rare Dis.* 2012;7:51.

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Surveillance of ALD

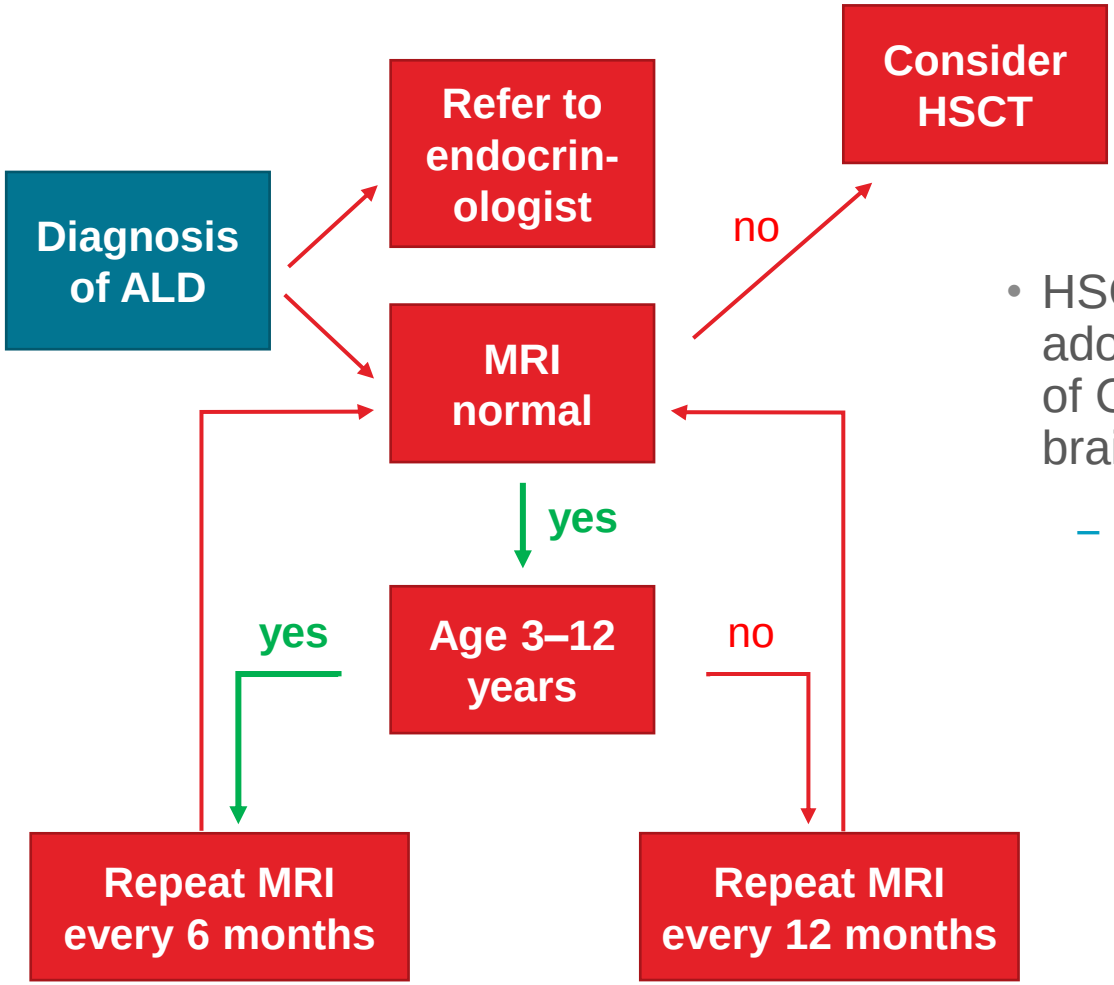

Chart adapted from: Engelen M et al. *Orphanet J Rare Dis.* 2012;7:51.[3]

- HSCT is an option for boys and adolescents in the early stages of CCALD who have evidence of brain involvement on MRI[1]
  - Due to high morbidity/mortality, HSCT is recommended only for individuals with radiographic evidence of brain involvement[1,2]

1. Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.
2. Resnick IB et al. *Clin Transplant.* 2005;19(6):840-847. **3.** Engelen M et al. *Orphanet J Rare Dis.* 2012;7:51.

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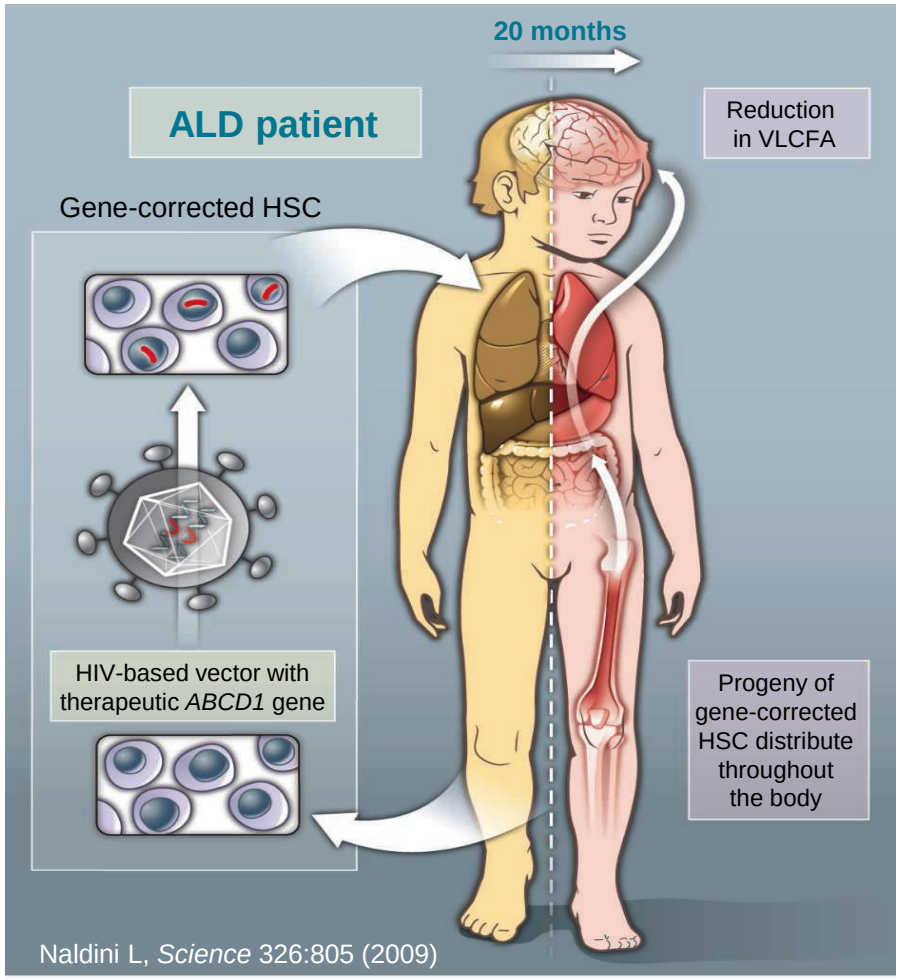
Gene therapy for ALD: *ex vivo* gene correction



- Addition of a functional *ABCD1* gene to autologous hematopoietic stem cells (HSC) via lentiviral vector[1,2]
- Autologous HSC are transplanted back to the patient[1,2]
  - Should reduce morbidity and mortality associated with transplant using allogeneic donor—eg, acute and chronic graft-versus-host disease (GVHD), graft rejection
  - Promising but early clinical proof-of-concept results reported in 2 CALD patients in a French study

1. Cartier N et al. *Methods Enzymol.* 2012;507:187-198. **2.** Cartier N et al. *Science.* 2009;326(5954):818-823.

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Summary

- PAI is a rare and potentially fatal condition in which irreversible damage to the adrenal cortex leads to insufficient production of glucocorticoids, mineralocorticoids, and androgens
- Once PAI is suspected, a sequence of diagnostic tests should enable clinicians to identify the underlying cause of the presenting PAI
  - In antibody-negative patients, it is critical for endocrinologists to work patients up for various genetic causes of PAI
    - Testing should include baseline VLCFA testing to test for ALD, especially in male patients
- ALD is a treatable condition, and timely diagnosis of patients with ALD has a considerable impact on the clinical outcomes of these patients and can be life saving
  - Early diagnosis enables comprehensive, long-term disease management and the opportunity to treat patients with early-stage CALD
  - Genetic counseling for affected family members may identify at-risk relatives and aid in family planning

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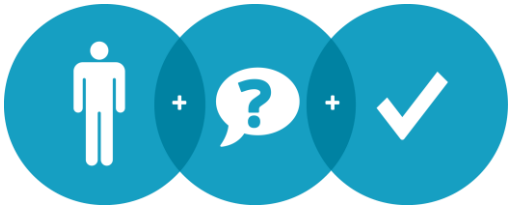

APPENDIX

PAI in the presence of psychiatric or developmental disorders

Suggestive findings of ALD in boys with primary adrenal insufficiency[1-3]
Attention deficit/hyperactivity disorder
Cognitive difficulties
Progressive behavioral disturbance: withdrawal or aggression
Difficulty in speaking or understanding spoken language
Seizures
Vision loss
Worsening handwriting
Disturbances of gait and coordination
Intermittent vomiting
Increased skin pigmentation

- ALD is a frequent cause of idiopathic PAI in children and adults[1]
- Clinical presentation of ALD is highly variable: psychiatric symptoms and symptoms of PAI are among the most common presenting features[2]
- Consideration should be given especially to young males presenting with antibody-negative PAI, particularly in the presence of psychiatric or developmental disorders[2,3]

1. Laureti S et al. *J Clin Endocrinol Metab*. 1996;81(2):470-474. **2.** Morell BK et al. *BMJ Case Rep*. 2010. doi:10.1136.bcr.11.2009.2419.
3. Steinberg SJ et al. In: Pagon RA et al (eds). *GeneReviews® [Internet].* Seattle, WA: University of Washington; 2015.

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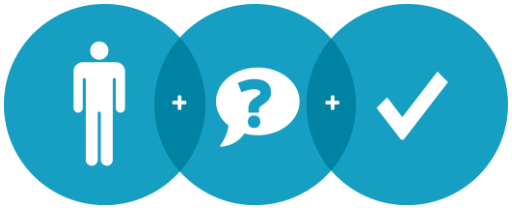
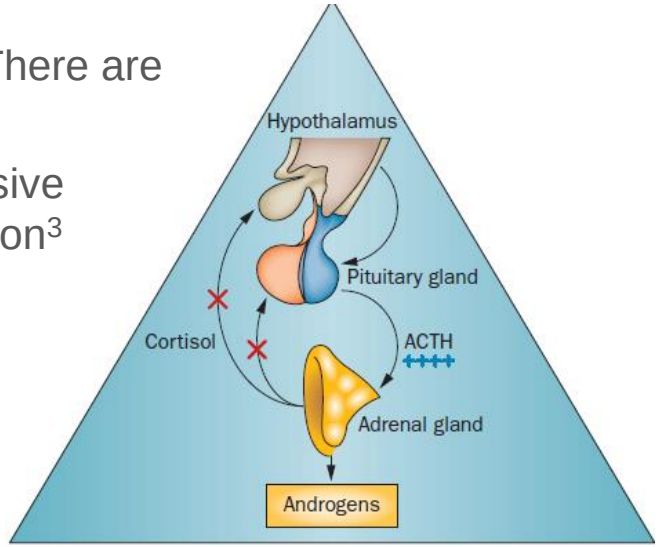


Additional Slides on Other Genetic Causes of PAI

CAH: the most common cause of PAI in children[1,2]

- CAH is the most common genetic endocrine disorder. There are several genetic variants of CAH[3]
- The most common form (~95%) is an autosomal recessive disorder affecting *CYP21A2* by either deletion or mutation[3]
  - Results in 21-OH enzyme deficiency
  - Found in 1 in 16,000 newborns[4]
- CAH can affect neonates, children, and adults[1,3]
  - Frequently diagnosed within the first weeks of life
  - Leads to inadequate production of cortisol and aldosterone and increased production of androgens
  - Girls present with virilized genitalia at birth
  - Boys present with a salt-wasting crisis at 2–3 weeks of age
- Mortality in first year following diagnosis is 5-fold higher than in general population[1]
- Diagnostic test: 17-OHP assay[5]
  - Levels >1000 ng/dL are diagnostic for 21-OH deficiency or CAH
- Patients with a negative test result and signs of AI need to be further evaluated for rare genetic syndromes[2]



Han TS et al. *Nat Rev Endocrinol*. 2014;10(2):115-124

1. Shulman DI et al. *Pediatrics.* 2007;119(2):e484-e494. **2.** PAI Draft Guideline. **3.** Han TS et al. *Nat Rev Endocrinol*. 2014;10(2):115-124. **4.** Brett EM, Auchus RJ. *Endocr Pract.* 2015;21(4):395-399. **5.** Speiser PW et al. *J Clin Endocrinol Metab*. 2010;95(9):4133-4160.

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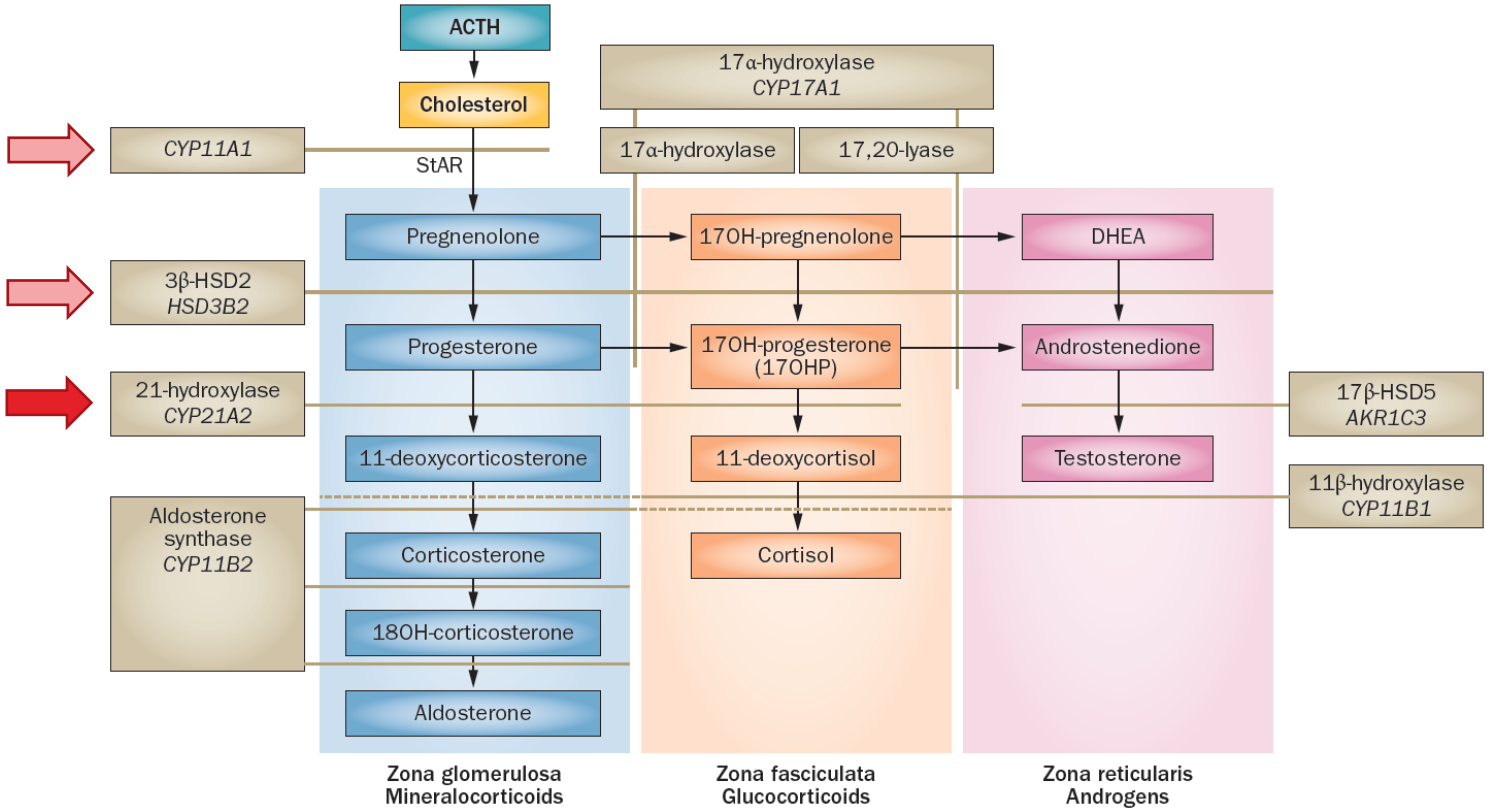
Steroid synthesis and enzymatic defect in CAH



- The 21-OH enzyme deficiency form of CAH causes a reduction in specific end products, accumulation of hormone precursors, and increased ACTH production[1,2]
  - Additional, genetically distinct variants of CAH causing PAI have been identified, including 3β-hydroxysteroid dehydrogenase deficiency and lipoid CAH[2]
- The clinical picture reflects the effects of inadequate production of cortisol and aldosterone and the increased production of androgens and steroid metabolites[1,2]

1. Han TS et al. *Nat Rev Endocrinol*. 2014;10(2):115-124. **2.** Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399.

3β-hydroxysteroid dehydrogenase deficiency

- Rare, less common form of CAH, that results in accumulation of DHEA, which is converted to testosterone in peripheral tissues[1,2]
- Mutations in the *HSD3B2* gene cause 3β-hydroxysteroid dehydrogenase deficiency[1]
  - This causes variable degrees of adrenal insufficiency and androgen excess
- Can cause virilization of female fetus and leads to ambiguous genitalia in the newborn[1]
  - Genital virilization in females is less severe than in the more common 21-OH deficiency
  - Males have androgen deficiency

1. Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399. **2.** Han TS et al. *Nat Rev Endocrinol*. 2014;10(2):115-124.

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Lipoid congenital adrenal hyperplasia (LCAH)

- Although uncommon, LCAH is the most severe form of CAH
- Most commonly caused by mutations in the *STAR* gene encoding the steroidogenic acute regulatory protein (StAR)
  - Severe defects in StAR lead to a block in the first step in steroidogenesis; complete deficiency in glucocorticoid, mineralocorticoid, and sex steroid hormones; and pathognomonic massive cholesterol ester accumulation in the adrenal cortex
- Most common in Palestinian and Japanese populations
- Within the last decade, a milder or “nonclassic” form of LCAH has been described in which glucocorticoid deficiency is the primary and usually only manifestation
- Mutations in the *CYP11A1* gene encoding the cholesterol side-chain cleavage enzyme cause a similar global loss of steroidogenesis and adrenal insufficiency
  - Main difference: adrenal tissue is not lipid laden as in patients with *STAR* mutations

Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399.

Non-CAH genetic causes of PAI: Adrenal hypoplasia congenita (AHC)

- AHC is an X-linked recessive disorder characterized by PAI or primary adrenal failure and hypogonadotropic hypogonadism with pubertal failure
- In AHC, there is a failure of development of the permanent adult adrenal cortex caused by a mutation in the *NR0B1* gene encoding nuclear receptor subfamily 0, group B, number 1
  - Also known as DAX1: dosage-sensitive sex reversal, adrenal hypoplasia congenita, X chromosome
- Affected individuals may present in infancy with severe salt wasting or may have a more insidious onset during childhood
- Rarely, AHC can present with adrenal failure later in adulthood
- Carrier females may very rarely have symptoms of adrenal insufficiency or hypogonadotropic hypogonadism

Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399.

Non-CAH genetic causes of PAI: Familial glucocorticoid deficiency (FGD)

- FGD, or hereditary unresponsiveness to ACTH, comprises a group of rare autosomal recessive disorders
  - Key defect: cells of the zona fasciculata within the adrenal cortex do not produce cortisol in response to ACTH stimulation
  - However, aldosterone production from the zona glomerulosa remains intact
- Caused by mutations in the melanocortin type 2 receptor (MC2R) or, more commonly, in its accessory protein (MRAP), which together yield the functional receptor for ACTH
- Loss of cortisol's negative feedback results in high ACTH, which leads to hyperpigmentation from overstimulation of melanocortin type 1 receptors
- Patients typically present in infancy with hypoglycemia, hyperpigmentation, and failure to thrive
  - Occasionally, diagnosis is not established until later in childhood
  - No hyperkalemia or severe hyponatremia due to intact aldosterone production
- Patients with FGD are often noted to have tall stature, but the underlying mechanisms are not clear

Brett EM, Auchus RJ. *Endocr Pract*. 2015;21(4):395-399.

