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The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders characterized by impaired cortisol synthesis. It results from the deficiency of one of the five enzymes required for the synthesis of cortisol in the adrenal cortex. Most of these disorders involve excessive or deficient production of hormones such as glucocorticoids, mineralocorticoids, or sex steroids, and can alter development of primary or secondary sex characteristics in some affected infants, children, or adults. It is one of the most common autosomal recessive disorders in humans. CAH can occur in various forms. The clinical presentation of each form is different and depends to a large extent on the underlying enzyme defect, its precursor retention, and deficient products. Classical forms appear in infancy, and nonclassical forms appear in late childhood. The presentation in patients with classic CAH can be further subdivided into three forms: salt-wasting, simple-virilizing, and non-classic (NC) depending on whether mineralocorticoid deficiency presents or absents, respectively. This subtyping is often not clinically meaningful, though, because all patients lose salt to some degree, and clinical presentations may overlap. “DISEASE DIAGNOSIS”section talks in-depth about CAH.

The other two segments, namely – “INTERPRETATION” and “TROUBLE SHOOTING” segments talk about another newborn screening important disorder namely “PKU”.

Tulip Diagnostics is proud to present two systems and platforms for newborn screening protocols – considered to be mandatory in many countries.

Yes, “BOUQUET” too is available within. Happy reading!

DISEASE DIAGNOSIS

CONGENITAL ADRENAL HYPERPLASIA

Background

The term congenital adrenal hyperplasia (CAH) encompasses a group of autosomal recessive disorders, each of which involves a deficiency of an enzyme involved in the synthesis of cortisol, aldosterone, or both.

Pathophysiology

The clinical manifestations of each form of congenital adrenal hyperplasia are related to the degree of cortisol deficiency and/or the degree of aldosterone deficiency. In some cases, these manifestations reflect the accumulation of precursor adrenocortical hormones. When present in supraphysiologic concentrations, these precursors lead to excess androgen production with resultant virilization, or because of mineralocorticoid properties, cause sodium retention and hypertension.
The phenotype depends on the degree or type of gene deletion or mutation and the resultant deficiency of the steroidogenic enzyme. The enzymes and corresponding genes are displayed below.

Enzyme Nomenclature

Enzyme Activity	Gene
• SCC / 20,22 desmolase	• StAR / 20,22 desmolase
• 17 alpha hydoxylase / 17,20 desmolase	• CYP17A
• 21 alpha hydroxylase	• CYP21A
• 11 beta hydroxylase	• CYP11B1
• Aldosterone synthetase	• CYP11B2

Enzymes and genes involved in adrenal steroidogenesis.

Two copies of an abnormal gene are required for disease to occur, and not all mutations and partial deletions result in disease. The phenotype can vary from clinically inapparent disease (occult or cryptic adrenal hyperplasia) to a mild form of disease that is expressed in adolescence or adulthood (nonclassic adrenal hyperplasia) to severe disease that results in adrenal insufficiency in infancy with or without virilization and salt wasting (classic adrenal hyperplasia). The most common form of adrenal hyperplasia (due to a deficiency of 21-hydroxylase activity) is clinically divided into 3 phenotypes: salt wasting, simple virilizing, and nonclassic. *CYP21A* is the gene that codes for 21-hydroxylase, *CYP11B1* codes for 11-beta-hydroxylase, and *CYP17* codes for 17-alpha-hydroxylase. Many of the enzymes involved in cortisol and aldosterone syntheses are cytochrome P450 (CYP)

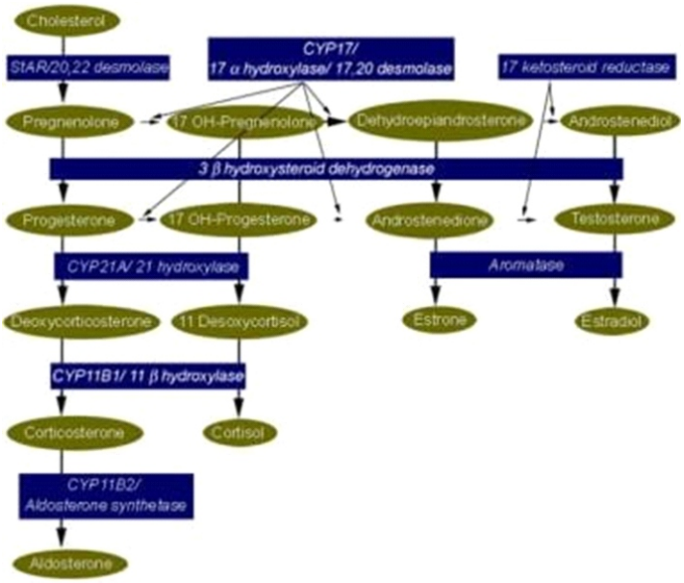
Frequency

International

Congenital adrenal hyperplasia caused by 21-hydroxylase deficiency is found in all populations. 11-beta-hydroxylase deficiency is more common in persons of Moroccan or Iranian-Jewish descent.

Mortality/Morbidity

The morbidity of the various forms of adrenal hyperplasia is best understood in the context of the steroidogenic pathway, shown below, used by the adrenal glands and gonads.



Steroidogenic pathway for cortisol, aldosterone, and sex steroid synthesis. A mutation or deletion of any of the genes that code for enzymes involved in cortisol or aldosterone synthesis results in congenital adrenal hyperplasia. The particular phenotype that results depends on the sex of the individual, the location of the block in synthesis, and the severity of the genetic deletion or mutation.

The clinical phenotype can be understood by analyzing the location of the enzyme deficiency, the accumulation of precursor hormones, the products of those precursors when one enzyme pathway is ineffective, and the physiologic action of those hormones (see History). A study by Halper et al of 42 children with congenital adrenal hyperplasia reported that total body bone mineral density was lower in these youngsters than in controls (0.81 g/cm vs 1.27 g/cm, respectively). However, no significant differences in body composition, including with regard to visceral adipose tissue and android:gynoid ratio, were found between the two groups. A study by Yang and White indicated that in children with the salt-wasting form of 21-hydroxylase deficiency congenital adrenal hyperplasia, the risk of postdiagnostic hospitalization is greater in patients younger than 2 years (possibly resulting from a higher susceptibility to viral infections and a lower ability to cope with stress and dehydration) and those who need a greater daily dosage of fludrocortisone (perhaps because these patients are likely to have more severe disease). The study also found that children with noncommercial insurance were more likely to be hospitalized, possibly because they are more likely to experience social barriers to treatment compliance. A study by Herting et al indicated that medial temporal lobe volumes are smaller in young people with congenital adrenal hyperplasia, with the lateral nucleus of the amygdala, along with the hippocampal subiculum and CA1 subregion, particularly being affected. A study by Lim et al of Asian adults with congenital adrenal hyperplasia found that males had a 2.7-fold greater risk for hypertension, while women had a 2.0-fold increased risk for obesity. Adrenal limb thickness was significantly greater in men with obesity, while 17-hydroxyprogesterone and dehydroepiandrosterone sulfate levels were significantly higher in women with obesity. Women with irregular periods also tended to have higher dehydroepiandrosterone sulfate levels. Severe forms of congenital adrenal hyperplasia are potentially fatal if unrecognized and untreated because of the severe cortisol and aldosterone deficiencies

that result in salt wasting, hyponatremia, hyperkalemia, dehydration, and hypotension.

Epidemiology

Race

Congenital adrenal hyperplasia occurs among people of all races. Congenital adrenal hyperplasia secondary to *CYP21A1* mutations and deletions is particularly common among the Yupik Eskimos.

Sex

Because all forms of congenital adrenal hyperplasia are autosomal recessive disorders, both sexes are affected with equal frequency. However, because accumulated precursor hormones or associated impaired testosterone synthesis impacts sexual differentiation, the phenotypic consequences of mutations or deletions of a particular gene differ between the sexes.

Age

Classic congenital adrenal hyperplasia is generally recognized at birth or in early childhood because of ambiguous genitalia, salt wasting, or early virilization. Nonclassic adrenal hyperplasia is generally recognized at or after puberty because of oligomenorrhea or virilizing signs in females.

Prognosis

With adequate medical and surgical therapy, the prognosis is good. However, problems with psychological adjustment are common and usually stem from the genital abnormality that accompanies some forms of congenital adrenal hyperplasia.

- Short stature and infertility are common.
- Gender identity in females with virilizing adrenal hyperplasia is usually female if female gender assignment is made early in life, if adequate medical and surgical support are provided, and if the family (and eventually the patient herself) is given adequate education to understand the disease.
- Females with virilizing adrenal hyperplasia may have more masculine interests.
- Females with adrenal hyperplasia have reduced fertility rates, but fertility is possible with good metabolic control.
- Early death may occur if patients are not provided with stress doses of glucocorticoid in times of illness, trauma, or surgery.

Patient Education

Educate the caretakers and patients about the nature of the disease in order for them to understand the importance of replacement of the deficient adrenal cortical hormones. Patients must also understand the need for additional glucocorticoids in times of illness and stress in order to avoid an adrenal crisis. Patients must know the importance of IM injections of glucocorticoids and be educated in the technique of IM administration. Useful Web sites for patients and parents include the **National Adrenal Diseases Foundation** and the **Congenital Adrenal Hyperplasia Research Education and Support (CARES) Foundation**.

Clinical Presentation

History

The clinical phenotype of congenital adrenal hyperplasia depends on the nature and severity of the enzyme deficiency. The most common form is 21-hydroxylase deficiency (CYP21). Approximately 50% of patients with classic congenital adrenal hyperplasia due to *CYP21A* mutations or deletions have salt wasting due to inadequate aldosterone synthesis. Although the information below is presented according to chromosomal sex, the sex of a neonate with congenital adrenal hyperplasia is often initially unclear because of genital ambiguity.

Clinical presentation in females

Females with severe forms of adrenal hyperplasia due to deficiencies of 21-hydroxylase, 11-beta-hydroxylase or 3-beta-hydroxysteroid dehydrogenase have ambiguous genitalia at birth due to excess adrenal androgen production in utero. This is often called classic virilizing adrenal hyperplasia. Mild forms of 21-hydroxylase deficiency in females are identified later in childhood because of precocious pubic hair, clitoromegaly, or both, often accompanied by accelerated growth and skeletal maturation due to excess postnatal exposure to adrenal androgens. This is called simple virilizing adrenal hyperplasia. Still milder deficiencies of 21-hydroxylase or 3-beta-hydroxysteroid dehydrogenase activity may present in adolescence or adulthood with oligomenorrhea, hirsutism, and/or infertility. This is termed nonclassic adrenal hyperplasia. Females with 17-hydroxylase deficiency appear phenotypically female at birth but do not develop breasts or menstruate in adolescence because of inadequate estradiol production. They may present with hypertension.

Clinical presentation in males

21-hydroxylase deficiency in males is generally not identified in the neonatal period because the genitalia are normal. If the defect is severe and results in salt wasting, these male neonates present at age 1-4 weeks with failure to thrive, recurrent vomiting, dehydration, hypotension, hyponatremia, hyperkalemia, and shock (classic salt-wasting adrenal hyperplasia). Patients with less severe deficiencies of 21-hydroxylase present later in childhood because of the early development of pubic hair, phallic enlargement, or both, accompanied by accelerated linear growth and advancement of skeletal maturation (simple virilizing adrenal hyperplasia). In male infants, the disease may be misdiagnosed as gastroenteritis or pyloric stenosis, with potentially disastrous consequences due to delayed treatment with glucocorticoids. Males with steroidogenic acute regulatory (StAR) deficiency, classic 3-beta-hydroxysteroid dehydrogenase deficiency, or 17-hydroxylase deficiency generally have ambiguous genitalia or female genitalia because of inadequate testosterone production in the first trimester of fetal life.

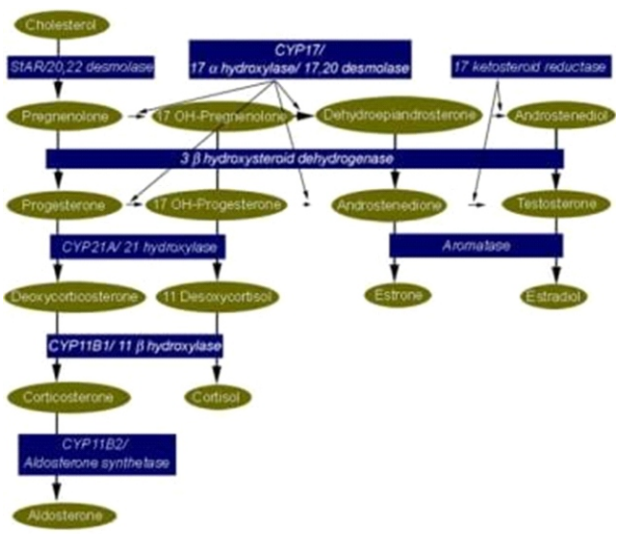
Other findings

Hyponatremia, hyperkalemia, and/or hypoglycemia suggests the possibility of adrenal insufficiency. Hypoglycemia and hypotension may, in part, be due to associated epinephrine synthesis in the adrenal medulla due to cortisol deficiency. Cortisol, perfusing the adrenal medulla from the cortex, normally stimulates phenylethanolamine *N* -methyltransferase, the last enzyme in epinephrine synthesis. Children with simple virilizing 21-hydroxylase deficiency or 11-hydroxylase deficiency have early pubic hair, phallic enlargement, and accelerated linear growth and advanced skeletal maturation. Two forms of adrenal hyperplasia (ie, 11-hydroxylase [CYP11B1] and 17-hydroxylase [CYP17] deficiency) result in hypertension due to the accumulation of supraphysiologic concentrations of deoxycorticosterone. This weak mineralocorticoid has little consequence at physiologic concentrations but causes sodium retention and hypertension at the supraphysiologic concentrations that occur in these conditions. One form of adrenal hyperplasia results in isolated aldosterone deficiency without affecting the synthesis of cortisol or sex steroids. This form is due to a defect in enzymatic activities that have variously been termed CMO I, CMO II, 18-

hydroxylase, or 18-hydroxycorticosterone dehydrogenase; however, it is currently thought to represent one protein called aldosterone synthetase (CYP11B2). A study by Carvalho determined that in 46,XX patients with CAH resulting from *CYP17A1* defects, diagnostic factors include amenorrhea, absence/sparseness of pubic hair, and ovarian macrocysts (risk factors for ovarian torsion), along with the aforementioned hypertension. *CYP17A1* defects are also indicated by high basal progesterone levels in patients with hypergonadotropic hypogonadism. Other forms of adrenal hyperplasia are characterized by disordered genital development in utero, lack of secondary sexual characteristics development, or hypertension. For example, 17-hydroxylase deficiency in females is rarely identified at birth, but these females seek medical attention later in life because of hypertension or failure to develop secondary sexual characteristics at puberty due to an inability to synthesize estrogens. Male patients with this disorder have ambiguous or female genitalia and may be raised as girls and seek medical attention later in life because of hypertension or a lack of breast development. Patients with aldosterone deficiency of any etiology may present with dehydration, hyponatremia, and hyperkalemia, especially with the stress of illness. Male or female patients with 11-hydroxylase deficiency may present in the second or third week of life with a salt-losing crisis. However, these patients develop hypertension, hypokalemic alkalosis, or both later in life. This paradox is explained by resistance to mineralocorticoids in infancy and the inability of the elevated deoxycorticosterone levels to replace the deficient serum concentrations of aldosterone in infancy. Upon maturation, mineralocorticoid responsiveness increases, and the elevated concentrations of deoxycorticosterone are sufficient to cause sodium retention, potassium excretion, and hypertension. Infants with StAR deficiency (lipoid adrenal hyperplasia) usually have signs of adrenal insufficiency (eg, poor feeding, vomiting, dehydration, hypotension, hyponatremia, hyperkalemia). Some patients do not receive medical attention until late infancy. Male patients with this form of adrenal hyperplasia have female or ambiguous genitalia. Female patients have normal female genitalia. A curious observation is that girls who survive develop breasts and menstruate at puberty, suggesting preservation of ovarian steroidogenesis.

Physical

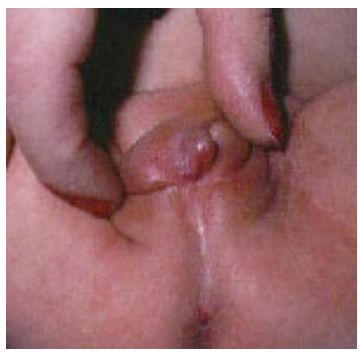
Physical findings depend on the nature and severity of the deficient enzyme activity. See the image below.



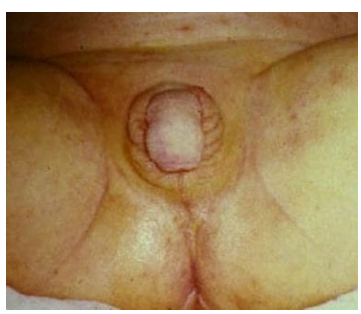
Steroidogenic pathway for cortisol, aldosterone, and sex steroid synthesis. A mutation or deletion of any of the genes that code for enzymes involved in cortisol or aldosterone synthesis results in congenital adrenal hyperplasia. The particular phenotype that results depends on the sex of the individual, the location of the block in synthesis, and the severity of the genetic deletion or mutation.

See the list below:

- Deficiencies of enzyme activity involved in cortisol synthesis result in elevations in concentrations of corticotropic hormone (previously adrenocorticotropic hormone [ACTH]) that often cause hyperpigmentation. This hyperpigmentation may be subtle and is best observed in the genitalia and areolae.
- In virilizing forms (ie, 21-hydroxylase deficiency, 11-beta-hydroxylase deficiency, and 3-beta-hydroxysteroid dehydrogenase deficiency), female patients have ambiguous genitalia at birth that range from complete fusion of the labioscrotal folds and a phallic urethra to clitoromegaly, partial fusion of the labioscrotal folds, or both, as shown in the images below.
- This virilization results from the abnormally high concentrations or steroidogenic precursors that are converted to potent androgens, testosterone, and dihydrotestosterone. Dihydrotestosterone is most potent in terms of virilizing the external genitalia and is synthesized from testosterone by 5-alpha reductase, an enzyme that resides in skin of genital tissue. Recently, human steroidogenic tissues have been shown to have the capability of converting precursors like progesterone and 17-OH progesterone to dihydrotestosterone through a "backdoor pathway" that does not involve testosterone in the pathway.



A female patient with the 46,XX karyotype with mild virilization due to congenital virilizing adrenal hyperplasia secondary to 21-hydroxylase deficiency. Despite the mild clitoromegaly, this patient has fusion of the labial-scrotal folds and salt wasting.



Severe virilization in a female patient with the 46,XX karyotype with congenital adrenal hyperplasia secondary to 21-hydroxylase deficiency. This patient also has salt wasting.

See the list below:

- In relatively nonsevere forms, genitalia may be normal at birth, but early pubic hair and clitoromegaly (often accompanied by tall stature) may appear in childhood.
- In mild forms, excess facial or body hair often appears.
- Male patients with 21-hydroxylase deficiency have normal genitalia but may develop signs of dehydration at age 1-4 weeks if they have salt wasting or may have no problems in infancy but develop a salt-wasting crisis with illness during childhood (classic salt-wasting adrenal hyperplasia). Less-severely affected males may present with precocious development of pubic hair, phallic enlargement, and accelerated growth and skeletal maturation in childhood (simple virilizing adrenal hyperplasia).
- Ambiguous genitalia or female genitalia are also observed in male patients with 3-beta-hydroxysteroid dehydrogenase deficiency, 17-hydroxylase deficiency, and StAR deficiency.
- High blood pressure and, sometimes, hypokalemia may be observed in individuals with 11-beta-hydroxylase deficiency and 17-hydroxylase deficiency. These findings are due to the accumulation of the mineralocorticoid deoxycorticosterone.

Causes

The defects that cause congenital adrenal hyperplasia are autosomal recessive disorders due to deficient activity of a protein involved in cortisol synthesis, aldosterone synthesis, or both.

- In most cases, this disorder is due to a mutation or deletion of the gene that codes for the involved protein. When both genes carry the same mutation or deletion, the condition is homozygous. When the 2 affected genes carry different mutations or deletions, the patient is said to be a compound heterozygote. In general, the clinical severity reflects the least affected allele. Carriers or heterozygotes who carry only one abnormal gene are asymptomatic.
- Many of the genes involved in cortisol and aldosterone synthesis code for CYP proteins. The best-studied gene is the 21-hydroxylase gene (*CYP21, CYP21A*). The 21-hydroxylase gene is located on chromosomal band 6p21.3 among genes that code for proteins that determine human leukocyte antigen (HLA) types. The gene for 21-hydroxylase has a pseudogene (*CYP21P*) 30 kb away from *CYP21* that is 98% homologous in structure to *CYP21A*; however, it is rendered inactive because of minor differences in the gene. The proximity of *CYP21P* with *CYP21A* is thought to predispose the *CYP21A* gene to crossovers in meiosis between *CYP21A* and *CYP21P*, resulting in loss of genetic function.
- Other defects occur because of gene deletions or mutations. Among abnormalities of *CYP21A*, approximately 95% are thought to be due to recombinations with *CYP21P*, 20% are thought to represent deletions, and 70% are point mutations. The phenotype depends on the function of the less-severely affected gene rather than on the more severely affected gene because the former determines the level of enzyme activity. In general, genotype-phenotype correlations are strong, although exceptions occur. Because aldosterone secretion is approximately 1000-fold less than cortisol secretion, the enzyme activity required for aldosterone synthesis is less than that required for cortisol synthesis. Therefore, patients with only the most severe loss of function of *CYP21A* have salt wasting.
- The 11-beta-hydroxylase gene (*CYP11B1*) is on chromosomal band 8q21. *CYP11B1* has no pseudogene, and no HLA association is found. CYP11B1 catalyzes the conversion of 11-deoxycortisol to cortisol in the glucocorticoid pathway and the conversion of deoxycorticosterone to corticosterone in the mineralocorticoid pathway. A neighboring gene codes for CYP11B2, or aldosterone synthetase, which catalyzes the conversion of corticosterone to aldosterone in the zona glomerulosa. Mutations and deletions of the *CYP11B2* gene result in diminished aldosterone synthesis. Therefore, individuals with CYP11B2 deficiency develop hyponatremia, hyperkalemia, and dehydration. Sexual differentiation occurs normally because sex steroid synthesis and cortisol synthesis are not impaired. The genes for CYP11B1 and CYP11B2 share 95% sequence homology for coding sequences. Nonetheless, gene conversion from chromosomal crossover at meiosis does not appear to play a major role in the mutations and deletions thatrendereither gene inactive.
- Two tissue forms of 3-beta-hydroxysteroid dehydrogenase are described. Type I occurs primarily in the adrenal and gonad, whereas type II occurs primarily in the placenta and liver. The genes for both forms reside on chromosomal band 1p13. The classic form of 3-beta-hydroxysteroid dehydrogenase deficiency results from mutations or deletions in the gene for the adrenal form of the enzyme.
- Some patients appear to have nonclassic forms of this disease, as evidenced by symptoms and signs of virilization such as hirsutism, oligomenorrhea, and infertility. Laboratory studies may reveal mildly abnormal precursors-to-product ratios (ie, increased ratio of 17-hydroxypregnenolone to 17-hydroxyprogesterone and of dehydroepiandrosterone to androstenedione). These patients have not had mutations or deletions in any of the genes that code for adrenal 3-beta-hydroxysteroid dehydrogenase. The molecular basis for this disorder remains undefined. Clinical and hormonal findings of this condition and polycystic ovary disease overlap considerably. Some patients benefit from suppression of adrenal steroidogenesis with dexamethasone.
- 17-alpha-hydroxylase activity and 17,20-desmolase activities are thought to be due to a single protein (CYP17) with separate enzymatic activity sites.
- Some patients with lipoid adrenal hyperplasia, which was originally thought to be due to deficiency of CYP450 side-chain cleavage (scc) enzyme activity, have had mutations in a gene that codes for StAR. This protein appears to be involved in the transport of cholesterol across the mitochondrial membrane, where CYP450 scc can act on it. This enzyme converts cholesterol to pregnenolone, which is then processed in the various steroidogenic tissues into cortisol, aldosterone, or sex steroids. Thus, a deficiency of StAR results in a global steroid deficiency state. Affected 46 XY individuals may have female external genitalia, and affected 46 XX individuals have normal female genitalia. Both develop signs of adrenal insufficiency with onset from early infancy to age 6 months.
- A curious observation is that females with this disorder who survived as the result of early replacement of glucocorticoids and mineralocorticoid have developed breasts and spontaneous nonovulatory menses at puberty. Researchers postulate that the accumulation of cholesterol esters in steroidogenic cells, which results from StAR deficiency, is eventually toxic to the steroidogenic cells. According to this theory, some ovarian function is preserved because ovarian steroidogenesis does not occur until puberty, and then steroidogenesis occurs in only one follicle at a time, thereby allowing some preservation of steroidogenesis.
- Mutations in the gene that code for CYP oxidoreductase were recently found to cause deficiencies of several enzymes involved in

steroidogenesis. CYP oxidoreductase facilitates electron transfer from nicotinamide adenine dinucleotide phosphate (NADPH) reduced form to the 21-hydroxylase and 17-hydroxylase enzymes required in steroidogenesis (Online Mendelian Inheritance in Man [OMIM] 201750 and 124015). Some individuals with these mutations have craniosynostosis and skeletal abnormalities known as the Antley-Bixler syndrome (OMIM 207410). However, mutations in the fibroblast growth factor receptor-2 can also cause the phenotypic picture of Antley-Bixler syndrome without problems in steroidogenesis.

Differential Diagnoses

Differential Diagnoses

- 3-Beta-Hydroxysteroid Dehydrogenase Deficiency
- 5-Alpha-Reductase Deficiency
- Adrenal Hypoplasia
- Androgen Insensitivity Syndrome
- Bilateral adrenal hemorrhage
- Congenital Adrenal Hyperplasia
- Defects in testosterone synthesis
- Denys-Drash Syndrome
- Differences (Disorders) of Sex Development (DSDs)
- Familial Glucocorticoid Deficiency
- Fluid, Electrolyte, and Nutrition Management of the Newborn
- Gender Identity
- Hyperkalemia in Emergency Medicine
- Hyponatremia in Emergency Medicine
- Mixed gonadal dysgenesis
- Nutritional Considerations in Failure to Thrive
- Obstructive uropathy
- Pediatric Adrenal Insufficiency (Addison Disease)
- Pediatric Cryptorchidism Surgery
- Infantile Hypertrophic Pyloric Stenosis
- Pediatric Hypokalemia
- Polycystic Ovarian Syndrome
- Pseudohypoparathyroidism
- Kidney Disease and Pregnancy
- Sexual Orientation
- Sinonasal Manifestations of Cystic Fibrosis
- Small-Bowel Obstruction Imaging and Diagnosis
- WAGR Syndrome

Workup

Laboratory Studies

The diagnosis of congenital adrenal hyperplasia depends on the demonstration of inadequate production of cortisol, aldosterone, or both in the presence of accumulation of excess concentrations of precursor hormones. For example, the distinguishing characteristic of 21-hydroxylase deficiency is a high serum concentration of 17-hydroxyprogesterone (usually >1000 ng/dL) and urinary pregnanetriol (metabolite of 17-hydroxyprogesterone) in the presence of clinical features suggestive of the disease (eg, salt wasting, clitoromegaly or ambiguous genitalia, precocious pubic hair, excessive growth, premature phallic enlargement in the absence of testicular enlargement, hirsutism, oligomenorrhea, female infertility). Likewise, 11-beta-hydroxylase deficiency is indicated by excess concentrations of 11-deoxycortisol and deoxycorticosterone or by an elevation in the ratio of 24-hour urinary tetrahydrocompound S (metabolite of 11-deoxycortisol) to tetrahydrocompound F (metabolite of cortisol). Both forms of adrenal hyperplasia are accompanied by elevated levels of 24-hour urinary 17-ketosteroids, the urinary metabolites of adrenal androgens. 3-beta-hydroxysteroid dehydrogenase deficiency is indicated by an abnormal ratio of 17-hydroxypregnenolone to 17-hydroxyprogesterone and dehydroepiandrosterone to androstenedione. Salt-wasting forms of adrenal hyperplasia are accompanied by low serum aldosterone concentrations, hyponatremia (see Serum Sodium), hyperkalemia (see Potassium), and elevated plasma renin activity (PRA), indicating hypovolemia. In contrast, hypertensive forms of adrenal hyperplasia (ie, 11-beta-hydroxylase deficiency and 17-alpha-hydroxylase deficiency) are associated with suppressed PRA and, often, hypokalemia. Subtle forms of adrenal hyperplasia (as in nonclassic forms of 21-hydroxylase deficiency and nonclassic 3-beta-hydroxysteroid dehydrogenase deficiency) often require a synthetic corticotropin (Cortrosyn) stimulation test to demonstrate the abnormal accumulation of precursor steroids. Nomograms are available for interpreting the results.

Imaging Studies

Imaging studies of the adrenal gland are generally not useful in the evaluation of patients with suspected adrenal hyperplasia. However, CT scanning of the adrenal gland can be useful in excluding bilateral adrenal hemorrhage in patients with signs of acute adrenal failure without ambiguous genitalia or other clues of adrenal hyperplasia. Pelvic ultrasonography may be performed in an infant with ambiguous genitalia to demonstrate a uterus or associated renal anomalies, which are sometimes found in other conditions that may result in ambiguous genitalia (eg, mixed gonadal dysgenesis, Denys-Drash syndrome). Urogenitography is often helpful in defining the anatomy of the internal genitalia. A bone-age study is useful in evaluating a child who develops precocious pubic hair, clitoromegaly, or accelerated linear growth. Patients who have these symptoms because of adrenal hyperplasia have advanced skeletal maturation.

Other Tests

A karyotype is essential in the evaluation of an infant with ambiguous genitalia to establish the patient's chromosomal sex. Genetic testing is rarely necessary to diagnose classic forms of adrenal hyperplasia but is essential for genetic counseling and prenatal diagnosis of adrenal hyperplasia. Newborn screening programs for 21-hydroxylase deficiency should be encouraged as they may be lifesaving in an affected male infant who would otherwise be undetected until presentation with a salt-wasting crisis.

Histologic Findings

Histologic features of congenital adrenal hyperplasia include hyperplasia of the adrenal cortex and disorganized architecture of both the adrenal cortices and medullae. Lipoid deposits in the adrenal cortical cells characterize lipoid adrenal hyperplasia due to a deficiency of StAR. Lipoid deposits are thought to represent cholesterol esters that have accumulated from the inability of the cell to transport cholesterol into the mitochondria. With salt wasting, hypertrophy of the juxtaglomerular apparatus of the kidney occurs due to hypovolemia stimulating enhanced renin activity.

Treatment & Management

Management

Newborns with ambiguous genitalia should be closely observed for symptoms and signs of salt wasting while a diagnosis is being

established. Clinical clues include abnormal weight loss or lack of expected weight gain. Electrolyte abnormalities generally take from a few days to 3 weeks to appear, but in mild forms of salt-wasting adrenal hyperplasia, salt wasting may not become apparent until an illness stresses the child.

Management is as follows:

- Patients with dehydration, hyponatremia, or hyperkalemia and a possible salt-wasting form of CAH should receive an IV bolus of isotonic sodium chloride solution (20 mL/kg or 450 mL/m) over the first hour, as needed, to restore intravascular volume and blood pressure; this may be repeated if the blood pressure remains low
- Dextrose must be administered if the patient is hypoglycemic and must be included in the rehydration fluid after the bolus dose to prevent hypoglycemia
- After samples are obtained to measure electrolyte, blood sugar, cortisol, aldosterone, and 17-hydroxyprogesterone concentrations, the patient should be treated with glucocorticoids; treatment should not be withheld while confirmatory results are awaited
- After the patient's condition is stabilized, treat all patients who have adrenal hyperplasia with long-term glucocorticoid or aldosterone replacement (or both), depending on which enzyme is involved and on whether cortisol and/or aldosterone synthesis is affected
- Patients who are sick and have signs of adrenal insufficiency should receive stress dosages of hydrocortisone (50-100 mg/m or 1-2 mg/kg IV administered as an initial dose), followed by 50-100 mg/m /day IV divided every 6 hours

The Endocrine Society's 2010 clinical practice guidelines note the following :

- Prenatal treatment for CAH should be regarded as experimental
- Glucocorticoid therapy should be carefully titrated to avoid Cushing syndrome
- Mineralocorticoid replacement is encouraged; in infants, mineralocorticoid replacement and sodium supplementation are encouraged
- Use of agents to delay puberty and promote growth are experimental
- Psychiatric support should be encouraged for patients with adjustment problems
- Medication should be used judiciously during pregnancy and in symptomatic patients with nonclassical CAH.

Surgical care

Infants with ambiguous genitalia require surgical evaluation and, if needed, plans for corrective surgery, as follows:

- The traditional approach to the female patient with ambiguous genitalia due to adrenal hyperplasia is clitoral recession early in life followed by vaginoplasty after puberty
- Vocal groups of patients with disorders of sexual differentiation (eg, Intersex Society of North America) have challenged this approach
- Some female infants with adrenal hyperplasia have only mild virilization and may not require corrective surgery if they receive adequate medical therapy to prevent further virilization

The Endocrine Society's 2010 clinical practice guidelines note the following :

- Adrenalectomy should be avoided
- Surgical reconstruction may not be necessary during the newborn period in mildly virilized girls but may be appropriate in severely virilized girls; it should be a single stage genital repair, performed by experienced surgeons.

INTERPRETATION

What Is Newborn Screening?

Newborn screening is a public health service done in each U.S. state. Every newborn is tested for a group of health disorders that aren't otherwise found at birth. With a simple blood test, doctors can check for rare genetic, hormone-related, and metabolic conditions that can cause serious health problems. Newborn screening lets doctors diagnose babies quickly and start treatment as soon as possible.

Which Screening Tests Are Offered?

Screening varies by state. Tests offered can change as technology advances and treatments improve. Although there are national recommendations for newborn screening, it is up to each state to decide which tests to include.

Newborn screening includes tests for:

Metabolic problems. Metabolism is the process that converts food into energy the body can use to move, think, and grow. Enzymes are special proteins that help with **metabolism** by speeding up the chemical reactions in cells. Most metabolic problems happen when certain enzymes are missing or not working as they should. Metabolic disorders in newborn screening include:

- **phenylketonuria (PKU)**
- methylmalonic acidemia
- **maple syrup urine disease (MSUD)**
- tyrosinemia
- citrullinema
- medium chain acyl CoA dehydrogenase (MCAD) deficiency

Hormone problems. Hormones are chemical messengers made by glands. Hormone problems happen when glands make too much or not enough hormones. Hormone problems in newborn screening include:

- **congenital hypothyroidism**
- **congenital adrenal hyperplasia**

Hemoglobin problems: Hemoglobin is a protein in red blood cells that carries oxygen throughout the body. Some of the hemoglobin problems included in newborn screening are:

- **sickle cell disease** and **sickle cell trait**
- hemoglobin SC disease
- **beta thalassemia**

Other problems. Other rare but serious medical problems included in newborn screening are:

- **galactosemia**
- biotidinase deficiency
- **cystic fibrosis**
- **severe combined immunodeficiency (SCID)**
- **Pompe disease** (glycogen storage disease type II)
- mucopolysaccharidosis type 1
- X-linked adrenoleukodystropy
- **spinal muscle atrophy (SMA)**

Most states also screen for **hearing loss** and critical congenital heart disease, which are not done by testing the blood. Talk to your doctor if you think your baby may need other newborn screening tests not offered through your state program.

How Is Newborn Screening Done?

A small blood sample taken by pricking the baby's heel is tested. This happens before the baby leaves the hospital, usually at 1 or 2 days of age. Talk to your doctor about newborn screening if your baby was not born in a hospital. The blood sample should be taken after the first 24 hours of life. Some babies are tested within the first 24 hours, though, because sometimes moms and newborns are discharged within 1 day. If this happens, experts recommend taking a repeat sample after the baby is more than 24 hours old. Some states routinely do two tests on all infants.

When Are the Results Ready?

Results of newborn screening for hearing loss and heart disease are available as soon as the test is done. Blood test results usually are ready by the time a baby is 5–7 days old. Often, parents won't hear about results if screening tests were normal. They are contacted if a test was positive for a condition. A positive newborn screening test does not mean a child definitely has the medical condition. Doctors order more tests to confirm or rule out the diagnosis. Parents can talk to their child's doctor about the newborn screening results. If a diagnosis is confirmed, doctors might refer the child to a specialist for more testing and treatment. When treatment is needed, it's important to start it as soon as possible. Treatment may include special formula, diet restrictions, supplements, medicines, and close monitoring.

TROUBLESHOOTING

Why is newborn screening important for PKU?

Most children born with these problems appear healthy at birth and are from healthy families. Parents who already have healthy children, or who are "silent carriers" of a genetic condition, do not expect any problems. Because every baby is tested soon after birth, children who may have one or more of these disorders can be identified early and get early care. This can make a tremendous difference in health outcomes for the child. The blood sample for phenylketonuria (PKU) screening should be obtained at least 12 hours after the infant's birth. Newborn screening for PKU has largely eliminated mental retardation caused by this disease. If the first phenylalanine test demonstrates positive results, a repeat test should be performed. Treatment to prevent sequelae from this disorder is best carried out in cooperation with an experienced PKU center. Dietary care is expensive, and financial assistance may be necessary for many families. A phenylalanine-restricted diet should be started as soon as possible. Occasionally, cases of PKU are missed by newborn screening. Thus, a repeat PKU test should be performed in an infant who exhibits slow development. Phenylketonuria (PKU) is caused by an autosomal recessive defect in the enzyme phenylalanine hydroxylase, which is required for converting phenylalanine to tyrosine. (Five percent of natural protein is composed of phenylalanine.) The mutation that causes PKU is located on chromosome 12. The specific type of mutation varies, resulting in variable severity in the clinical course of the disorder. Untreated, PKU results in severe mental retardation, but the exact pathogenesis of the mental defect is still not clear. Progressive neurologic damage occurs as the child grows older. Because treatment with a phenylalanine-restricted diet prevents the development of mental retardation, it seems likely that an increased concentration of phenylalanine in the blood is associated in some way with progressive neurologic deterioration if PKU remains untreated. The symptoms of untreated PKU develop gradually, so they may not be noticed until irreversible mental retardation has occurred. Hence, newborn screening is essential for prevention of harmful effects. When PKU is identified in the first few weeks of life and a phenylalanine-restricted diet is instituted, intellectual development is substantially better than when the disorder is diagnosed at three to five years of age. When PKU is not diagnosed until this late age, serious damage has usually already occurred. Initial estimates of the frequency of PKU (one per 25,000 live births) were based on institutionalized populations with profound mental retardation caused by defects that completely prevented the production of phenylalanine hydroxylase. The frequency of PKU in the United States is currently considered to be one per 10,000 to one per 12,000 live births. The frequency varies in different ethnic groups. For example, it is one per 2,500 live births in Turkey and one per 4,000 live births in Ireland. In the United States, the frequency of PKU is one per 20,000 live births in California, compared with a frequency of one per 12,000 live births in Massachusetts. The large population of Americans of Irish ancestry in Massachusetts may explain the increased frequency in that state.

PKU Screening Procedure

Newborn PKU screening in the United States is performed with the Guthrie inhibition assay or the McCamon-Robins fluorometric test. Both tests can be performed on blood spotted on filter paper, and both are highly accurate. The laboratory that performs the PKU test has the responsibility of reporting the results to the physician and to the facility where the infant was born. Some laboratories also notify the parents. The blood specimen for PKU screening must be obtained at least 12 hours after birth, but in recent years this has proved rather difficult because of changing obstetric practices in which patients are sometimes discharged a few hours after delivery. The American Academy of Pediatrics recommends that a PKU screening test be repeated by two weeks of age if it was performed before the newborn was 24 hours of age. However, a second test is not necessary if the initial PKU screening is performed with the McCamon-Robins fluorometric test when the newborn is between 12 and 24 hours of age. With either the McCamon-Robins test or the Guthrie test, the test should be repeated if it was performed when the newborn was less than 12 hours of age.

TABLE 1

Follow-up Testing in Response to the Initial Results of Newborn Phenylketonuria Screening

Infant's age at initial testing	*Follow-up testing*
<12 hours	Repeat the test regardless of the results on initial screening. Repeat the test if McCamon-Robins fluorometric assay was used.
12 to 24 hours	If Guthrie inhibition assay was used, repeat the test if the results were positive; repeat testing is not required if the results were negative.
>24 hours	Repeat the test if the results were positive; repeat testing is not required if the results were negative.

NORMAL AND ABNORMAL PHENYLALANINE LEVELS IN INFANTS

The blood phenylalanine concentration in newborns is normally 0.5 mg to 1 mg per dL (30 to 60 µmol per L). In general, few infants with PKU will remain unidentified when a phenylalanine cut-off value of 2 mg per dL (121 µmol per L) in the first 24 hours is used. Not all states, however, use 2 mg per dL as the cut-off value. Forty states and jurisdictions use 4 mg per dL (242 µmol per L) as the screening cut-off value for PKU, six states use 3 mg per dL (182 µmol per L) and seven states use 2 mg per dL. Some newborns without PKU have transiently elevated phenylalanine levels of more than 6 mg per dL (363 µmol per L) related to delayed maturation of enzymes required for amino acid metabolism. Blood phenylalanine levels are generally slightly lower in breast-fed infants than in bottle-fed infants. Breast milk contains only 12 to 14 mg of phenylalanine per ounce, compared with 24 to 28 mg per ounce in formula. Because PKU is a heterogeneous disorder, phenylalanine levels vary greatly in infants with the disease. However, treatment is usually not necessary in infants who persistently demonstrate blood phenylalanine concentrations of less than 10 mg per dL (605 µmol per L). Such cases are mild and permit production of phenylalanine hydroxylase, which converts phenylalanine to tyrosine, as in the normal person. Persons with severe mutations have much higher phenylalanine levels. To be completely certain of the diagnosis, follow-up testing is recommended in male infants with initial phenylalanine levels between 3 and 10 mg per dL and in female infants with values between 4 and 10 mg per dL. Affected females should be followed through their reproductive years to prevent impaired fetal neurologic development as a result of maternal PKU.

FALSE-POSITIVE RESULTS

Although initial PKU screening demonstrates positive results in 1 percent of infants, there is only a 10 percent chance that an infant with an initial positive result has the disorder (false-positive rate of 90 percent). A repeat test must be performed if the initial test is positive. False-negative results are rare. Infants should be given a normal diet until a definitive diagnosis is made.

VERIFICATION OF POSITIVE RESULTS

When the initial newborn screening test and the second test show positive results, a confirmatory quantitative test, such as the McCamon-Robins fluorometric test, should be performed by a laboratory at a referral metabolic center. The infant should be referred to a center capable of providing the required medical, nutritional and laboratory services for infants with PKU. While the phenylalanine-restricted diet requires the assistance of health care professionals experienced in the management of PKU, the family physician should continue to provide routine care, including immunizations. Follow-up developmental testing before kindergarten is recommended. If it becomes evident during regular follow-up of an infant that the infant's development is delayed, it is useful to examine the report of PKU screening, and consideration may be given to repeating the test. There may be instances in which the blood specimen was obtained during the first six hours after the infant's birth.

BOUQUET

In Lighter Vein

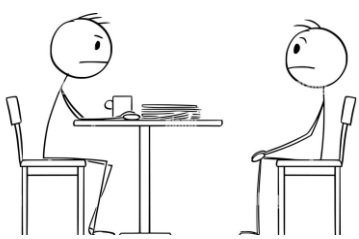
A pair of cows were talking in the field. One says, "Have you heard about the mad cow disease that's going around?"

"Yeah," the other cow says. "Makes me glad I'm a penguin."

At a party, a young wife admonished her husband

"That's the fourth time you've gone back for ice cream and cake. Doesn't it embarrass you?"

"Why should it?" answered her spouse. "I keep telling them it's for you.



Recruiter: "Can you work overtime for our company without Overtime Claim?"

Candidate: "I can work for this company without any pay."

Recruiter: "Ha.Ha… You must be Joking."

Candidate: "Well…You started First."

Wisdom Whispers

**"Be there for others but
Never leave yourself behind"**

**"Do Not Go Where The Path may lead,
Go instead where there is no Path
and Leave a Trail."**

**" Life is short, but it is Wide.
This too shall pass ."**

**"Not in doing what you like,
But in liking what you do
Is the secret of happiness"**

Brain Teasers

1. **PAH deficiency is termed as _____**
a. PKU
b. G6PD
c. 17 OHP
d. Total Gal

2. **Metabolic Disorders in new-born screening include:**
a. PKU
b. MSUD
c. MCAD
d. All above

3. **Rare but serious medical problems included in new-born screening include:**
a. Galactosemia
b. SCID
c. SMA
d. All above

4. **SKIN findings in PKU are:**
a. Fair skin and hair
b. Eczema
c. Light sensitivity
d. All above

ANSWER: 1: a, 2: d, 3: d, 4: d

Newborn Screening Solutions from Tulip

BornSafe™

ELISA based Assays for the detection of Congenital disorders in Newborns

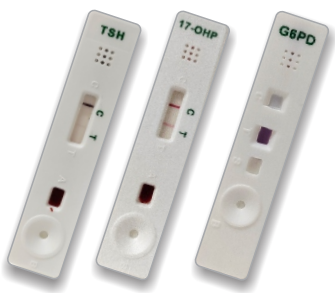
• G6PD • Total Galactosemia • MSUD • PKU • Biotinidase • TSH • 17-OHP

BabySafe™

A Unique Rapid Immunoassay Test for Newborn Screening

BabySafe™ is a unique range of Rapid Immunoassay Test for NBS which works with BabySafe™ Analyzer for reading and interpretation of test results detecting the most important analytes

• 17-OHP • TSH • G6PD



Benefits:

- Convenient and easy to perform with small heel stick puncture.
- Bedside point of care testing with fresh whole blood.
- Affordable, advanced technology.
- Immediate results.

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