Congenital Adrenal Hyperplasia (CAH)
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The term congenital adrenal hyperplasia (CAH) summarizes several metabolic diseases that lead to a cortisol deficiency and inadequate synthesis of adrenal steroid hormones. Autosomal, recessive inherited defects of the cortisol synthesis can occur in all six enzymes, involved in the synthesis of steroid hormones. Only cortisol, the end product of this metabolic pathway, is able to deactivate the CRH or ACTH secretion in the hypothalamus and pituitary gland. If the cortisol deficiency is not treated, it will lead to a permanent stimulation of the hormone synthesis in the adrenal cortex and consequently to the development of hyperplasia. Depended on the step of hormone synthesis that is catalyzed by the defect enzyme the intermediates accumulate. This leads to an inadequate concentration of mineral corticoids and androgen steroids. Thus, dependent on the defective enzyme, hormone excess or deficiency syndromes might occur.

Classical forms
The most important symptoms are already present at birth in the classical forms of CAH. The excess of androgen leads to prenatal virilisation in girls and to pseudopubertas praecox in both genders. The decreased production of oestrogen causes pubertas tarda with primary amenorrhoea in girls. The decreased production of cortisol leads to tiredness, apathy, reduced tolerance to stress, hypoglycaemia, increased susceptibility to infections and Addison-like crisis. The decreased production of aldosterone results in hyperkalemia, hyponatremia, salt wasting syndrome, metabolic acidosis and hypotension.

Nonclassical forms
Depending on the severity of the genetic defect, the age of onset of the symptoms of nonclassical forms of CAH related to excessive androgen varies. The disease can manifest itself in girls before puberty in form of a premature pubarche or adrenarche, accelerated bone age, microsomia and clitoromegaly. Typical symptoms in adult women are hirsutism, acne, seborrhoea, deep voice, clitoromegaly, temporary loss of hair, bald brow, primary or secondary amenorrhoea and oligomenorrhoea.
### Clinical and biochemical changes due to congenital adrenal hyperplasia

<table>
<thead>
<tr>
<th>Metabolic Disease</th>
<th>Intersexual Genitals</th>
<th>Salt Wasting</th>
<th>Postnatal Virilisation</th>
<th>Increased Plasma Steroids</th>
<th>Decreased Plasma Steroids</th>
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<tbody>
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<td>Boys</td>
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<td>No</td>
<td>None</td>
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Lipoid congenital hyperplasia (20,22-desmolase deficiency)

Enzyme: 20,22-desmolase (synonym: STAR-protein), [STAR]
Chromosome: 8p11.2
MIM: 201710, 600617
GeneID.: 6770

Biochemistry and molecular biology
In lipoid congenital hyperplasia, the elimination of the side chain between C20 and C22 of cholesterol, leading to pregnenolone is disturbed. This reaction is catalyzed by the side-chain-cleavage-enzyme (P450scc). However no patient with this form of CAH showed any mutations in the gene encoding this enzyme. In 1995 mutations in the STAR-protein (steroidogenic acute regulatory protein) gene have been shown. This protein is in charge of the transport of cholesterol to the outer mitochondrial membrane. It seems to be of such importance for the first step of steroid hormone synthesis that an inactivating mutation causes this form of the disease. The STAR-gene consists of 7 exons. Missense and nonsense mutations as well as small deletions and insertions are detected as pathogenic mutations in patients with lipoid congenital hyperplasia.

Clinical relevance
Boys develop phenotypic female or intersexual genitalia, while girls exhibit normal genitalia. The adrenal glands are massively enlarged at birth and interspersed with cholesterol ester encrustations that have given this disease its name. Even though a steroid hormone level is still detectable right after birth, a complete deficiency of all adrenal gland steroids and clinically an acute Addison crisis is imminent if no treatment is administered. This form of CAH is very rare.

3-beta-hydroxysteroid-dehydrogenase deficiency

Enzyme: 3-beta-hydroxysteroid-dehydrogenase, [HSD3B2]
Chromosome: 1p13.1
MIM: 201810
GeneID.: 3284

Biochemistry and molecular biology
The 3-beta-hydroxysteroid-dehydrogenase is an essential key-enzyme for the synthesis of mineral corticoids, the cortisol as well as for the synthesis of gonadal steroids. It has properties of an isomerase and can catalyze the reactions in both directions. There are two genes that encode enzymes with 3-beta-hydroxysteroid-dehydrogenase activity: HSD3B1, expressed in the placenta, skin and adipose tissue and HSD3B2 that is active in the adrenal gland and the gonads. Inactivating mutations in the latter gene can cause CAH. The HSD3B2 gene consists of four exons with only three coding for the actual protein. Pathogenic mutations might occur in all coding exons.

Clinical relevance
The disease can manifest itself in different forms. The severe forms of progression (classical CAH) arise with salt wasting during the first months after birth, while the mild forms (non-classical CAH, late onset CAH) manifest themselves not until puberty and frequently affect girls.
The severe HSD3B2 deficiency leads to cortisol and aldosterone deficiency in both genders as well as to the disruption of testosterone biosynthesis that might cause an incomplete masculinisation of the male external genitalia. Usually this form of CAH does not lead to a masculinisation of female lipids and can remain undiscovered in girls until salt wasting is diagnosed. Male newborns exhibit intersexual genitalia due to the androgen deficiency. The high DHEA concentrations associated with this disease can cause slight virilisation (clitoromegaly) in female newborns due to the androgen-like effect of this metabolite. The HSD3B2 deficiency is responsible for 1–10% of classical CAH cases. Several authors are discussing, whether the nonclassical forms of HSD3B2 deficiency are the cause of premature puberty, hirsutism, menstrual disorder and polycystic ovaries.

Contrary to defects in the CYP21 and steroid-11-beta-hydroxylase genes, the defect in the HSD3B2-gene is not limited to a malfunction of the adrenal gland, but additionally prevents the synthesis of steroids in the adrenal gland as well as in the gonads. Thus the excretion of cortisol, aldosterone, progesterone, androgens and oestrogens is diminished in these tissues. For the diagnosis, an increased ratio of the plasmatic concentrations of 17-hydroxypregnenolone to hydroxyprogesterone or DHEA to androstenedione are mentioned as biochemical markers.

**Steroid-21-hydroxylase deficiency**

Enzyme: 21-hydroxylase, [CYP21A2]
Chromosome: 6p21.3
MIM: 201910
GeneID: 1589

**Biochemistry and molecular biology**

The CYP21-gene is located on the short arm of chromosome 6, right in the middle of the gene location of the HLA histocompatibility complex and the complement factor C4. Its structure is completely solved. In close proximity (30 kb) to the active gene a pseudogene is located for the CYP21-gene (CYP21P) as well as for the complement factor C4 gene (C4A). Both have probably emerged together by duplication during the early evolution of mankind and have since then become inactive due to the accumulation of mutations. The homology of the sequences of the gene and the pseudogene is 98 % in the exon location. The CYP21 gene consists of 10 exons and has a size of 3.1 kb.

The presence of the pseudogenes in direct proximity and the high homology of sequences are the reasons for the prevalence of inactivating mutations in the CYP21-gene.

Two gene effects are responsible for the pathogenic mutations: (1) In about 20–25% of all cases of classical CAH 30 kb of the CYP21 and C4 gene have been deleted. This kind of mutation probably originates from unequal crossing-over during meiosis. The break point is usually located somewhere between exon 3 and 8 of CYP21 and includes the region of the CYP21 and C4 gene up to
The corresponding location on CYP21P. (2) In 75% of all cases one or more mutations are present that result from pseudogene conversion.

The several mutations originating from the pseudogene are biochemically characterized according to the remaining activity of the enzyme. Thus rules for the genotype-phenotype correlation can be derived. Mutations that prevent active enzymes from being made result, if they are homozygous, in severe forms of CAH with salt wasting (SW). A remaining activity of 2–4% is enough to produce sufficient aldosterone to prevent the salt wasting syndrome. Such mutations lead in the homozygous state to phenotypically simple virilising (SV) forms of CAH. If the mutation results in a protein with a high remaining activity, nonclassical CAH (NC) can be expected in the homozygous state. In mixed heterozygous gene carriers the rule that the phenotype complies with the milder mutation, thus the allele that codes for an enzyme with a higher activity, can be applied.

**Clinical relevance**

In over 90% of all classical CAH cases a CYP21 deficiency is present. Due to the great clinical relevance this form of CAH will be discussed in more detail.

The frequency of the classical forms of 21-hydroxylase deficiency is claimed to be 1:12000 births in Caucasians. However in some ethnic groups the frequency is substantially higher (e.g.: 1:700 in Yupik Inuit, Alaska).

The heterozygous frequency of the German population is about 1:50. This value corresponds with other European countries and northern America.

The frequency of the nonclassical forms is probably around 1:350, however it needs to be considered that this disease is probably under-diagnosed.

The CYP21 deficiency can manifest itself in three forms:

- Classical CAH with salt wasting syndrome
- Classical simple virilising CAH
- Nonclassical (late-onset) CAH

Classical simple virilising CAH leads to virilisation of female foetuses already in utero. Depending on the severity of the enzyme defect, the changing of the outer genitalia can reach from clitoromegaly to a complete fusion of the labioscrotal folds with a penis-like enlargement of the clitoris and an extension of the urethra to the glans penis. The deformities are classified according to Prader. The internal genital is always female. Female newborns can be mistaken for boys at birth. The genital of male newborns is except for an occasional pigmentation of the scrotum inconspicuous.

Without treatment a pseudopubertas praecox with early growth of pubic hair and clitoromegaly or penis hypertrophy will occur in CAH affected children of both genders. The excess of androgens results at first in accelerated bone growth. This leads on the other hand to a premature closing of the epiphyseal cartilage resulting in microsomia. If not treated, CAH affected girls will remain primarily amenorrhoeic.
The classical CAH with salt wasting occurs if a (nearly) complete loss of CYP21 activity is present. This results in addition to the virilisation in a life threatening salt wasting syndrome. This usually manifests during the second or third week after birth in sucking weakness, vomiting, changes of electrolytes, dehydration metabolic acidosis and increasing apathy. If the treatment is not adjusted in due time, salt wasting crisis can occur at an older age under stressful situations (infections, fever, gastroenteritis, surgery).

The manifestations of nonclassical CAH are very variable. Severe forms lead to premature pubarche, macrosomia and clitoromegaly already during adolescence. Mild forms usually only emerge in female carriers. Affected men with the corresponding genetic predisposition develop clinical symptoms only in rare cases. In adult women the effects of the hyperandrogenaemia result in symptoms such as hirsutism, acne, seborrhoea, deep voice, mild clitoromegaly, temporary loss of hair, bald brow, primary or secondary amenorrhoea or oligomenorrhoea. It is important to distinguish between the mild, nonclassical form of CYP21 deficiency and the syndrome of polycystic ovaries (PCO) by differential diagnosis.

A prenatal diagnosis is subscribed, if there is a substantial suspicion that a female foetus is affected by a classical form of CAH. Gender and mutation can be determined at an early stage (chorionic villus sampling, amniocentesis). Thus the prenatal therapy will only be continued until the end of pregnancy if the girl is affected.

**Steroid-11-beta-hydroxylase deficiency**

Enzyme: 11-beta-hydroxylase [CYP11B1]
Chromosome: 8q21
MIM: 202010, 610613
GeneID.: 1584

**Biochemistry and molecular biology**

CYP11B1 catalyses the conversion of 11-deoxycortisol to cortisol as well as the conversion of deoxycorticosterone (DOC) to corticosterone. The CYP11B1 gene has a size of approx. 7 kb and consists of 9 exons. The coding region is 93% homologous to the aldosterone synthase gene.

Mutations in the CYP11B1 gene result in a complete loss of activity or at least in a decrease of enzyme activity and thus to a disturbance of the synthesis of adrenal steroids.

**Clinical relevance**

About 5% of all cases of classical CAH are caused by a CYP11B1 deficiency. This form of adrenal hyperplasia is characterized by an excess of androgens and mineral corticoids. In newborns there is a risk of hyperkalemia, hyponatremia and substantial retardation of growth. Girls are born with intersexual outer genitalia. Affected boys develop a pseudopubertas praecox. The increased production of deoxycorticosterone has a mineral corticoid effect and compensates the aldosterone deficiency. There usually occurs an arterial hypertonia during the first years of life. This can result in a later development of left ventricle hypertrophy and/or retinopathy. As the virilisation pathology hardly
differs from the one related to CYP21 deficiency and the hypertension requires treatment, it is essential that this situation is clarified by means of differential diagnoses. In addition to the severe classical form of CYP11B1 deficiency, also nonclassical forms, similar to the CYP21 deficiency, are postulated.

A complete sequence analysis of the CYP11B1 gene is subscribed, if a steroid 21-hydroxylase deficiency has been biochemically or genetically excluded and the presumptive diagnosis of an adrenal enzyme defect remains or other biochemical parameters support the existence of a CYP11B1 deficiency.

**17-alpha-hydroxylase/17,20-lyase deficiency**

Enzyme: 17-alpha-hydroxylase/17,20-lyase, [CYP17A1]
Chromosome: 10q24.3
MIM: 202110, 609300
GeneID.: 1586

**Biochemistry and molecular biology**
The enzyme CYP17A1 is a protein with two catalytic activities. It has a 17-alpha-hydroxylase as well as an 17,20-lyase activity and thus can catalyze the 17-alpha-hydroxylation of pregnenolone and progesterone to 17-alpha-hydroxypregnenolone and 17-alpha-hydroxyprogesterone as well as the further conversion of DHEA or androstenedione by means of the 17,20-lyase activity. Most defects in this gene prevent the hydroxylation at C17 and the 17,20-lyase activity. Consequently the synthesis of the precursors of cortisol and DHEA is blocked. Neither cortisol nor testosterone and oestrogen can be produced. In rare cases only the 17,20-lyase activity is impaired by mutations, resulting in a normal cortisol level with testosterone and oestrogen deficiency. The CYP17B1 gene consists of eight exons. Pathogenic mutations can occur in all exons.

**Clinical relevance**
About 1% of all cases of classical CAH are based on defects in the CYP17A1 gene. Patients with CYP17A1 deficiency cannot synthesize gonadal steroids. Male newborns are noticed due to intersexual genitalia. In girls a spontaneous development of puberty fails to appear and there is a primary amenorrhoea. The blockade in the metabolic pathway of steroid synthesis leads to increased deoxycortisol and corticosterone concentrations. The mineral corticoid effect of those leads to hypertonia, hyperkalemia, hyponatremia and metabolic alkalosis. The plasma renin activity is lowered.

**Aldosterone synthase deficiency**

Enzyme: steroid-18-oxidase, [CYP11B2]
Chromosome: 8q21
MIM: 203400, 610600, 124080
GeneID.: 1585

**Biochemistry and molecular biology**
The aldosterone synthase (CYP11B2) differs from the isoenzyme CYP11B1 by having in addition to the 11-hydroxylase activity a 18-oxidase activity and being able to convert 11-deoxycorticostero-
ne at first to corticosterone and then to aldosterone. The CYP11B2 gene is highly homologous (93% in the coding region) to the CYP11B1 gene. Both genes are situated at the same gene locus, however they are regulated separately. The CYP11B2 gene consists of nine exons.

Clinical relevance
Hypoaldosteronism is an autosomal recessive inherited disease that is caused by a CYP11B2 deficiency. A distinction is drawn between the hypoaldosteronism type 2 (CMO II), where no aldosterone can be detected despite an increased plasma level of 18-OH-corticosterone, and type 1 (CMO I), where not sufficient aldosterone is produced due to the loss of the precursor protein 18-OH-corticosterone. The cortisol and androgen levels are not affected.

Newborns with this defect suffer from a life threatening salt wasting crisis, recurring vomiting and failure to thrive during the first few weeks after birth. With increasing age the severity the disease decreases and is during adulthood usually asymptomatic even if it is not treated. This clinical picture is rare. Only in some segments of the population a certain frequency is observed due to consanguine marriages.

Both forms of hypoaldosteronism are caused by mutations in the CYP11B2 gene.

Cytochrome-P450-oxidoreductase deficiency

Enzyme: Cytochrome-P450-oxidoreductase, [POR]
Chromosome: 7q11.2
MIM: 201750, 124015
GeneID.: 5447

Biochemistry and molecular biology
The cytochrome-P450-oxidoreductase is of strategic importance for the metabolic functions of a multitude of cytochrome-P450-enzyme proteins. The POR enzyme has binding sites for the coenzymes FAD and FMN and mediates the transfer of the coenzyme NADPH to all microsomal P450-enzymes. This transfer is an indispensable prerequisite for the function of the CYP-enzymes. The POR gene consists of 15 exons and has a size of 32.9 kb. Mutations of the POR gene cause several distinct impairments of function in the POR-protein that can consequently lead to partial or complete loss of function of the associated CYP-enzymes. 20,22-desmolase, 17-alpha-hydroxylase/17,20-lyase and steroid-21-hydroxylase are POR dependent enzymes. Depending on the mutation, the enzymes can be affected individually or all three at the same time.

Clinical relevance
The cytochrome-P450-oxidoreductase deficiency results in combination with a CYP17, CYP21 and/or STAR deficiency in the development the clinical symptoms of CAH. Depending on the enzyme that is impaired in its function, the symptoms of classical and nonclassical CAH occur. In the least favourable case all three enzymes have lost their function.
References


Tusié-Luna MT, White PC (1995) Gene conversions and unequal crossovers between CYP21 (steroid 21-hydroxylase gene) and (CYP21P involve different mechanisms. Proc Natl Acad Sci USA 92:10796-10800


Specimen

- 2 – 5 ml EDTA blood
- Cultured amnion and chorion cells
- Approx. 20 mg (moist mass) chorionic villi

Turnaround time

- 1 – 2 weeks

Methodology

- Detection of mutations by DNA-sequencing after a polymerase chain reaction.
- If necessary, analysis of the ratio active gene copy number to pseudogene copy number (CYP21).
- Analysis of deletions by means of the MLPA (multiplex ligation-dependent probe amplification) method
Congenital Adrenal Hyperplasia

- Steroid-21-Hydroxylase-Deficiency (CYP21A2, MIM 201910)
- Steroid-11-beta-Hydroxylase-Deficiency (CYP11B1, MIM 202010)
- Steroid-17-alpha-Hydroxylase-Deficiency (CYP17, MIM 202110)
- Steroid-3-beta-Dehydrogenase-Deficiency (3HSD, MIM 201810)
- Aldosterone-Synthase-Deficiency (CYP11B2, MIM 203400)
- Cytochrome-P450-Oxidoreductase-Deficiency (POR, MIM 201750)

For genetic tests 3–5 ml EDTA-blood is required. Please use the service of one of the world wide logistic companies (e.g. DHL). The material is stable for several days at ambient temperature. Cooling or freezing is not necessary. Do not forget to label the tubes with the patients name and date of birth.

Cut or copy this orderform for shipment of blood or DNA samples to BioGlobe GmbH. The complete orderform is available at www.bioglobe.net.
## Facts – gene and protein structure

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<th>gene symbol</th>
<th>gene</th>
<th>chromosome</th>
<th>gene size (Kb)</th>
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