MEDICAL GRANDS ROUNDS

"CONGENITAL ADRENAL HYPERPLASIA"

June 29, 1989

Michael J. McPhaul, M.D.

Steroid hormone action is a complex process that is only recently beginning to be understood. Steroid hormones interact with specific protein receptors and cause an alteration in the genetic material of the nucleus. As a result of these processes, gene activity is modulated and a response occurs (1). The interactions depicted here are very specific and are dependent on the presence of the hormone, binding by its specific receptor, and the presence of the genetic machinery to mediate the response.

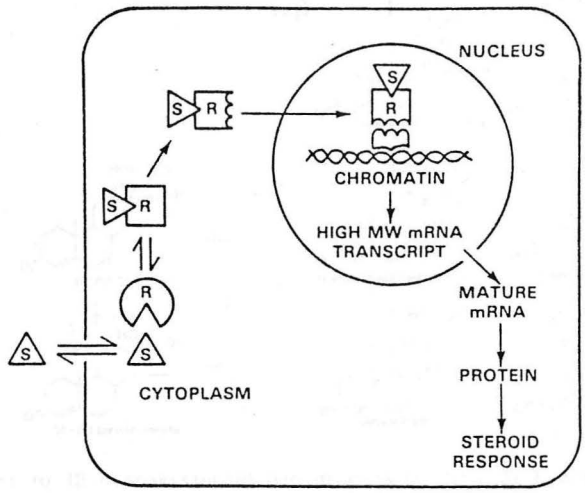
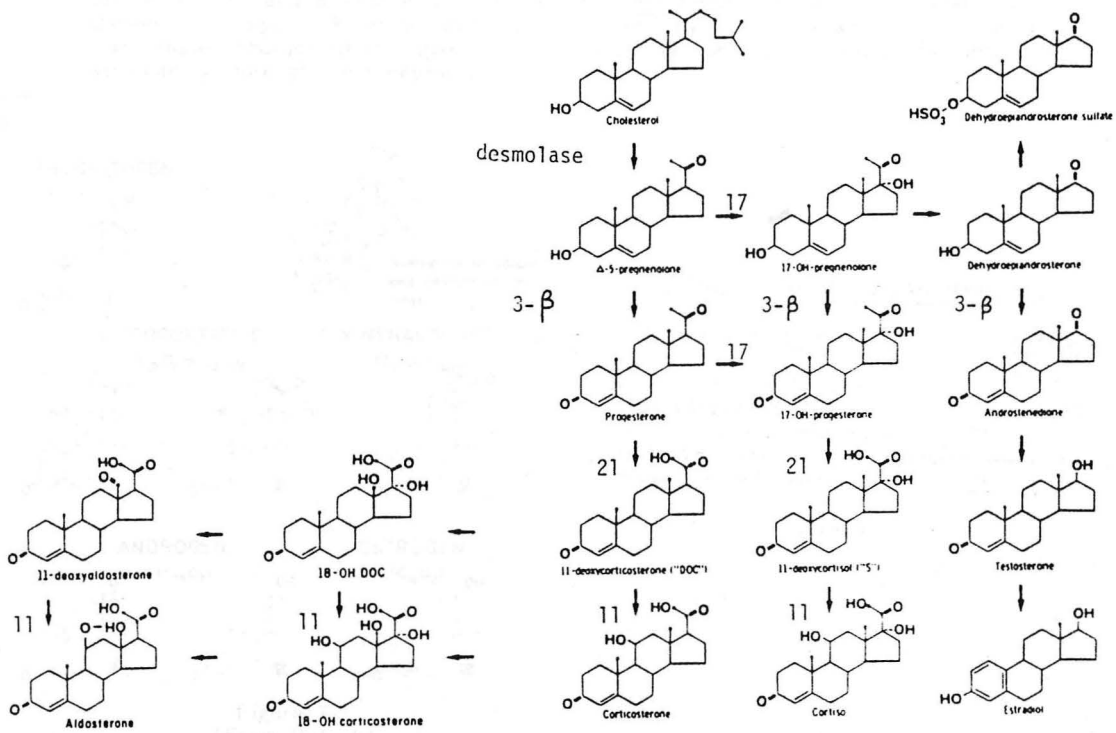


Figure 1
(from Ref. 64)

It is obvious that abnormalities at any step in this process will interfere with the action of a particular hormone. Defects in the receptor or post-receptor machinery will lead, for the most part to abnormalities in the action, of one specific hormone (2). Abnormalities of steroid hormone production, however, can result in far more profound effects. A blockade in the pathways of normal steroid hormone synthesis leads to the accumulation of compounds that can activate other members of the steroid hormone receptor family. Congenital adrenal hyperplasia is a group of genetic disorders that is characterized by a hyperplastic growth of the adrenal glands and disordered steroid hormone metabolism. The pathogenesis of these disorders has been worked out in the recent past and will be the subject of today's review. These disorders touch on many aspects of steroid hormone biochemistry and action, human genetics, and molecular biology.



3β refers to 3β-hydroxysteroid dehydrogenase, 21 = 21-hydroxylase, and 11 = 11β-hydroxylase

Figure 2
(From Ref. 65)

The biosynthesis of steroid hormones is a fascinating process. In this process, the neutral lipid cholesterol, a normal constituent of lipid bilayers, is transformed via a series of hydroxylations, oxidations, and reductions into a vast array of biologically active steroid hormones (3). The majority of the steroidogenic transformations occur in the adrenal, testes, and ovary, although other tissues, particularly the liver and kidney, are also quite active.

The chemical nature of each steroid molecule determines the type of hormonal activity that it will possess. The nature of this reactivity is determined by the ability of each to interact with the specific receptors. Thus, each of the five major classes of steroid hormone is characterized by the invariant placement of certain functional groups on the steroid hormone

backbone. Glucocorticoids: hydroxyl groups on 11β and 21, ketones at positions 3 and 20, and a double bond between carbons 4 and 5. Androgens: hydroxyl or ketone at C17 and a ketone at position #3. Estrogens: oxygen at carbon 17, a phenolic A ring. Although not yet visualized by crystallography, it is likely that these characteristics give the molecule an intrinsic ability to interact with one or more of the receptors.

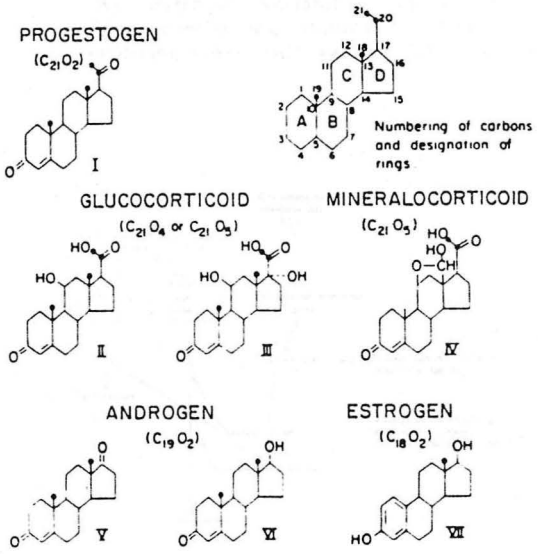


Figure 3
(From Ref. 4)

CONGENITAL ADRENAL HYPERPLASIA

Deficient Enzyme	Frequency
•21-hydroxylase deficiency	>90%
•11β-hydroxylase	5-10%
•17-hydroxylase deficiency	rare
•3β-hydroxysteroid dehydrogenase	rare
•desmolase deficiency	rare

Figure 4

Congenital adrenal hyperplasia has been shown to be the result of defects in one of several discrete steps in the steroid hormone biosynthetic pathway. A listing of these defects is shown in Fig. 4. 21-Hydroxylase deficiency is by far the most common cause of congenital adrenal hyperplasia, accounting for over 90% of cases. A defect in 11β-hydroxylase is much less common, accounting for perhaps 5-10% of cases. 17-Hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase, and desmolase deficiency are less common. I will focus, for the most part on the various forms of 21-hydroxylase and 11β-hydroxylase deficiency.

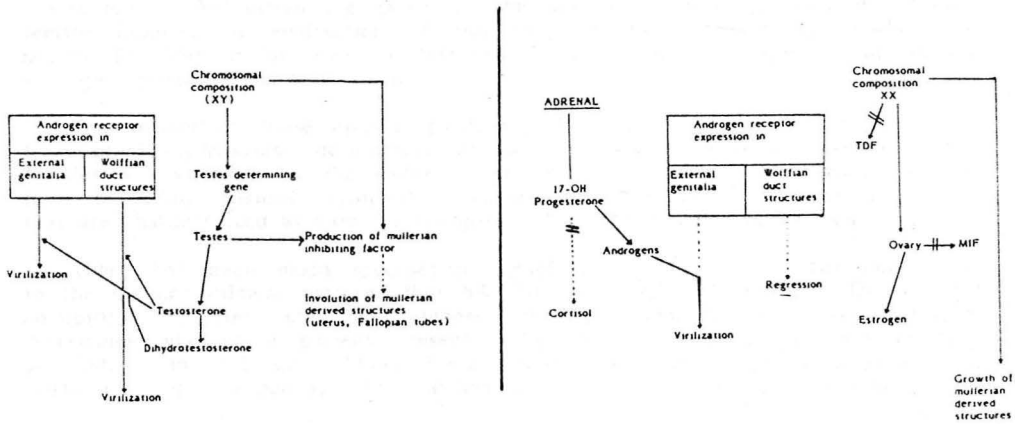
21-Hydroxylase Deficiency

Deficiency of 21-hydroxylase results in an inability to synthesize cortisol, which requires a hydroxyl group at position 21 to be active as a glucocorticoid. This inability to synthesize cortisol leads to a loss of feedback inhibition on the pituitary and hypothalamus and a massive increase in ACTH. This in turn results in an outpouring of adrenal steroids. Among the principal products that accumulate as a result of this metabolic block are

17-hydroxy pregnenolone and 17-hydroxy progesterone. Note that while a block in 21-hydroxylase prevents the normal synthesis of aldosterone and cortisol, the conversion of these steroids to the androgens, androstenedione and testosterone, occurs readily.

Clinical Spectrum

While the biochemical result of a block in 21-hydroxylation is predictable, the extreme variability in clinical presentation that results is remarkable. Four overlapping clinical pictures have been described and designated simple virilizing CAH, salt-wasting CAH, late-onset and "cryptic" CAH (5, 6, 7).



Normal Male Sexual Differentiation

Virilization of Females With 21- Hydroxylase Deficiency

Figure 5

Salt Wasting and Simple Virilizing CAH

In the virilizing form of CAH, virilization of affected females and premature virilization of affected males is the predominant feature. The simplified scheme of sexual differentiation shown in Figure 5 demonstrates the genesis of the abnormalities that are encountered. In normal male sexual differentiation, TDF (8) testosterone and dihydrotestosterone act to stimulate the wolffian ducts to form the epididymis, vas deferens and seminal vesicle (9). Testosterone and dihydrotestosterone also act in the lower urogenital sinus to effect the growth and differentiation of the prostate and the virilization of the external genitalia. Note that a second product of the fetal testes, mullerian-inhibiting substance (10), is responsible for the involution of the mullerian-derived structures - that is, the uterus and fallopian tubes. Normal female development can be conceived as the same process in which the three major mediators of male sexual development (mullerian-inhibiting

substance, testosterone, dihydrotestosterone) have been removed. Thus, in the absence of mullerian-inhibiting substance, the mullerian rudiments develop into the uterus and fallopian tubes. Likewise, in the absence of testosterone and dihydrotestosterone, the wolffian structures and external genitalia do not virilize. In female fetuses with 21-hydroxylase deficiency, the enzymatic blocks lead to accumulation of steroid precursors, particularly 17-hydroxyprogesterone and 17-hydroxypregnenolone. These in turn are converted to androstenedione and dihydroepiandrosterone (DHEA). Thus, while the genetic machinery is set default parameters (XX genotype, the presence of ovaries, the absence of mullerian-inhibiting substance), the extragonadal production of androgens effects the virilization of the external genitalia. Furthermore, as there is no production of mullerian-inhibiting substance, the development of mullerian-derived structures such as the uterus and fallopian tubes is unaffected. An interesting point is the invariant absence, even in virilized female infants, of virilization of the wolffian ducts structures. While the reason for this is unknown, differences in the timing or quantity of adrenal androgens produced has been invoked.

The second of these clinical pictures is termed "salt-losing" CAH. In this form of 21-hydroxylase deficiency, as in the "simple virilizing" form of CAH, females are virilized by the excessive production of adrenal androgens. In this form of CAH, patients manifest a mineralocorticoid deficient state and crises that are characterized by vascular collapse, hyponatremia and hyperkalemia.

There has been much speculation regarding the differences that could lead to the distinct clinical pictures that SWCAH and SVCAH represent. The simplest possibility is that the completeness of the blockade in 21-hydroxylation determines whether a patient presents with the virilizing or salt-wasting form of CAH. In this work (11), these patients were examined to ascertain the production of compounds that accumulate as the result of 21-hydroxylase

ACTH tests: 60-min ACTH-stimulated hormone concentrations of 17-OHP and Δ^4

	Classical CAH patients		
	SW	SV	SW + SV
60-min 17-OHP (ng/dl)			
n*	3	3	6
Average	27,520	38,276	39,457
SD	14,963	13,740	12,354
Minimum	16,260	23,300	16,260
Maximum	44,500	50,300	50,300
60-min Δ^4 (ng/dl)†			
n*	3	4	7
Average	1,987	1,057	1,456
SD	1,903	430	1,244
Minimum	480	621	480
Maximum	4,126	1,604	4,126

Figure 6
(From Ref. 11)

deficiency. As is evident here, the levels of 17-hydroxyprogesterone and Δ^4-androstenedione are similar in both the SV and SWCAH forms of CAH when ACTH

stimulated levels of these hormones are examined. Thus, it does not appear to be simply the amount of residual 21-hydroxylase activity that is demonstrable in any fashion. Since the major clinical difference between the SV and SW is in the amounts of mineralocorticoid activity present, the expectation would be that a difference would be most evident when mineralocorticoid production is assessed. This point was examined by Kowarski and co-workers (12) using a double label technique to directly examine the production of aldosterone in patients with SW and SVCAH. In the SV form of CAH, the patients had elevated aldosterone production rates when compared to normal controls. These elevated levels were hypothesized to result from the antimineralocorticoid activity of progesterone and 17-hydroxyprogesterone (13, 14). Furthermore, these elevated

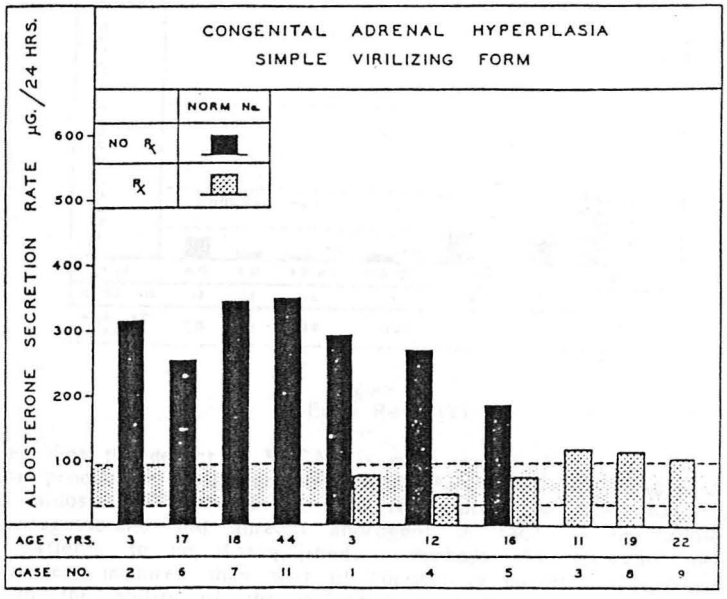


Figure 7
(From Ref. 12)

levels of aldosterone production suppressed into the normal range following treatment with replacement doses of glucocorticoids. These results are in contrast to the findings in patients with SWCAH. The patients with SWCAH had low levels of aldosterone secretion. Equally important was the observation that these patients were unable to stimulate aldosterone production under conditions of salt restriction, a stimulus to aldosterone production in normal individuals.

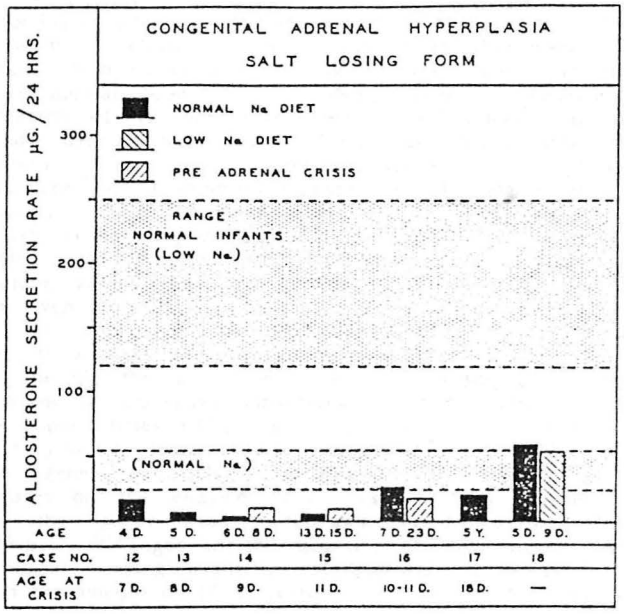


Figure 8
(From Ref. 12)

This implies that the defect in SWCAH is more severe than that present in SVCAH, in that the production of aldosterone in SWCAH is low and unable to respond to stimuli of aldosterone secretion. This is despite the fact that the levels of 17-hydroxyprogesterone and adrenal androgens in fact do not allow these two types of patients to be distinguished. Perhaps the formation of aldosterone synthesis is less impaired than that of cortisol in patients with SVCAH due, for example, to the ability of the defective 21-hydroxylases to retain the ability to one substrate preferentially to another.

Late Onset or Attenuated CAH

While the incidence of the simple virilizing and salt wasting forms of CAH preceded a precise understanding of their pathogenesis, the prevalence of the late onset form of CAH was appreciated only as a result of adequate biochemical tests. The successful treatment of the more severe forms of CAH with cortisone, led to trials of glucocorticoids in women with virilization, particularly hirsutism, and oligomenorrhea. While these therapies led to clinical improvement in many cases, the suspicion that these disease entities were in fact a mild form of CAH was disproven when biochemical specific markers of CAH such as blood levels of 17-hydroxyprogesterone or urinary excretion of pregnanetriol were employed.

While accounting for only a portion of women that present with hirsutism, infertility or menstrual irregularities, it is clear that these can be caused by 21-hydroxylase deficiency (15, 16, 17). Affected patients typically present with oligomenorrhea, acne, or hirsutism, or a combination thereof. Onset of symptoms is often in adulthood but can occur in childhood. Cases of clearly defined partial 21-hydroxylase deficiency have been described that have presented after normal pregnancies. Measurement of adrenal steroids indicate increased secretion of 17-hydroxyprogesterone and adrenal androgens, albeit at times with some overlap with normal individuals, particularly when measured in the basal states. Thus, the clearest definition of this syndrome is dependent on the measurement of 17-hydroxyprogesterone following infusion of ACTH (see diagnosis, below). While the mean levels of 17-hydroxyprogesterone are lower in patients with late onset when compared to the SW and SV forms of CAH, there are clearly patients with the late onset form that have 17-hydroxyprogesterone and Δ^4-androstenedione values that are in the range described for SV and SWCAH. This raises an even more basic problem. Why do these patients not exhibit more virilization? This point has not been elucidated. A number of theories have been advanced to explain the pathogenesis of this syndrome. These center on processes that permit the increased production of adrenal androgens but do not lead to the signs of excessive virilization: 1) the defect is in fact acquired and that the abnormalities of 21-hydroxylase are not present in early life; and 2) a distinct genetic locus (loci) influences the ability of the defective 21-hydroxylase gene to effect virilization in utero. One interesting possibility focuses on the enzyme 17-20 lyase. This enzyme controls entry of steroids into the accumulated precursors (17-hydroxyprogesterone) into the androgen pathway. Changes in the quantity of this enzyme within the adrenal gland would permit varying quantities of androgen to be produced. It is clear that changes in the amount of 17,20 lyase activity occur at adrenarche (18).

Despite the controversies surrounding its pathogenesis, current evidence suggests that this form of 21-hydroxylase deficiency is inherited as an autosomal recessive trait that is allelic to the classic forms of CAH (19, 20). Thus, this abnormality would appear to be caused by mild abnormalities of the 21-hydroxylase gene itself.

Cryptic 21-Hydroxylase Deficiency

The final form of 21-hydroxylase deficiency was not appreciated until specific biochemical tests were available. In 1980, Levine et al reported the characterization of individual members within 8 families with classic CAH (21). In this study, several individuals were found to have hormonal levels consistent with mild 21-hydroxylase deficiency. However, signs of excessive virilization, hirsutism, oligomenorrhea, and infertility were absent. These patients were detected by the presence of high 17-hydroxyprogesterone levels in the unstimulated state. Further examination of these patients (22) showed that their hormonal profiles were distinct from those of the normal population and from patients believed to be CAH heterozygotes. Further investigations using ACTH stimulated hormone measurements disclosed hormonal profiles that were distinct from classic CAH (homozygous and heterozygous) and the normal population (see diagnosis, below). However, even using hormonal measurements obtained in the stimulated state, cryptic CAH and late onset CAH cannot be distinguished.

Diagnosis

In patients that present with the full blown picture of SV or SWCAH, the diagnosis is not difficult to establish. Serum samples are examined for the concentration of 17-hydroxyprogesterone and for the levels of adrenal androgens, specifically testosterone, dihydrotestosterone, and DHEA. In the late onset and cryptic variants, however, the diagnosis may not be quite as evident from baseline hormonal measurements. The reason for this is to be found in an examination of the hormonal profiles present in these individuals throughout the course of a day. Thus, while baseline measurements may show overlap with normal individuals, levels of 17-hydroxyprogesterone and its metabolites vary with the normal ACTH driven circadian rhythm (23, 24). In these individuals, the diagnosis is established by examination of the hormonal profile following stimulation of steroidogenesis by the infusion of ACTH (25). In this protocol, which has received much use, ACTH (cotrosyn 0.25 mg) is injected as a bolus between 0800-1000 AM. Blood samples are collected for 17-hydroxyprogesterone and Δ^4-androstenedione at 0 and 60 min. A second protocol uses slightly higher doses of ACTH (1-24) (0.4 mg) infused continuously over a 6 h period. Using these criteria patients with all forms of 21-hydroxylase deficiency can be differentiated from unaffected individuals.

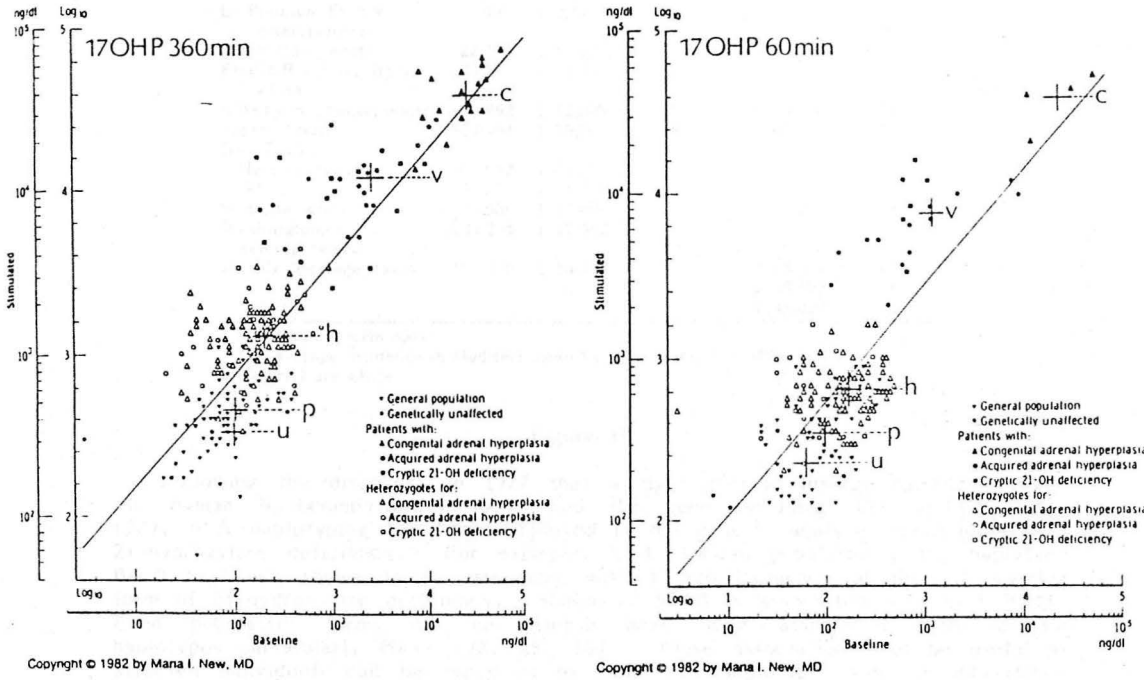


Figure 9

Genetics

The incidence of CAH in the general population has varied from among different reports. A summary of incidences in several different populations based on case surveys is indicated in Figure 9 (26). This summary reveals a wide range of incidence, but that appears to be approximately 1 in 10-20,000. Note that this is an estimate based on the identification of clinically affected individuals and may thus represent an underestimation of its true incidence. In 1977, a rapid screen was developed, similar to that in use for phenylketonuria that allows the determination of 17-hydroxyprogesterone levels in small samples of blood. The application of this technique to the measurement of 17-hydroxyprogesterone has revealed an incidence that is similar in all populations, except certain Alaskan Eskimos and a small town in France where a founder effect and inbreeding have resulted in a quite high frequency.

Comparison of Classical Congenital Adrenal Hyperplasia Determined by Screening and by Case Survey

Geographic Area and Population	Screening (1978–1988)			Case Survey (1965–1985)	
	No. Tested	Incidence	Reference No.	Incidence	Reference No.
Alaska, Yupik Eskimo	1,131	1:282*	22	1:490*	11
La Reunion, France, heterogeneous	14,987	1:2,141*	25		
Rome, Italy, white	22,400	1:5,580†	24		
Emilia-Romagna, Italy, white	73,000	1:14,600†	23		
Lille/Lyon, France, white	173,662	1:12,000†	25	1:23.000	25
Japan, Asian	253,494	1:15,800	26	1:43,674	20
New Zealand					
Heterogeneous	97,552	1:16,258	28		
White	87,000	1:14,500			
Scotland, white	119,960	1:17,098		1:20,907	19
Washington, heterogeneous‡	233,244	1:17,942			
Illinois, heterogeneous‡	120,000	1:13,333		1:15,000	10
				1:26,792	9
				1:40,000	12

* Salt-wasting form only.
† The average incidence in Mediterranean Europeans was 1:10,866 births.
‡ Majority are white.

Figure 10

Following the discovery in 1977 that a tight genetic linkage existed between the human histocompatibility locus and the gene encoding the 21-hydroxylase (27), HLA haplotyping has been employed in the genetic analysis of patients with 21-hydroxylase deficiency. For example, in Caucasian populations, the haplotype Bw60 has been shown to be associated with a high incidence of the salt-wasting form of 21-hydroxylase deficiency. Similarly, SVAH is associated with HLA Bw51. Even nonclassic forms of the disease have been associated with specific haplotypes particularly Bw14 (28, 29, 30). These associations can be useful as affected individuals can be typed as to their HLA haplotypes and the inheritance of the affected alleles traced. This association has been used successfully, particularly in combination with measurements of 17-hydroxyprogesterone in amniotic fluid, to diagnose CAH in utero (31, 32).

Molecular Genetics

As the HLA linkage data implied a structural relationship between the histocompatibility locus and the gene encoding the 21-hydroxylase enzyme, it was natural to examine the nature of this linkage. This has been performed in both mice and humans. Following the successful isolation of the bovine 21-hydroxylase, Perrin White used his cDNA probe to examine overlapping fragments gene fragments derived from the mouse histocompatibility locus (33). In this way, he was able to demonstrate that two distinct regions of the genome were identified. Subsequent analysis (34) indicated that in the mouse two separate 21-hydroxylase genes could be identified. Each 21-hydroxylase gene was located adjacent to the 3' terminal segment of the genes encoding a functional (C4) and a nonfunctional (Slp) component of complement. Earlier work had indicated that Slp and C4 had arisen by a direct duplication of approximately 55 kb of chromosomal DNA. As the 21-hydroxylase genes reside within this region, they were presumably duplicated at the same time. Interestingly, subsequent work has demonstrated that only one of these two 21-hydroxylase is functional (35) - that is, in the mouse only the A gene is expressed and encodes a functional protein, while the B gene is not expressed and carries mutations that have inactivated it (36) (see Fig. 11).

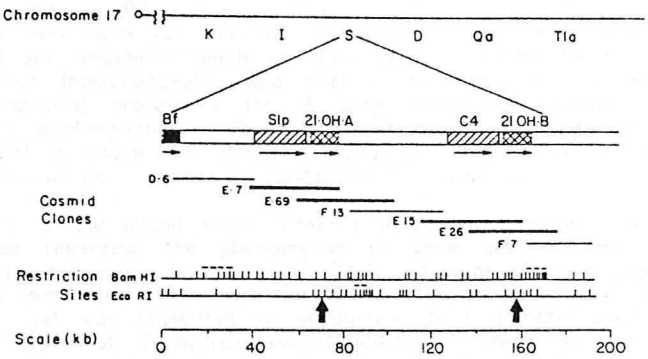


Figure 11

The results of this analysis of the mouse 21-hydroxylase genes have had numerous parallels in the analysis of the human 21-hydroxylase genes. As I mentioned, the extended haplotype HLA-(A3); BW47; DR7 is strongly associated with the severe form of 21-hydroxylase deficiency. Furthermore, it was known that alleles of this haplotype also carry a null allele at the locus encoding one of two forms of the fourth component of complement (C4). In 1984 Perrin White (37) examined the structure of the 21-hydroxylase gene in patients known by haplotyping to be homozygous for this type of severely abnormal 21-hydroxylase allele. When analyzed in this manner DNA from a patient homozygous for the normal HLA haplotype and without CAH shows two distinct bands. By contrast, DNA prepared from a patient homozygous for the HLA-BW47 associated form of 21-hydroxylase deficiency shows only a single band. This was felt to be consistent with the model shown in this figure. It was suggested that in this patient with severe CAH and C4A deficiency that there was a

deletion of a segment of DNA that encoded one complement locus and one 21-hydroxylase gene. This hypothesis was advanced without a clear understanding of the structure of the two duplicated 21-hydroxylase alleles. In particular, this idea did not explain how the deletion of only one of two 21-hydroxylase genes leads to a deficiency state.

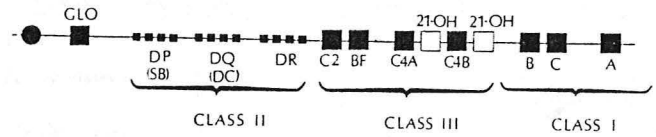


Figure 12

In 1985, Carroll et al (38) elucidated the structures and relative positions of the 21-hydroxylase and C4 genes in the human chromosome 18 (Fig. 12). They found that as in the mouse, there are in fact two 21-hydroxylase genes, situated at the 3' terminal side of the two functional complement genes. These have been designated the A and B genes. The nature of these two 21-hydroxylase genes is therefore critical. Do these in fact represent interchangeable, functional genes, or do they each subserve distinct functions? A year later Higashi et al (39) isolated and sequenced each of these genes. They found that these two genes were not interchangeable and that in fact only the B gene is able to encode a functional protein - the A gene containing insertions and deletions that render it nonfunctional. Thus, a simple model was apparent for this form of severe CAH in which the affected individual contains two chromosomes, each containing deletions that remove the functional 21-hydroxylase B gene.

Subsequent to the initial study referred to above, however, many papers have been published regarding the phenomenon of gene deletion that was originally described in reference as the cause of CAH. Several of these studies have cast doubt on this mechanism as a cause for CAH. The confusion that has centered on the evidence that was presented as supporting gene deletion as a cause of the more severe types of 21-hydroxylase deficiency. Many of the initial studies used only a small number of restriction enzymes to analyze the DNA of these patients. Subsequently, several analyses indicated that instead that a process termed gene conversion (40) might be occurring. This term, first coined in yeast genetics, indicates a process whereby two closely related genes become identical. Although a number of mechanisms have been proposed, that the end result of such a conversion would be the creation of two nonfunctional genes. Note that the examination of the DNA of such a patient if not performed with multiple restriction enzymes would lead to a pattern consistent with a deletion. It is clear from the more recent examinations of this region of the genome using both 21-hydroxylase and C4 probes that this region of the genome is highly polymorphic. I would say that the evidence taken as a whole (summarized in Table I) suggests that both processes occur and that the frequency of each process varies in the population examined. It is extremely interesting to note that even in those cases where single mutations have been identified within the 21-hydroxylase B gene, these point mutations are normally found within the nonfunctional 21-hydroxylase A pseudogene. This implies that the process of gene conversion may in fact be quite difficult to detect as it may affect only

parts of the normally functional B gene. No studies have yet been performed to analyze the alleles associated with the less severe forms of CAH (adult onset, cryptic 21-hydroxylase deficiencies).

TABLE I

DELETIONS/INSERTIONS

References	Number	Family Study	Type of Investigation	Remarks
(37)	1	Yes	Southern, HLA	Deletion of 21OH-B Gene
(41)	22 (unrelated)	No	Southern, HLA	Frequent deletion and duplications
(42)	126 haplotypes	Yes	Southern, HLA	-looked at C4 locus as well; deletions identified
(43)	20 patients	No	Southern	-C4 & 21OH examined -2OH-B deletions identified
(44)	6 patients	Yes	Southern, multiple enzymes, HLA, hormonal	Gene conversion inferred
(45)	51	Yes	Southern, multiple enzymes, HLA	No gene deletions All due to gene conversion
(46)	12	Yes	Southern, DNA sequencing	Homozygous haplotype cloned, found 21OH-B gene converted to 21OH-A gene
(47)	35	No	Southern (4/21OH)	13/15 chromosomes show absent TAQI fragment; analysis indicates deletions
(48)	33		Southern (C4/21OH)	Deletions of C4, 21OH-A, and 21OH-B detected
(49)		Review		
(50)	11	No	Southern (oligo)	Frequent gene converisons
(51)	68	Yes (extensive)	Southern	Extensive Analysis Small mutations (65%) alterations of 21OH-A gene conversion (11%), 21OH-B deletion (10%)

POINT MUTATIONS

Reference	Patient Type	Number	Family Study	Type of Investigation	Remarks
(52)	SWCAH	1	No	-DNA sequencing -oligonucleotide Southerns	-3 mutations, one is premature termination -mutations shared with 21-OH-A pseudogene
(53)	Classic CAH	1	No	-DNA sequencing -oligonucleotide Southerns	-ile 472 converted to Asn -mutation is present in 21-OH-A pseudogene
(54)	SWCAH	1	No	-DNA sequencing	-several point mutations in 21-OH-B gene - many shared with pseudogene -C4B converted to C4A
(55)	Classic CAH	3	No	-DNA sequencing	-point mutations (missense, splicing defects) found in 21OH B gene - these also found in 21OH A pseudogene

Summary

Current biochemical and genetic evidence (see below) suggests that CAH represents a spectrum of clinical disease that is caused by variations in severity of the enzymatic defect. Thus, SWCAH would appear to be caused by defects severe enough to impair synthesis of cortisol and aldosterone, SVCAH would represent disease that is less severe impairing cortisol synthesis but leaving sufficient aldosterone synthesis intact to allow sodium homeostasis. It is clear that on the whole, the disturbances that cause the late onset and cryptic forms of CAH are milder still. It does appear that in these latter two syndromes that other factors may be affecting the degree of virilization present in affected individuals.

11β-Hydroxylase Deficiency

Deficiency in 11-hydroxylase of steroids is an autosomal recessive disorder that accounts for approximately 5% of individuals with CAH. As with 21-hydroxylase deficiency, the enzymatic defect leads to the accumulation of steroid precursors proximal to the enzymatic block and the overproduction of adrenal androgens. Thus, as in CAH due to 21-hydroxylase deficiency, virilization of affected female infants and signs of premature sexual maturation in male infants can be observed. A distinct difference between this form of CAH and the 21-hydroxylase deficiency form of CAH is the overproduction of 11-deoxycortisol or DOC. In this situation, as in the deficiency of 21-hydroxylase, ineffective steroidogenesis drives the pituitary to produce high levels of ACTH which drive the production of DOC. These inappropriately elevated DOC levels in turn lead to volume expansion and hypertension. Hypertension, in fact, is the only clinical finding that would lead to the diagnosis of 11β-hydroxylase deficiency, as opposed to 21-hydroxylase deficiency.

Figure 13 (56) demonstrates the range of findings encountered in patients with 11β-hydroxylase deficiency in a series of 25 patients. Patients with the more sever forms tended to be diagnosed earlier in life. As is evident, a wide range of abnormalities were found at presentation, from genetic females with male external genitalia (patient 10) to adult women with hirsutism and oligomenorrhea (patients 24 and 25).

The diagnosis of 11β-hydroxylase has not been as well standardized as has been done for 21-hydroxylase deficiency. The usual studies include measurement of 11-deoxycortisol, which should be elevated. Measurement of plasma renin (which is suppressed due to the 11-deoxycorticosterone overproduction) is also performed. Finally, the production of 11-deoxycorticosteroids can be inferred by the quantitation of tetrahydro-11-deoxycortisol (THS) in the urine.

Clinical data on patients with classic congenital adrenal hyperplasia due to 11β-hydroxylase deficiency (group A)

Patient no.	CA at diagnosis (yr)	BA at diagnosis (yr)	Height SDS		Pubic hair[a]	Testicular volume (ml)[b]	Breast development[a]	Blood pressure (mm Hg)	Chief complaint
			CA	BA					
Males									
1	0		−1.2		1	1		n	Salt wasting
2	0		−0.3		1	1		n	Salt wasting
3	6.4	13.2	+4.1	−2.1	3	2		130/80	Pseudoprecocious puberty
4	6.5	13.5	+2.7	−3.0	3	4	2	130/90	Tall stature, early puberty, gynecomastia
5	10.9	17.0	+1.4	−3.4	4	10	2	145/100	Pseudoprecocious puberty, hypertension, hypokalemia
6	18.2	Adult	−2.7	−2.7	6	13		165/110	Hypertension, hypokalemia, muscular weakness and cramps
Females									
7	0		+0.6		1		1	n	Ambiguous genitalia 3[c]
8	0.1		−1.5		1		1	n	Ambiguous genitalia 3
9	2.0		−1.4		1		1	n	Ambiguous genitalia 2
10	5.5	11.0	+3.4	−3.0	3		1	150/110	Male external genitalia (hypospadia)
11	5.8		+4.1		3		1	130/70	Ambiguous genitalia 1
12	6.2	10.8	+0.5	−4.1	3		1	n	Ambiguous genitalia 1
13	12.7	14.0	+0.8	−0.1	3		1	160/80	Clitoris hypertrophy

n, Normal blood pressure (45) on repeated measurements; SDS, standard deviation scores; CA, chronologic age; BA, bone age.
[a] Pubic hair and breast development stages according to Tanner (40).
[b] Testicular volume according to Zachmann *et al.* (42).
[c] Rating of ambiguous genitalia according to Prader (41).

Clinical data on patients with mild congenital adrenal hyperplasia due to 11β-hydroxylase deficiency (group B)

Patient no.	CA at diagnosis (yr)	BA at diagnosis (yr)	Height SDS		Pubic hair	Testicular volume (ml)	Breast development	Blood pressure (mm Hg)	Chief complaint
			CA	BA					
Males									
14	8.1	10	+2.2	+0.5	1	1		105/65	Tall stature
15	9.4	12.8	+1.9	−0.8	3	4		105/65	Early puberty
16	15.6	Adult	−1.8	−2.6	5			140/80	Bilateral cryptorchidism, acne
17	17.5	Adult	−2.9	−3.0	5	10		155/90	Acne
Females									
18	0		−1.3		1		1	80/40	Ambiguous genitalia 1
19	2.9	2.5	+0.1	+1.6	1		1	145/85	Ambiguous genitalia 1
20	7.5	11.0	+3.0	−0.9	4		1	95/60	Ambiguous genitalia 1
21	7.6	10.2	+2.3	−0.8	3		1	95/60	Tall stature, premature pubarche
22	8.3	11.0	+2.9	−0.2	2		1	110/70	Premature pubarche
23	8.5	10.0	+2.7	+0.9	1		1	110/70	Tall stature
24	19.7	Adult	−0.9	−0.9	6		5[a]	130/80	Hirsutism, oligomenorrhea
25	23.6	Adult			5		5[a]	130/80	Hirsutism, oligomenorrhea

[a] Previous estrogen treatment.

Figure 13

Figure 14 demonstrates the salient features of such patients (57). Note in the basal state, that the patient produces high levels of 11-deoxycortisol and DOC, compared to normal individuals, while cortisol production is impaired. Notably, despite the low levels of aldosterone assayed, plasma renin activity is low, presumably as a result of the volume expansion mediated by deoxycorticosterone, a weak mineralocorticoid.

Case reports: urinary steroid excretions and plasma cortisol, corticosterone and 11-deoxycortisol levels

		Jac.	Jos.
Urine	17-ketosteroids (mg/24 hr)	17.0	4.0
	17-ketogenic steroids (mg/24 hr)	31.5	11.2
	17-hydroxysteroids (mg/24 hr)	22.0	7.6
	Pregnanetriol* (mg/24 hr)	0.09	0.06
	Tetrahydrodeoxycorticosterone (μg/24 hr)	800	200
	Aldosterone (μg/24 hr)	1	1
Plasma	Cortisol (μg/100 ml)	0.3	0.3
	Corticosterone (μg/100 ml)	0	0
	11-deoxycortisol (μg/100 ml)	20	12.5

* Determinations were kindly performed by Dr. S. Saez, Unité de Recherches—INSERM, Lyon (France).

Figure 14

The changes caused in one of these patients by salt restriction, dexamethasone, and ACTH is shown in Figure 15. In response to salt restriction, plasma renin activity and aldosterone production remain low. If dexamethasone is then added to this regimen, DOC production drops and plasma renin begins to rise. Concomitant with this is a rise in aldosterone production, even prior to stimulation with ACTH. These studies suggest two things. First, they support the concept that overproduction of DOC maintains a volume expanded state. Second, is that the observed rise in aldosterone, itself an 11β-hydroxylated steroid, following suppression of ACTH production with dexamethasone has suggested to others that the 11β-hydroxylase mechanism in the glomerulosa might be distinct from that present in the fasciculata and reticularis.

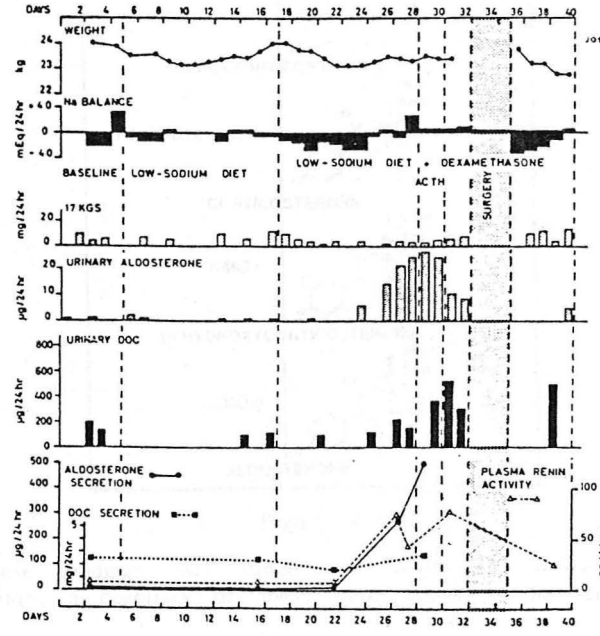


Figure 15

These observations have generated a great deal of interest in the enzyme that catalyzes steroid 11β-hydroxylation. This enzyme has been purified and extensively characterized (58). As expected, it is a typical cytochrome P-450 enzyme and is located within the mitochondria. What came as quite a surprise was the discovery that his single cytochrome P-450 was capable of catalyzing not only the 11β-hydroxylation of steroids but the 18-hydroxylation and 18-aldehyde synthesis reaction as well. Thus, it would appear that a single enzyme is responsible not only for the 11-hydroxylase reaction that is crucial to the synthesis of cortisol in the fasciculata and reticularis but also for the terminal steps in the synthesis of aldosterone as well. This poses a major difficulty in understanding adrenal steroid biosynthesis as it has been well accepted that the adrenal cortex is divided both morphologically and functionally in its production of steroids: the glomerulosa producing aldosterone and the fasciculata and reticularis producing cortisol. Thus, even though 11β-hydroxylase is present in both the glomerulosa and fascularis, the synthesis of aldosterone occurs predominately in the glomerulosa.

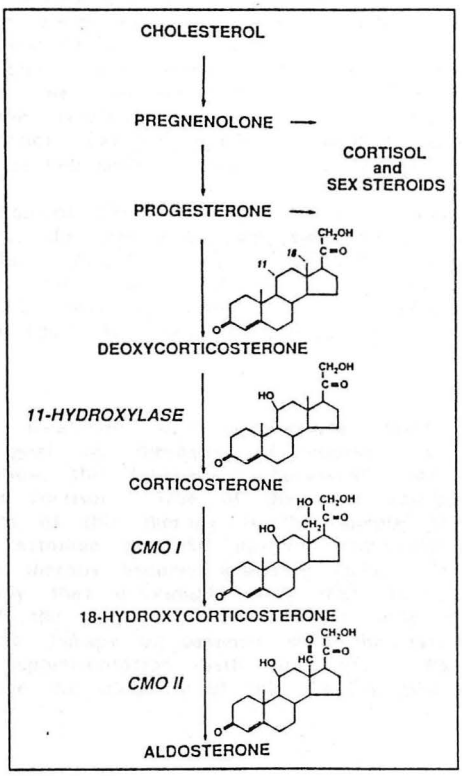


Figure 16

While these findings may cloud the issue of compartmentalization of 11β-hydroxylation, a corollary of these observations is that defects in distinct

functional sites of this single polypeptide might lead to different clinical pictures. Thus, a defect leading to an abnormal 11-hydroxylase function would lead to the picture of classic CAH caused by 11-hydroxylase deficiency. Likewise, according to this scheme, specific defects in the more terminal steps catalyzed by the 18-hydroxylase or of the 18-hydroxyl oxidase function of the P-450-11β could lead to a selective disorder in aldosterone synthesis.

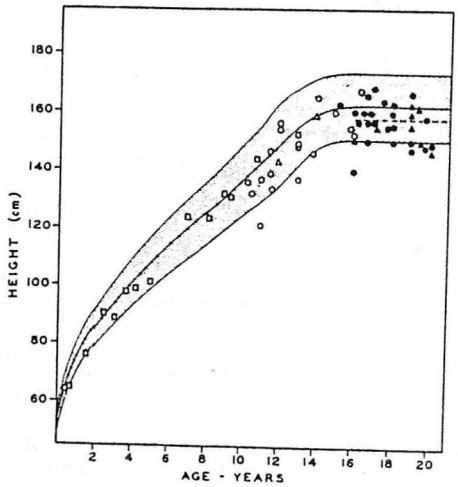
Molecular Biology

Recently, cDNA clones encoding the bovine and human 11β-hydroxylase have been isolated (59, 60). Early indications were that a relatively uncomplicated picture would emerge - that is, that a single gene encoded the 11β-hydroxylase that was located on human chromosome 8 (59) However, more recent reports have indicated that this may not be so simple. Kirita and coworkers have recently reported the nucleotide sequence of two related P-450 (11β) molecules derived from their analysis of several adrenal 11-hydroxylase cDNA bovine sequences (61). By analysis of bovine adrenal RNA and DNA they were able to conclude that at least two distinct genes exist and are expressed. More recently, these investigators have shown that these mRNAs result in the production of two proteins. This then still leaves open the possibility that distinct 11β-hydroxylase genes may be involved in the synthesis of 11-hydroxylated glucocorticoids in the reticularis and for aldosterone in the glomerulosa. Notably, a recent article (3) references unpublished observations that indicate the existence of at least two tandemly repeated 11β-hydroxylase genes.

The characterization of the defects in the 11β-hydroxylase are as yet quite rudimentary. In fact, the only study published thus far on the subject is one that supports the multifunctional catalytic activity ascribed to 11β-hydroxylase. In this study (62) an RFLP was uncovered using the 11β-hydroxylase as probe that segregates with a defect in the terminal step in aldosterone biosynthesis (corticosterone methyl oxidase II).

Treatment

The effects of treatment with suppressive doses of glucocorticoids is miraculous. The goal of therapy, of course, is to provide adequate glucocorticoids to allow the feedback suppression that is impossible without effective synthesis of cortisol. One of the most impressive representations of the physiologic results of this therapy is this simple graph, which shows the average final height attained of CAH patients stratified by their ages at the time that suppressive therapy became available (63). It is evident that as a result of this therapy that individuals with that were previously destined to premature closure of the epiphyses, instead are able to attain their normal heights. Replacement therapy of patients with the salt-wasting form of CAH would also include supplementation with fluorinef. Patients with the salt-losing form should have the adequacy of this therapy assessed by measurement of plasma renin activity.



Current height of Patients in Treatment Groups III, IV, and V. Shaded area represents the third to ninty-seventh percentile for normal girls. The fiftieth percentile is designated by a solid line. The mean final height for Group IV patients who have attained final height is represented by a dashed line. Solid figures denote final heights. Squares represent patients now less than 10 years of age. Triangles represent patients in Group III, and circles denote patients in Group IV.

Figure 17

Conclusions

Heterogeneity characterizes both the phenotypes and enzymatic defects that underlie congenital adrenal hyperplasia. A variety of molecular defects have been described that are thought to be responsible for CAH caused by 21-hydroxylase deficiency, ranging from deletions to point mutations to gene conversions. This, in turn, appears to result from a remarkable degree of plasticity at the 21-hydroxylase loci within the general population.

Although less common than 21-hydroxylase deficiency, CAH due to 11β-hydroxylase deficiency appears to be caused by defects in a cytochrome P-450 that catalyzes multiple steps of steroidogenesis in addition to 11β-hydroxylation. Recent studies at the protein and DNA levels provide direct evidence of a link that has long been suspected between the 11β-hydroxylase and the catalysis of the terminal steps in the synthesis of aldosterone.

REFERENCES

1. Evan RM. The steroid and thyroid hormone receptor superfamily. Science 240:889-895, 1988.

2. Wilson JD. Disorders of steroid and thyroid receptors. Internal Medicine Grand Rounds, UT Southwestern Med Ctr, March 2, 1989.

3. Miller WL. Molecular biology of steroid hormone synthesis. Endocr Rev 9:295-318, 1988.

4. Bondy PK. The adrenal cortex. In Metabolic Control and Disease, PK Bondy, LE Rosenberg, eds. WB Saunders, Philadelphia, 1980, pp 1427-1499.

5. White PC, New MI, DuPont B. Congenital adrenal hyperplasia. N Engl J Med 316:1519-1524, 1987.

6. Blankstein J, Faiman G, Reyes FI, Schroeder ML, Winter JSD. Adult-onset familial adrenal 21-hydroxylase deficiency. Am J Med 68:441-448, 1980.

7. Levine LS et al. Cryptic 21-hydroxylase deficiency in families of patients with classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 51:1316-1324, 1980.

8. Page DC, Mosher R, Simpson EM, Fisher EMC, Mardon G, Pollack J, McGillivray B, de al Chappelle A, Brown LG. The sex-determining gene of the human Y chromosome encodes a finger protein. Cell 51:1091-1104, 1987.

9. Schultz FM, Wilson JD. Virilization of the wolffian duct in the rat fetus by various androgens. Endocrinology 94:979-986, 1974.

10. Donahoe PK, Cate RL, MacLaughlin DT, et al. Mullerian inhibiting substance: gene structure and mechanism of action of a fetal regressor. Recent Prog Horm Res 43:431, 1987.

11. New MI et al. Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 57:320-327, 1983.

12. Kowarski A, Finkelstein JW, Spaudling JS, Holman GH, Migeon CJ. Aldosterone secretion rate in congenital adrenal hyperplasia. A discussion on the theories on the pathogenesis of the salt-losing form of the syndrome. J Clin Invest 44:1505-1513, 1965.

13. Landau RL, Lugibihl K. Inhibition of the sodium-retaining influence of aldosterone by progesterone. J Clin Endocrinol 18:1237, 1958.

14. Jacobs DR, van der Poll J, Gabrilove JL, Soffer LJ. 17α-hydroxyprogesterone - a salt-losing steroid: relation to congenital adrenal hyperplasia. J. Clin. Endocrinol. 21:909, 1961.

15. Chrousos GP, Loriaux DL, Mann DL, Cutler GB. Late-onset 21-hydroxylase deficiency mimicking idiopathic hirsutism or polycystic ovarian disease. Ann Intern Med 96:143-148, 1982.

16. Birnbaum MD, Rose LI. Late-onset adrenocortical hydroxylase deficiencies assocaited with menstrual dysfunction. Obstet Gynecol 63:445-451, 1984.

17. Lee PA, Rosenwaks Z, Urban MD, Migeon CJ, Bias WD. Attenuated forms of congenital hyperplasia due to 21 hydroxylase deficiency. J Clin Endocrinol Metab 55:866-871, 1982.

18. Schiebinger RJ, Albertson BD, et al. The developmental changes in plasma adrenal androgens during infancy and adrenarche are associated with changing activities of adrenal microsomal 17-hydroxylase and 17,20-desmolase. J Clin Invest 67:1177-1182, 1981.

19. Migeon CJ, Rosenwaks Z, Lee PA, Urban MD, Bias WB. The attenuated form of congenital adrenal hyperplasia as an allelic form of 21-hydroxylase deficiency. J Clin Endocrinol Metab 51:647-649, 1980.

20. Kohn B, et al. Late-onset steroid 21-hydroxylase deficiency: A variant of classical congenital adrenal hyperplasia. J Clin Endocrinol Metab 55:817-827, 1982.

21. Levine LS, et al. Cryptic 21-hydroxylase deficiency in families of patients with classical congenital hyperplasia. J Clin Endocrinol Metab 51:1316-1324, 1980.

22. Levine LS, et al. Genetic and hormonal characterization of cryptic 21-hydroxylase deficiency. J Clin Endocrinol Metab 53:1193-1198, 1981.

23. Atherden SM, Barnes ND, Grant DB. Circadian variations in plasma 17-hydroxyprogesterone in patients with congenital adrenal hyperplasia. Arch Dis Child 47:602-604, 1972.

24. Fiet J, et al. Comparison of basal and adrenocorticotropin-stimulated plasma 21-deoxycortisol and 17-hydroxyprogesterone values as biological markers of late-onset adrenal hyperplasia. J Clin Endocrinol Metab 66:659-667, 1988.

25. New MI, et al. Genotyping steroid 21-hydroxylase deficiency: hormonal reference data. J Clin Endocrinol Metab 57:320-326, 1983.

26. Pang S, Wallace MA, et al. Worldwide experience in newborn screening for classicla congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 31:866-874, 1988.

27. DuPont B, Oberfield SE, Smithwick EM, Lee TD, Levine LS. Close genetic linkage between HLA and congenital adrenal hyperplasia. Lancet 2:1309-1311, 1977.

28. DuPont B, Virdis R, Lerner AJ, Nelson C, Pollack MS, New MI. Distinct HLA-B antigen associations for the salt wasing and simple virilizing forms of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. In Histocompatibility Testing, ED Albert, MP Baur, WR Mayr, eds. Springer-Verlag, Berlin, 1984, p 660.

29. Holler W, Scholz S, et al. Genetic differences between the salt wasting, simple virilizing, and nonclassical types of congenital adrenal hyperplasia. J Clin Endocrinol Metab 60:757-763, 1985.

30. Fleischnick E, Awdeh ZL, et al. Extended MHC haplotypes in 21-hydroxylase deficiency congenital adrenal hyperplasia: Shared genotypes in unrelated patients. Lancet 1:152-156, 1983.

31. Grosse-Wilde H, et al. HLA-A, B, C, DR typing and 17OHP determination for second trimestern prenatal diagnosis of 21 hydroxylase deficient CAH prenatal diagnosis. Prenatal Diagnosis 8:131-143, 1988.

32. Gueux B, et al. Prenatal diagnosis of 21-hydroxylase deficiency congenital adrenal hyperplasia by simultaneous radioimmunoassay of 21-deoxycortisol and 17-hydroxyprogesterone in amniotic fluid. J Clin Endocrinol Metab 66:534-537, 1988.

33. White PC, Chaplin DD, Weis JH, DuPont B, New MI, Seidman JG. Two steroid 21-hydroxylase genes are located in the murine S region. Nature 312:465-468, 1984.

34. Amor M, Tosi M, Duponchel C, Steinmetz M, Meo T. Liver mRNA probes disclose two cytochrome P-450 genes duplicated in tandem with the complement C4 loci of the mouse H-2S region. Proc Natl Acad Sci USA 82:4453-4457, 1985.

35. Parker KL, Chaplin DD, Wong M, Seidman JG, Smith JA, Schimmer BP. Expression of murine 21-hydroxylase in mouse adrenal glands and in transfected Y1 adrenocortical tumor cells. Proc Natl Acad Sci 82:7860-7864, 1985.

36. Chaplin DD, Galbraith LJ, Seidman JG, White PC, Parker KL. Nucleotide sequence analysis of murine 21-hydroxylase genes: Mutations affecting expression. Proc Natl Acad Sci USA 83:9601-9605, 1985.

37. White PC, New MI, DuPont B. HLA-linked congenital adrenal hyperplasia results from a defective gene encoding a cytochrome P-450 specific for steroid 21-hydroxylation. Proc Natl Acad Sci USA 81:7505-7509, 1984.

38. Carroll MC, Campbell RD, Porter RR. Mapping of steroid 21-hydroxylase genes adjacent to complement component C4 genes in HLA, the major histocompatibility locus in man. Proc Natl Acad Sci USA 82:521-525, 1985.

39. Higashi Y, Yoshioka H, Yamane M, Gotoh O, Fujii-Kuriyama Y. Complete nucleotide sequence of two steroid 21-hydroxylase genes tandemly arranged in human chromosome: A pseudogene and a genuine gene. Proc Natl Acad Sci USA 83:2841-2845, 1986.

40. Radding CM. Genetic recombination: Strand transfer and mismatch repair. Ann Rev Biochem 47:847, 1978.

41. Werkmeister JW, New MI, Dupont B, White PC. Frequent deletion and suplication of the steroid 21-hjydroxylase genes. Am J Hum Genet 39:461-469, 1986.

42. Schneider PM, Carroll MC, Alper CA, Rittner C, Whitehead AS, Yunis EJ, Colten HR. Polymorphism of the human complement C4 and steroid 21-hydroxylase genes. Restriction fragment length polymorphisms revealing structural deletions, homoduplications, and size variants. J Clin Invest 78:650-657, 1986.

43. Rumsby G, Carroll MC, Porter RR, Grant DB, Hjelm M. Deletion of the steroid 21-hydroxylase and complement C4 genes in congenital adrenal hyperplasia. J Med Genet 23:204-209, 1986.

44. Donohoue PA, van Dop C, McLean RH, White PC, Jospe N, Migeon CJ. Gene conversion in salt-losing congenital adrenal hyperplasia iwth absent complement C4B protein. J Clin Endocrinol Metab 62:995-1002, 1986.

45. Matteson KJ, Phillips JA III, Miller WL, Chung B-C, Orlando PJ, Frisch H, Ferrandez A, Burr IM. P450XXI (steroid 21-hydroxylase) gene deletions are not found in family studies of congenital adrenal hyperplasia. Proc Natl Acad Sci USA 84:585-5862, 1987.

46. Harada F, Kimura A, Iwanaga T, Shimozawa K, Yata J, Sasazuki T. Gene conversion-like events cause steroid 21-hydroxylase deficiency in congenital adrenal hyperplasia. Proc Natl Acad Sci USA 84:8091-8094, 1987.

47. White PC, Vitak A, Dupont B, New MI. Characterization of frequent deletions causing steroid 21-hydroxylase deficiency. Proc Natl Acad Sci USA 85:4436-4440, 1988.

48. Rumsby G, Fielder AHL, Hague WM, Honour JW. Heterogeneity in the gene locus for steroid 21-hydroxylase deficiency. J Med Genet 25:596-599, 1988.

49. Miller WL. Gene conversions, deletions, and polymorphisms in congenital adrenal hyperplasia. Am J Hum Genet 42:4-7, 1988.

50. Higashi Y, Tanae A, Inoue H, Fujii-Kuriyama Y. Evidence for frequent gene conversion in the steroid 21-hydroxylase P-450 (C21) gene: Implications for steroid 21-hydroxylase deficiency. Am J Hum Genet 42:17-25, 1988.

51. Morel Y, André J, et al. Rearrangements and point mutations of P450c 21 genes are distinguished by five restriction endonuclease haplotypes identified by a new probing strategy in 57 families with congenital adrenal hyperplasia. J Clin Invest 83:527-536, 1989.

52. Globerman H, Amor M, Parker KL, New MI, White PC. Nonsense mutation causing steroid 21-hydroxylase deficiency. J Clin Invest 82:139-144, 1988.

53. Amor M, Parker KL, Globerman H, New MI, White PC. Mutation in the CYP21B gene (Ile-172→Asn) causes steroid 21-hydroxylase deficiency. Proc Natl Acad Sci USA 85:1600-1604, 1988.

54. Rodrigues NR, Dunham I, Yu CY, Carroll MC, Porter RR, Campbell RD. Molecular characterization of the HLA-linked steroid 21-hydroxylase B gene from an individual with congenital adrenal hyperplasia. EMBO J 6:1653-1661, 1987.

55. Higashi Y, Tanae A, Inoue H, Hiromasa T, Fujii-Kariyama Y. Aberrant splicing and missense mutations cause steroid-21-hydroxylase [P450(21)]

deficiency in humans: possible gene conversion products. Proc Natl Acad Sci USA 85:7486-7490, 1988.

56. Zachman M, Tassinari D, and Prader A. Clinical and biochemical variability of congenital adrenal hyperplasia due to 11β-hydroxylase deficiency. A study of 25 patients. J Clin Endocrinol Metab 56:222-229, 1983.

57. Sizonenko P-C, Riondel A-M, Kohlberg IJ, Paunier L. 11β-hydroxylase deficiency: Steroid response to sodium restriction and ACTH stimulation. J Clin Endocrinol Metab 35:281-287, 1972.

58. Yanagibashi K, Haniu M, Shively JE, Shen WH, Hall P. The synthesis of aldosterone by the adrenal cortex. J Biol Chem 261:3556-3562, 1986.

59. Chua SC, Szabo P, Vitek A, Grzeschik K-H, John M, White PC. Cloning of cDNA encoding steroid 11β-hydroxylase (P450c11). Proc Natl Acad Sci USA 84:7193-7197, 1987.

60. Morohashi K, Yoshioka H, Gotoh O, Okada Y, Yamamoto K, Miyata T, Sogawa K, Fujii-Kuriyama Y, Omura T. Molecular cloning and nucleotide sequence of DNA of mitochondrial cytochrome P-450 (11β) of bovine adrenal cortex. J Biochem 102:559-568, 1987.

61. Kirita S, Morohashi K, Hashimoto T, Yoshioka H, Fujii-Kuriyama Y, Omura T. Expression of two kinds of cytochrome P-450 (11β) mRNA in bovine adrenal cortex. J Biochem 104:683-686, 1988.

62. Globerman H, Rösler A, Theodor R, New MI, White PC. An inherited defect in aldosterone biosynthesis caused by a mutation in or near the gene for steroid 11-hydroxylase. N Engl J Med 319:1193-1197, 1988.

63. Klingensmith GL, Garcia SC, et al. Glucocorticoid treatment of girls with congenital adrenal hyperplasia: Effects on height, sexual maturation and fertility. J Pediatr 6:996-1004, 1977.

64. Felig P, Baxter JD, Broadus AE, Frohman LA, eds. Endocrinology and Metabolism, McGraw-Hill, New York, 1987, p 138.

65. Bondy PK. Disorders of the adrenal cortex. In Williams Textbook of Endocrinology, JD Wilson, DW Foster, eds. WB Saunders, Philadelphia, 1985, p 816.