

# Disorders of Androgen Synthesis – from Cholesterol to Dehydroepiandrosterone

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## Key Words

P450c17 · Dehydroepiandrosterone · Polycystic ovary syndrome · Adrenarche · Steroidogenic acute regulatory protein · Steroids · P450 oxidoreductase · Hormones · Antley-Bixler syndrome · Congenital lipid adrenal hyperplasia

## Abstract

Androgens and estrogens are primarily made from dehydroepiandrosterone (DHEA), which is made from cholesterol via four steps. First, cholesterol enters the mitochondria with the assistance of the steroidogenic acute regulatory protein (StAR). Mutations in the StAR gene cause congenital lipid adrenal hyperplasia (lipoid CAH), a potentially lethal disease in which virtually no steroids are made. Lipoid CAH is common among Palestinian Arabs and people from eastern Arabia, and among Korean and Japanese people. Second, within the mitochondria, cholesterol is converted to pregnenolone by the cholesterol side chain cleavage enzyme, P450scc; disorder of this enzyme is very rare, probably due to embryonic lethality. Third, pregnenolone undergoes 17 $\alpha$ -hydroxylation by microsomal P450c17. 17 $\alpha$ -Hydroxylase deficiency, manifesting as female sexual infantilism and hypertension, is rare except in Brazil. Finally, 17-OH pregnenolone is converted to DHEA by the 17,20 lyase activity of P450c17. The ratio of the 17,20 lyase to 17 $\alpha$ -hydroxylase activity of P450c17 determines the ratio of

C21 to C19 steroids produced. This ratio is regulated posttranslationally by at least three factors: the abundance of the electron-donating protein P450 oxidoreductase (POR), the presence of cytochrome b<sub>5</sub> and the serine phosphorylation of P450c17. Mutations of POR are a new, recently described disorder manifesting as the Antley-Bixler skeletal dysplasia syndrome, and a form of polycystic ovary syndrome.

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## Steroid Hormones

Steroid hormones are essential for life and reproduction. There are several classes of steroid hormones, defined according to their physiologic actions and the receptors to which they bind. Mineralocorticoids instruct the kidney to retain sodium. In the absence of mineralocorticoids, the kidney discards Na<sup>+</sup> and retains K<sup>+</sup> and H<sup>+</sup>, leading to hyponatremia, hyperkalemia, acidosis, dehydration and death. Thus, mineralocorticoids play a central role in salt and water metabolism and in regulating blood pressure [1]. Glucocorticoids help to maintain euglycemia, mediate stress responses and regulate a variety of immune responses. Survival is possible in the absence of glucocorticoids (Addison's disease) until the time of severe physiologic stress (febrile illness, major surgery, hypovolemia) [2]. Three distinct classes of sex steroids are required for sexual differentiation and repro-

duction: *progestins* are required for normal menstrual cycles and for the maintenance of pregnancy; *estrogens* for female sexual development and reproduction; and *androgens* for male sexual differentiation in fetal life, male sexual development, and reproduction. While the individual can survive without sex steroids, their loss would eventually be lethal to the species. Thus, steroid hormones play a central, crucial and irreplaceable role in human life.

### **Mitochondria, Steroidogenic Acute Regulatory Protein and P450scc**

The first, rate-limiting and hormonally regulated step in the biosynthesis of all steroid hormones is the conversion of cholesterol to pregnenolone, described in the next paragraph. Steroidogenic cells do not store significant quantities of steroid hormones; hence steroid secretion is directly related to steroid synthesis. Steroid synthesis is regulated both acutely and chronically. The acute stimulation of steroidogenesis, as in the rapid rise in cortisol in response to intravenous administration of ACTH, is mediated at the level of cholesterol import into mitochondria, which is facilitated by the steroidogenic acute regulatory protein (StAR) [3, 4]. Chronic stimulation, over a period of hours to weeks, such as in the hypercortisolism of Cushing's disease, is mediated by increased transcription of the genes encoding the various steroidogenic enzymes. Thus, the acutely regulated step is the availability of substrate to, and the chronically regulated step is the amount of, those enzymes.

The conversion of cholesterol to pregnenolone is accomplished by the cleavage of the cholesterol side chain, catalyzed by a mitochondrial cytochrome P450 enzyme termed P450scc, where scc designates side chain cleavage. This single enzyme, encoded by a single gene [5, 6] (sometimes termed CYP11A) on chromosome 15q23–q24 [7], catalyzes three distinct sequential reactions on a single active site: cholesterol sequentially undergoes 20-hydroxylation, 22-hydroxylation, and scission of the 20,22 carbon-carbon bond to yield pregnenolone and isocaproaldehyde [8]. The expression of the gene for P450scc enables a cell to become steroidogenic (able to make steroids). The level of P450scc gene transcription is hormonally regulated by multiple pathways, permitting independent regulation of steroidogenesis in multiple cell types [9–11], and the amount of P450scc mRNA present in a cell directly correlates with its steroidogenic capacity [12, 13]. Each of the three reactions catalyzed by P450scc requires a pair of

electrons. A flavoprotein (ferredoxin reductase) receives the electrons from NADPH, then passes them to an iron/sulfur protein (ferredoxin), which in turn passes them to the P450. Ferredoxin reductase and ferredoxin are generic electron transfer proteins that can donate electrons to any of the various mitochondrial P450 enzymes [14–17]. P450scc can only function within the mitochondria [18]; hence, delivery of cholesterol to the inner mitochondrial membrane by StAR is a crucial step in steroidogenesis.

Early work showed that ACTH could induce adrenal steroidogenesis very rapidly, and that this induction could be inhibited by cycloheximide or other inhibitors of protein synthesis [19–22], suggesting that a short-lived protein was an essential trigger of the acute response. The responsible protein, StAR, was first identified in rat adrenals and corpus luteum as a phosphoprotein, seen on 2-dimensional gels that appeared rapidly when cells were stimulated with cAMP [23–25]. Stocco's laboratory then cloned the mouse cDNA and found that expression of StAR in mouse Leydig MA-10 cells increased steroidogenesis 6-fold [26]. StAR similarly increased steroidogenesis in COS-1 cells cotransfected with the P450scc system [27, 28], but the definitive proof that StAR is essential in human physiology came from finding StAR mutations in congenital lipid adrenal hyperplasia (lipoid CAH) [27, 29].

### **Congenital Lipoid Adrenal Hyperplasia**

Lipoid CAH is a rare autosomal recessive disorder that severely disrupts the synthesis of all adrenal and gonadal steroids [4]. A severe defect in fetal testicular biosynthesis is evident because affected 46,XY genetic males are born with wholly female external genitalia, reflecting an absence of testosterone synthesis between 6 and 12 weeks of gestation. At birth, the adrenals are engorged with cholesterol ester deposits. Affected newborns have low but measurable levels of steroid hormones. They soon die from glucocorticoid and mineralocorticoid deficiency if hormonal replacement therapy is not initiated, but properly treated patients can survive to adulthood [30, 31].

Although StAR is needed for a rapid and maximal steroidogenic response, there are also low levels of StAR-independent steroidogenesis in cells that express StAR [27, 32]. However, some steroidogenic cells, such as those of the placenta [28], do not express StAR. The brain also expresses low levels of StAR in apparently steroidogenic cells that also contain P450scc [33–35], but the role of StAR in the brain is not clear, as there is no known tropic

**Table 1.** Lipoid CAH in Palestinians

Nucleotide	Amino acid	Families	Alleles	Countries of origin
A632G	E169G	1	1	Israel
G671T	R182L	4	7	Denmark, Jordan
ΔC650	frameshift	1	2	Kuwait
Δ2T593	frameshift	2	3	Jordan
C703T	R193X	1	2	Israel

Seven patients have been reported from 6 families, 1 of which was consanguineous, thus representing 11 unique alleles. Several alleles carried more than 1 mutation, so that the total number of affected alleles is greater than 11. Data are from Bose et al. [32, 43].

stimulus to neurosteroidogenesis [36], and a specific CNS defect in StAR-deficient patients with lipoid CAH has not been reported [36]. The demonstration of StAR-independent steroidogenesis led to formulation of the two-hit model of lipoid CAH [32]. The first hit is the mutation in the StAR gene, ablating StAR-dependent steroidogenesis but permitting StAR-independent steroidogenesis to persist. StAR-independent placental steroidogenesis and placental synthesis of progesterone remain normal, permitting term gestation. Low levels of adrenal StAR-independent steroidogenesis account for the low, but detectable, levels of steroid hormones seen in the sera of lipoid CAH patients in the first month of life [31, 32]. The presence of these steroids appears to explain why untreated lipoid CAH infants may survive without treatment for several months [31, 32, 37, 45], whereas patients with other forms of salt-wasting CAH do not. However, these steroid hormone concentrations are too low to suppress secretion of ACTH, gonadotropins and angiotensin II to normal levels. Excess amounts of these tropic hormones stimulate cellular uptake of low-density lipoprotein cholesterol and increased production of cholesterol from acetate. This tropic hormone stimulation results in the accumulation of cholesterol esters, which eventually disrupts the cells, either via physical engorgement of the cell with droplets of cholesterol esters or by a chemical action of cholesterol oxidation products, or both. This second hit thus destroys the low levels of StAR-independent steroidogenesis, leading to the unmeasurable levels of steroid in the serum of older children with lipoid CAH, and to the absence of circulating testosterone in affected 46,XY fetuses [4, 32].

The fetal ovaries do not express the genes for the steroidogenic enzymes and thus do not make steroids [38]; unlike the testes and adrenals, the ovaries only start to

make steroid hormones at the onset of puberty. Consequently, the ovaries of 46,XX females affected with lipoid CAH do not receive the second hit until the onset of puberty, when LH stimulates low levels of StAR-independent steroidogenesis. With each monthly cycle, another follicle is recruited and stimulated by gonadotropins, producing spontaneous age-appropriate breast development in affected 46,XX individuals. However, gonadotropin stimulation quickly results in cholesterol engorgement of these cells (the second hit in lipoid CAH) so that the later phase of ovarian steroidogenesis, the secretion of large amounts of progesterone, does not occur [39, 40]. Follicles that are not recruited remain unstimulated and constitute a reservoir of steroidogenic cells undamaged by the second hit of lipoid CAH, so that with each monthly cycle a new undamaged follicle is recruited. Estrogen is then produced, leading to cyclic uterine estrogen withdrawal bleeding that resembles normal menses, but, as there is no progesterone, these cycles are anovulatory. The predictions of the two-hit model have recently been confirmed by elegant experiments with StAR knockout mice [41, 42].

StAR mutations causing lipoid CAH have been described in about 70 patients from all over the world. Lipoid CAH is most common in Japan and Korea [27, 32, 37, 43, 44]. The mutation Q258X accounts for about 70% of affected alleles in Japan and 95% of the alleles reported to date from Korea. A second population in whom lipoid CAH appears to be common is Palestinian Arabs, among whom 7 cases have been reported to date. However, several different mutations are found in these patients, suggesting founder effects in limited gene pools, and consanguinity (table 1). A third group in whom lipoid CAH appears to be common is Arabs from the Eastern Province of Saudi Arabia and nearby Qatar, all of whom carry the mutation R182H, suggesting a founder effect [45].

### True P450scc Deficiency

When lipoid CAH was first described, long before the discovery of StAR, it was generally termed '20,22 desmolase deficiency' because it was thought to be a disorder in the enzyme (P450scc) that converts cholesterol to pregnenolone [31]. However, analysis of the gene for P450scc was normal in these patients [46, 47]. Finding StAR mutations in virtually all patients with the lipoid CAH phenotype thus posed the question: 'Why do we not see mutations in P450scc, as we do in all other steroidogenic

enzymes?' As P450scc is required for placental steroidogenesis, and placental progesterone is required to suppress uterine contractility to maintain pregnancy, we inferred that absence of P450scc and placental steroidogenesis would lead to spontaneous abortion, so that P450scc deficiency would result in embryonic lethality [48]. This view appears to be consistent with two recent reports of the lipid CAH phenotype in patients with partial deficiency of P450scc. One patient carried a *de novo* heterozygous mutation not found in either parent [49]; the other was a compound heterozygote, but one of the 'mutations' was simply a polymorphism having no impact on P450scc catalysis [50]. In these cases, the lipid CAH phenotype can again be attributed to a modification of the two-hit model of lipid CAH [49]. Thus, unusual patients provide definitive tests of models formulated from observations from experiments *in vitro*.

### The Biochemistry of StAR

StAR is a most unusual protein. It is initially synthesized as a 37-kDa protein that is cleaved to a 30-kDa form when it is imported into mitochondria. Cloning studies and analyses of DNA databases show that StAR is the first member of a new class of proteins that have so-called START (StAR-related lipid transfer) domains, which are structurally related to residues 62–285 of StAR [51–53]. The X-ray crystal structure of two of these proteins, N-218 MLN64 [54] and StarD4 [55], has been determined, showing that each has a helix-grip fold encompassing a hydrophobic pocket that appears to be able to bind a single molecule of cholesterol. While StAR/cholesterol binding curves also indicate that StAR binds a single cholesterol molecule [54], experiments with StAR-induced lipid transfer between liposomes show that each molecule of StAR can transfer several cholesterol molecules *in vitro* [56, 57] and experiments *in vivo* suggest that each molecule of StAR can promote the movement of hundreds of molecules of cholesterol from the outer mitochondrial membrane (OMM) to the inner mitochondrial membrane (IMM) [58]. This fostered the view that StAR functions in the mitochondrial intramembranous space (IMS) to shuttle cholesterol from OMM to IMM. Furthermore, the cleavage of the 37-kDa StAR to the 30-kDa StAR, similarly to other mitochondrial proteins, led to the mistaken notion that the 37-kDa form is a 'precursor' and the 30-kDa protein is the 'mature' form. However, it is now clear that StAR's activity is determined by its cellular location, and not by its proteolytic cleavage status. Hence the 37-

kDa form is 'mature' (i.e. active) and the 30-kDa form represents the first step in the intramitochondrial degradation of StAR [59]. Deleting the mitochondrial leader peptide prevents StAR from entering the mitochondria and confines it to the cytoplasm; yet this N-62 StAR molecule remains fully active [60], and is also able to transfer cholesterol to other membranes, such as the endoplasmic reticulum [61]. Thus, it appeared that StAR's mitochondrial leader peptide directed StAR's activity to the mitochondria and was confined to that organelle, preventing it from transferring cholesterol to other membranes, and that StAR's activity was confined to the OMM [62]. The amino-terminal domain of StAR (residues 63–193) is protease resistant, suggesting tight protein folding [63]. This would presumably slow mitochondrial import, prolonging contact with the OMM, and increasing activity. N-62 StAR undergoes a conformational change in acidic environments, both in solution and in synthetic membranes [63, 64]. This conformational change appears to foster association with the OMM, facilitating binding of OMM cholesterol for transfer to the IMM. However, until recently this view had been controversial.

Using novel fusion proteins that affix StAR to the OMM, confine it to the IMS, or affix it to the IMM, we have now proven that StAR acts exclusively on the OMM, and is wholly inactive in all other mitochondrial compartments [65]. By fusing StAR to a protein (Tom 20) that is a component of the OMM, we confined StAR to the cytoplasmic side of the OMM and prevented its mitochondrial entry, resulting in a dramatic increase in activity. This suggests that the level of StAR activity is proportional to the time it resides on the OMM. To test this, we built variants of StAR with altered mitochondrial leaders designed to either slow or speed its mitochondrial entry. Constructs designed for slow entry increased activity both in intact cells and in isolated mitochondria; constructs designed to speed entry similarly decreased activity. Using an *in vitro* cell-free transcription/translation system and *in vitro* assays of the kinetics of mitochondrial protein import, we could show that each construct either increased or decreased the speed of mitochondrial protein entry, as designed, and that the level of StAR activity was inversely proportional to its speed of entry [65]. Recent experiments have shown that only the carboxyl-terminal  $\alpha$ -helix of StAR interacts with the OMM [65a]. Thus, StAR acts exclusively on the OMM to facilitate the transfer of many molecules of cholesterol to the IMM. StAR then is imported into the mitochondria and cleaved to the 30-kDa form. This 30-kDa StAR protein is inactive, not because of the proteolytic



cleavage but because of its location in the mitochondrial matrix from where it cannot reach the cytoplasmic aspect of the OMM [65].

### **P450c17 – The Qualitative Regulator of Steroidogenesis**

Whereas the StAR/P450scc system is the rate-limiting and hormonally regulated step, and hence is the quantitative regulator of steroidogenesis, P450c17 is the qualitative regulator that determines which class of steroids that would be produced [8]. When P450c17 is absent, such as in the adrenal zona glomerulosa, the products are C21 (21-carbon) 17-deoxysteroids, such as aldosterone. When the 17 $\alpha$ -hydroxylase and 17,20 lyase activities of P450c17 are present, C19 precursors of sex steroids are produced. Because all steroid hormones are made from the pregnenolone produced by P450scc, the presence or absence of each of the activities of P450c17 directs this pregnenolone toward its final metabolic pathway. While all cytochrome P450 enzymes can catalyze multiple reactions on a single active site, P450c17 is the only one described to date in which these multiple activities are differentially regulated by a physiologic process. Unlike P450scc, which is found in mitochondria, P450c17 is found in the endoplasmic reticulum. These ‘microsomal’ P450 enzymes, which include all of the drug-metabolizing hepatic cytochrome P450 enzymes, receive electrons from NADPH via a flavoprotein intermediate called P450 oxidoreductase (POR).

### **P450c17 – One Enzyme with Two Activities**

Until the 1980s, observations of human physiology led to the mistaken belief that 17 $\alpha$ -hydroxylase and 17,20 lyase were separate enzymes, regulated independently. The adrenals of young children make very little dehydroepiandrosterone (DHEA) until the onset of adrenarche and the development of the adrenal zona reticularis. Adrenarche begins at about age 6–8, is independent of the gonads or gonadotropins, and continues after the end of puberty, since serum DHEA and DHEA-S concentrations are highest between ages 25 and 30 [66–68]. This profound increase in adrenal DHEA synthesis proceeds without changes in the serum concentrations of cortisol and ACTH [66], and thus appears to involve selective induction of 17,20 lyase activity, while 17 $\alpha$ -hydroxylase activity, as indicated by the age-independent cortisol con-

centrations, remains essentially unchanged. This phenomenon, adrenarche, is difficult to study, because it only occurs in large old-world primates [69, 70]. Searches for a hormonal trigger for adrenarche have been unsuccessful [71–73]. Early reports of familial, apparently autosomal recessive isolated 17,20 lyase deficiency, in which the patients had normal cortisol values and hence normal 17 $\alpha$ -hydroxylase activity [74–76], appeared to confirm that 17 $\alpha$ -hydroxylase and 17,20 lyase were separate enzymes encoded by separate genes. However, biochemical studies with the pig [77–80] and guinea pig [81, 82] showed that a single protein, cytochrome P450c17, catalyzed both reactions on single active site, and nonsteroidogenic cells transfected with vectors that expressed bovine [83] or human [84] P450c17 acquired both 17 $\alpha$ -hydroxylase and 17,20 lyase activities. Accordingly, genetic studies showed that there was a single species of P450c17 mRNA that was identical in the human adrenal and gonad [85], because it was encoded by a single-copy gene [86] (now termed CYP17) on chromosome 10q24.3 [7, 87, 88]. Thus, although the transcriptional regulation of human P450c17 is of central importance [89–91], to regulate the amount of P450c17 present, the differential regulation of the two activities of P450c17 must lie at one or more points downstream from its gene. All present data now indicate that this regulation is mediated by several factors, all of which influence electron flow from NADPH via POR to P450c17 [92].

### **Role of Redox Partners in Regulating 17,20 Lyase Activity**

Hall was first to note that POR is much more abundant in the porcine testis, which has a high ratio of lyase to hydroxylase activity, than in the porcine adrenal, which has low lyase activity [93, 94]. Combining these proteins in vitro showed that POR was needed for both activities, and had suggested that cytochrome b<sub>5</sub> could selectively augment the 17,20 lyase reaction [93–95]. It had generally been thought that cytochrome b<sub>5</sub> can serve as an alternative electron transfer protein, taking the place of POR in certain circumstances [96, 97]. Transfection of nonsteroidogenic monkey kidney COS-1 cells with a vector expressing human P450c17 permits the cells to catalyze 17,20 lyase activity that converts 17-OH pregnenolone to DHEA, but not 17-OH progesterone to androstenedione [84]. When these cells are cotransfected with a vector expressing human POR, conversion of 17-OH pregnenolone to DHEA increases, but 17-OH progester-

one is not converted to androstenedione [98]. Thus, qualitative data obtained in whole living cells indicated that human P450c17 catalyzes 17,20 lyase activity only with  $\Delta^5$  substrates, and that this activity could be augmented by excess POR.

To understand the enzymology of P450c17 in detail, we utilized yeast systems in which the endogenous yeast genes for POR or b<sub>5</sub> were disrupted, permitting rigorous kinetic analysis of the role of each potential redox partner on each of the four potential reactions of human P450c17 [99]. These data confirm that POR is required for both 17 $\alpha$ -hydroxylation and 17,20 lyase activity and that the 17,20 lyase reaction is much more severely impeded by low concentrations of POR than is the 17 $\alpha$ -hydroxylase reaction. Cytochrome b<sub>5</sub> selectively augments 17,20 lyase activity when POR is present, but b<sub>5</sub> alone cannot support catalysis. Apo-b<sub>5</sub>, which is the cytochrome protein without its heme group, is as effective at promoting 17,20 lyase activity as holo-b<sub>5</sub> which has the heme group, indicating that b<sub>5</sub> augments the 17,20 lyase activity of P450c17 as an allosteric facilitator, and not as an electron donor. The expression of b<sub>5</sub> in the adrenal is largely confined to the zona reticularis [100, 101] and this expression increases during adrenarche [102], so that adrenal levels of b<sub>5</sub> correlate with DHEA secretion. Surprisingly, 17-OH progesterone is a poor substrate for human P450c17. The K<sub>m</sub> of the 17,20 lyase reaction is 10 times higher, and the V<sub>max</sub> is reduced to 30% when  $\Delta^4$  17-OH progesterone is the substrate compared to the values with  $\Delta^5$  17-OH progesterone. Thus, the 17,20 lyase action of human P450c17 is about 30-fold greater with  $\Delta^5$  substrates than with  $\Delta^4$  substrates. As a result, most androstenedione is produced by the action of 3 $\beta$ -hydroxysteroid dehydrogenase on DHEA, and only minimal quantities derive from 17-OH progesterone, so that most human sex steroid production is from DHEA.

### Isolated 17,20 Lyase Deficiency

Several patients with apparent isolated 17,20 lyase deficiency were described in the 1970s and 1980s, but the demonstration that a single enzyme encoded by a single gene catalyzed both 17 $\alpha$ -hydroxylase and 17,20 lyase activities put the existence of such a disease in doubt. Further questions were raised when Waterman's group showed that a patient with apparent isolated 17,20 lyase deficiency in fact had P450c17 mutations that caused complete 17 $\alpha$ -hydroxylase deficiency [103]. No genetically and enzymologically proven cases of isolated 17,20

lyase deficiency had been reported until 1997, when we studied two patients from rural Brazil who had clinical and hormonal findings consistent with isolated 17,20 lyase deficiency [104].

DNA sequencing showed that each was homozygous for a different mutation; in one patient, the arginine at position 347 was replaced with histidine (R347H) and, in the other patient, the arginine at position 358 was replaced with glutamine (R358Q). Transfection of COS-1 cells with vectors expressing these P450c17 mutants showed that each retained only about 5% of normal 17,20 lyase activity but retained about 65% of normal 17 $\alpha$ -hydroxylase activity. Despite this loss of lyase activity, 17-OH pregnenolone acted as a strictly competitive inhibitor of the hydroxylase reaction, showing that these mutations did not affect the active site [105]. However, the 17,20 lyase activity of these mutants could be partially restored by adding excess b<sub>5</sub>, suggesting that they affect binding of redox partners and consequent electron transfer [105]. Molecular modeling of P450c17 based on the known X-ray crystallographic structures of several bacterial cytochrome P450 enzymes showed that the two mutations altered the distribution of electrostatic charge on the surface of the P450c17 protein in the region that interacts with POR [106]. Thus, changing a single positive charge to neutral or to a negative charge partially decreased the ability of P450c17 to interact with POR, so that 17 $\alpha$ -hydroxylation was relatively unaffected, but 17,20 lyase activity was severely disrupted, in fashion very analogous to the persistence of 17 $\alpha$ -hydroxylase activity but the relative absence of 17,20 lyase activity when the concentrations of POR are low. Thus, isolated 17,20 lyase deficiency is an important 'site-directed mutagenesis experiment of nature' that identifies redox partner interactions as crucial regulators of 17,20 lyase activity.

### POR Deficiency

A rare variant of 17,20 lyase deficiency seen in association with partial deficiency of steroid 21-hydroxylase was first described in 1985 by Peterson et al. [107] and is now understood in detail [108]. Mutations in the genes for P450c17 and P450c21 (steroid 21-hydroxylase) were sought but not found. In 1986, we suggested that this disorder was a deficiency of POR [109], but this seemed unlikely as POR knockout mice do not survive past the middle of fetal development [110, 111]. Furthermore, most children with apparent combined 17 $\alpha$ -hydroxylase and 21-hydroxylase deficiencies also have the Antley-Bixler

skeletal malformation syndrome (ABS), consisting of craniosynostosis, radioulnar or radiohumeral synostosis, and mid-face hypoplasia, and half also have genital anomalies. About half of the children with ABS have mutations in fibroblast growth factor receptor 2 (FGFR2), as do most patients with the Apert, Crouzon, Jackson-Weiss and Pfeiffer craniosynostosis syndromes, but the ABS patients who have FGFR2 mutations do not have abnormal steroidogenesis, and those who lack FGFR2 mutations have abnormal steroidogenesis [112]. The availability of the human POR gene sequence provided by the human genome project permitted the examination of the POR gene in this disorder. We found POR mutations in 3 children with ABS and abnormal steroidogenesis, and also in an adult woman with amenorrhea, hypertension, cystic ovaries and hormonal findings suggesting partial combined 17 $\alpha$ -hydroxylase and 21-hydroxylase deficiencies [113]. We found five different POR missense mutations, and tested the ability of each to receive electrons from NADPH and to donate them to both a test substrate (cytochrome c) and a real substrate (P450c17). There was substantially better phenotype/genotype correlation with the P450c17 assays than with the cytochrome c assays [113]. This indicates that POR mutants will donate electrons to different substrates with different efficiencies; thus, a POR mutant may severely affect some P450 enzymes but not others. As POR donates electrons to hepatic drug-metabolizing P450 enzymes, minor POR sequence variants may contribute to genetic variations in drug metabolism. The similarity between the hormonal phenotypes in POR deficiency and mutations in the POR (redox partner) binding site of P450c17 further underscores the crucial role of electron donation in regulating 17,20 lyase activity and hence regulating androgen synthesis. We recently reported a large series of 32 such patients, confirming and extending these observations [113a].

### **Serine Phosphorylation of P450 Increases 17,20 Lyase Activity**

Radiolabeling studies of COS-1 cells transfected with a human P450c17 expression vector or of untransfected human adrenal NCI-H295 cells [114], which express all human steroidogenic enzymes [115], showed that human P450c17 is a phosphoprotein [116]. This phosphorylation is rapidly induced by cAMP, occurs on serine and threonine but not on tyrosine residues, and increases the 17,20 lyase activity of P450c17. Conversely, dephosphor-

ylation of microsomes containing P450c17 ablates 17,20 lyase activity without reducing 17 $\alpha$ -hydroxylase activity and without affecting steroid binding to the enzyme. The mechanism by which serine phosphorylation promotes 17,20 lyase activity is not yet known. Phosphorylated serine residues near the redox partner binding site may increase the affinity of P450c17 for POR or b<sub>5</sub> (or both), thus effectively increasing the flow of electrons to the P450. The kinase responsible for P450c17 serine phosphorylation has not been identified yet, but recent data indicate that the degree of phosphorylation in vivo is regulated by the counterbalancing actions of the kinase and protein phosphatase 2A, which in turn is regulated by phosphoprotein SET [117].

### **Serine Phosphorylation, Adrenarche and the Polycystic Ovary Syndrome**

The polycystic ovary syndrome (PCOS) is a heterogeneous disorder characterized by hirsutism, virilism, hyperandrogenism, menstrual irregularities, chronic anovulation, obesity, insulin resistance, acanthosis nigricans, high concentrations of LH and ovarian cysts. Hyperandrogenism and insulin resistance appear to be primary lesions, and the other findings are secondary events [118]. The hyperandrogenism in women with PCOS is of both ovarian and adrenal origin [119–122]. The adrenal hyperandrogenism of PCOS resembles an exaggerated form of adrenarche, and girls with premature adrenarche are more likely to develop PCOS [123, 124]. A gain-of-function disorder in the pathway leading to the serine/threonine phosphorylation of P450c17 could account for such increases in both adrenal and ovarian androgen secretion and an earlier age of adrenarche [116], but such mutations have not yet been reported. The hyperinsulinism and insulin resistance of PCOS is at the level of insulin receptor signal transduction [125, 126]. Serine phosphorylation of the  $\beta$  chain of the insulin receptor interferes with the tyrosine phosphorylation of the receptor that normally follows binding of insulin [127–129]. Furthermore, some PCOS women appear to have insulin receptors in their fibroblasts that are hyperphosphorylated [130]. Thus, a gain-of-function mutation in a serine-threonine kinase or its signal transduction pathway might increase the serine hyperphosphorylation of both P450c17 and the  $\beta$  chain of the insulin receptor, thus accounting for both the hyperandrogenism and insulin resistance of PCOS with a single molecular lesion.

## Conclusions

Defects in the synthesis of DHEA cause deficiencies in the synthesis of androgens and estrogens. In the first stage, mutations in the StAR gene cause potentially lethal lipid CAH. Defects in the cholesterol side chain cleavage enzyme, the second step, are rare. Defects in the third

stage, 17-hydroxylation lead to hypertension and female sexual infantilism. Defects in the final stage of DHEA synthesis, 17,20 lyase activity, are due to disorders of electron transfer to P450c17, including mutations in POR. Of these, the defect in StAR is common among Palestinian Arabs and in Eastern Arabia.

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