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Defects in Steroidogenic Enzymes. Discrepancies Between Clinical Steroid Research and Molecular Biology Results*

M. Zachmann

Department of Pediatrics, University of Zurich, Kinderspital, CH-8032 Zurich, Switzerland

Molecular biology has clarified the understanding of steroidogenic enzyme genetics. Nevertheless, there are discrepancies between fundamental and clinical experience. (1) Why do patients with "pure" 17α-hydroxylase or 17,20-desmolase deficiency exist, when one cytochrome regulates both steps? A case of interest is discussed, who had "pure" 17,20-desmolase deficiency until adolescence, but additional 17α-hydroxylase deficiency thereafter. (2) In 11β-hydroxylase deficiency, it was puzzling to find 18-hydroxylated compounds, and, in isolated hypoaldosteronism, normal cortisol, since 11β- and 18-hydroxylation were thought to be regulated together. This has now been explained by differences in the fasciculata and glomerulosa. The occurrence of 11β-hydroxylase deficiency of 17-hydroxylated steroids only, however, remains enigmatic. (3) 3β-Hydroxysteroid dehydrogenase deficiency does not only seem to exist in classic (mutations of type II gene), but also in late-onset cases. In them, no molecular basis could be found. (4) Also, in cholesterol side-chain cleavage, there is an inequity: while evidently one cytochrome regulates 20- and 22-hydroxylation, pregnenolone is formed when 20αOH-cholesterol, but not when cholesterol, is added to adrenal tissue of deficient patients. Other factors (promoters, fusion proteins, adrenodoxin, cAMP-dependent expression of genes, and/or proteases), or hormonal replacement in patients may be responsible for these discrepancies.

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INTRODUCTION

Understanding of the genetics and regulation of steroidogenic enzymes and of their disorders has made much progress as a consequence of new developments in molecular biology. Nevertheless, there are some discrepancies between newly acquired fundamental knowledge and older clinical experience, which can at present not be completely understood.

In the following, selected examples are discussed to illustrate some apparent differences between clinical observation and molecular findings.

17α-HYDROXYLASE AND 17,20-DESMOLASE (OR 17,20-LYASE) DEFICIENCIES

Classic deficiency of 17α-hydroxylase originally described in adults [1] blocks the biosynthesis of cortisol, androgens and estrogens. Among steroids not hydroxylated in position 17, DOC and its 18-hydroxylated metabolites may cause hypertension. Deficiency of 17,20-desmolase [2] allows efficient 17-hydroxylation, but prevents the side-chain cleavage of 17αOH-pregnenolone and/or 17αOH-progesterone. Androgens and estrogens are lacking, whereas cortisol and aldosterone are normal. Male pseudohermaphroditism (XY-individuals) or absent pubertal development (XX-individuals) are the only symptoms [2–8].

In spite of these two clearly separate clinical entities, biochemical studies with 17-(bromoacetoxy) progesterone already performed years ago suggested that a cytochrome *P*450 from testicular microsomes consisted of a single protein with both enzymatic activities [9]. Estradiol-17β was also found to inhibit both activities [10]. Later, it was shown that most mature cytochromes are derived from a precursor with a higher mass [11]. The enzyme was active with Δ4 and Δ5 substrates as a 17α-hydroxylase and with the corresponding 17α-hydroxysteroids as a 17,20-desmolase. It was, however, observed that the activities were not

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evenly distributed, but that e.g. the 17,20-desmolase activity was largely suppressed in the microsomes.

Kinetic comparison of 17α-hydroxylase and 17,20-desmolase activities have been carried out in human adrenal microsomes [12]. The median maximum velocity of the 17α-hydroxylase was significantly greater than that of the 17,20-desmolase. Most steroids with the exception of cortisol competitively inhibited both enzyme activities. The inhibition constant was, however, always higher for the 17α-hydroxylase than for the 17,20-desmolase activity. It thus became evident that both, steroid 17α-hydroxylase and 17,20-desmolase activities reside within the same polypeptide chain, but that there are still some differences of reaction. Various genetic causes have thereafter been discovered as molecular bases for the defects [13–16].

The questions clinicians ask in this context are: why do clinical cases with "pure" 17α-hydroxylase [1] or "pure" 17,20-desmolase deficiency [2] exist separately within separate families, when a single cytochrome regulates both steps? Why do combined cases not always occur [17, 18]? What are the factors that lead to the clinical and biochemical picture of 17α-hydroxylase deficiency in one, and to that of 17,20-desmolase in another patient? Does one of the two defects remain unchanged during the whole life of a patient, or is there a developmental aspect to it?

A patient of interest in this context, whose data have been presented [19], has to be briefly discussed. This XY-individual with female external genitalia and compound heterozygous mutations [16] was followed from adolescence to adulthood. While up to the age of 16 years her 17α-hydroxylating capacity was efficient, and steroids not hydroxylated in position 17 were low, drastic steroid changes were observed thereafter. Simultaneously, the previously normal blood pressure increased. While conventional antihypertensives had no effect, dexamethasone normalized the pressure. The only iatrogenic difference after age 17 years was that estrogen and gestagen replacement at low doses had been started. The changes of integrated values of urinary steroids are shown in Table 1. The 17α-hydroxylated compounds (cortisol metabolites) decreased drastically, while compounds not hydroxylated in position 17 increased. The high quantities of compounds not hydroxylated in position 17, however, remained readily dexamethasone suppressible.

Table 1. Urinary steroids (μmol/d) in a patient with 17,20-desmolase/17α-hydroxylase deficiency from adolescence to adulthood

Age (yr)	Cortisol metabolites	Pregnanediol	THB + allo-THB
16.9	13.1	6.1	1.9
20.7	5.0	7.7	25.5
25.2	<0.1	26.5	76.8

From this observation we conclude that, in the same individual, 17α-hydroxylase activity was normal throughout childhood and adolescence, but decreased in adulthood under estrogen supplementation. 17,20-desmolase activity, by contrast, was already completely absent in the fetus, leading to complete absence of masculinization. This case thus demonstrates that previously pure 17,20-desmolase deficiency can with time be complicated by additional 17α-hydroxylase deficiency. The factors leading to this change are unknown. A modifying effect of estrogen replacement on the cytochrome *P*450 C17α-activity is, however, a possibility. Also insulin inhibits adrenal 17α-hydroxylase activity in man [20], but there is no evidence in our patient that there might have been newly acquired insulin hypersecretion. It is thus conceivable that, for unknown reasons, fetuses, infants, and children with a deficiency of cytochrome *P*450 C17α present the signs of "pure" 17,20-desmolase deficiency, while adults show the signs of the combined defect. This might be the reason why we as pediatric endocrinologists have originally described patients with 17,20-desmolase deficiency [2], while 17α-hydroxylase deficiency (which in retrospect rather has to be regarded as "combined" deficiency) was first described in adults [1].

11β- AND 18-HYDROXYLASE DEFICIENCIES

Steroid 11β-hydroxylase deficiency is, after 21-hydroxylase deficiency, the second most common cause of congenital adrenal hyperplasia. The conversion of 11-deoxycortisol (compound S) to cortisol, and that of DOC to corticosterone is blocked. In addition to androgen excess similar to that in 21-hydroxylase deficiency, hypertension may develop because of the hypersecretion of DOC, and salt-wasting is usually absent. 18-Hydroxylase deficiency has been postulated to be a cause of isolated hypoaldosteronism, where the formations of cortisol and corticosterone are normal.

The enzyme is encoded by the CYP11B1 gene on chromosome 8q22. Two mutations in CYP11B1 have previously been reported in patients with the defect (Arg-448→His and a 2 bp insertion in codon 394). In Jews of Morocco and in corticosterone methyl-oxidase (CMO) II deficiency in Jews of Iran, steroid 11β-hydroxylase has been found to be encoded by two homologous genes, CYP11B1 and CYP11B2. CYP11B1 encodes a specific cytochrome necessary for cortisol biosynthesis, with predominantly 11β-hydroxylase and moderate 18-hydroxylase activity, whereas CYP11B2 encodes another isozyme necessary for the biosynthesis of aldosterone with 11β-hydroxylase, 18-hydroxylase and 18-oxidase activities [21].

CYP11B2 has been shown to be the same gene as that for CMO type I and II [22]. Molecular genetic analysis of CYP11B2 of three patients affected with CMO I deficiency has revealed that deletion of 5 nucleotides occurs exclusively in exon 1, resulting in a frameshift

to form a stop codon in the same exon. Thus, *P*450 C18 is not produced, causing a complete lack of aldosterone biosynthesis in the patients. Additional, previously uncharacterized mutations causing 11β-hydroxylase deficiency have been reported by others [23, 24]. Some are point mutations (nonsense and missense), and one is a single base pair deletion causing a frameshift.

It has originally been puzzling for clinicians to find 18-hydroxylated compounds in patients with congenital adrenal hyperplasia due to 11β-hydroxylase deficiency, and conversely in isolated hypoaldosteronism to find normal cortisol formation, since 11β- and 18-hydroxylation were thought to be regulated together [25]. This problem has been partly solved by the observation of different regulations in the zona fasciculata and glomerulosa of the adrenal cortex: it is now known that, in the rat, there are two distinct species of cytochrome *P*450 C11β/18, aldosterone synthase and 11β-hydroxylase. Whereas aldosterone synthase is located exclusively in the zona glomerulosa, the distribution and site of production of 11β-hydroxylase is not entirely clear [26, 27]. Four forms of rat CYP11B genes were isolated and characterized [28], which were named CYP11B1,-B2,-B3, and -B4. Genomic Southern analyses indicated that the members of the rat CYP11B gene subfamily were confined to these four genes. Among them, CYP11B1 and -B2 encoded steroid 11β-hydroxylase and aldosterone synthase, respectively, while CYP11B3 was a gene homologous to CYP11B1 without a known expression product. Müller and Oertle [29, 30] identified two isozymes of cytochrome *P*450 C11β, CYP11B1 and CYP11B2 in the rat adrenal cortex. CYP11B1 is found in all zones and catalyzes a single hydroxylation of DOC in 11β- or 18-position. CYP11B2 is produced exclusively in the zona glomerulosa and catalyzes all three reactions involved in the conversion of DOC to aldosterone. *In vivo* and *in vitro*, the expression of the genes encoding CYP11B1 and CYP11B2 is regulated by two separate control systems which appear to operate both independently and interdependently. Nomura *et al.* [31] isolated three genomic clones of rat CYP11B. Two of them corresponded to 11β-hydroxylase gene (CYP11B1) and aldosterone synthase gene (CYP11B2). The third was a novel gene resembling CYP11B1 and CYP11B2, and was named CYP11B3.

When patients with congenital adrenal hyperplasia due to 11β-hydroxylase deficiency are studied, there are, however, still observations to be made, which are not completely clarified by the known genetic aspects [32]: some patients have a block of 11β-hydroxylation in the 17α-hydroxylated pathway only [33–35], while the 11β-hydroxylation of DOC is functioning normally. The respective steroid secretion rates of such a patient are shown in Table 2. In others, no 18-hydroxylated compounds can be found, while in most classic cases, 18OH-DOC is present or increased. Especially, the clinical fact that 11β-hydroxylase deficiency may be present in the 17α-hydroxylated pathway only remains completely unexplained by the molecular findings.

Table 2. Steroid secretion rates in a patient with congenital adrenal hyperplasia due to 11β-hydroxylase deficiency of 17α-hydroxylated steroids only (from [34])

Compound	Secretion rate—patient (mg/m^2/day)	Secretion rate—normal mean (mg/m^2/day)
S	82.6	0.31
DOC	0.13	0.05
F	3.9	8.7
B	2.4	2.3

3β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY

In this rare defect, steroid biosynthesis is blocked at the stage of the Δ5-compounds, and Δ4-compounds cannot be formed in the adrenals, but only by nonspecific reductases in the liver. DHEA is the only increased androgen. Because its action is weak, no or only mild virilization occurs. 46XY-patients are incompletely masculinized, while the genitalia in females show slight hypertrophy of the clitoris or are normal. In most cases, there is salt-wasting, but cases without it have been observed [36, 37]. Since the defect is also located in the gonads, no or insufficient pubertal development occurs [38].

The molecular basis of classic 3β-hydroxysteroid dehydrogenase deficiency has consistently been found to be a mutation of the type II gene [39–44].

Clinically, the syndrome does not only seem to exist in the mentioned classic form, but also in mild or late-onset cases, be it in children with premature adrenarche or advanced bone maturation [45, 46], or in hirsute women [47–50]. In them, unlike in the different types of 21-hydroxylase deficiency, a molecular basis could, however, so far not be found. The question from the clinicians therefore is: do such genetically determined mild cases really exist and if yes, how frequent are they? Most likely, they are much less frequent than has been suspected, and the diagnosis of mild or late-onset 3β-hydroxysteroid dehydrogenase deficiency has probably been made too often solely on the basis of increased DHEA or other Δ5-steroids. Since many different molecular causes have been found in late-onset 21-hydroxylase deficiency, and none in "mild" 3β-hydroxysteroid dehydrogenase deficiency, it seems that the latter has less variable causes, which could be limited to classic cases.

CHOLESTEROL SIDE-CHAIN CLEAVAGE

The incapability to cleave the side chain of cholesterol causes the syndrome of lipoid adrenal hyperplasia

(synonyms: Prader syndrome [51], deficiency of cytochrome *P*450scc). With the exception of some inactive cholesterol metabolites, no steroids can be produced [52]. Newborns of both chromosomal sexes have female external genitalia, adrenal insufficiency, and salt-wasting.

So far, no molecular basis of this syndrome could be found. Southern blotting patterns of the *P*450scc gene were normal, and PCR of all exons showed normal sequences on multiple amplifications and sequencing reactions, indicating normal *P*450scc genes [53]. Also, Northern blots of testicular RNA from a 6-month-old patient and from a control fetus showed normal *P*450scc mRNA, indicating a normal *P*450scc promoter. Reprobing with cloned human cDNAs for adrenodoxin reductase and adrenodoxin showed that these electron transport cofactors used by *P*450scc were also normal, as was probing with cDNAs for all three known factors involved in cholesterol transport to the mitochondria-sterol carrier protein 2, endozepine, and steroidogenesis activator peptide. These results suggest that the lesion in lipoid CAH is not in the *P*450scc system or in any step upstream from *P*450scc. In other studies, similar results were obtained [54–57].

Also concerning the clinical lack of this enzyme, there is a biochemical observation, which is difficult to understand: while it has been sufficiently demonstrated that one single cytochrome *P*450scc regulates the 20α- and 22-hydroxylation of cholesterol simultaneously, it has been shown in earlier, but still valid and careful studies, that pregnenolone could be formed when 20αOH-cholesterol, but not when cholesterol, was incubated with adrenal tissue of patients with lipoid adrenal hyperplasia (Table 3). 20α- and 22-hydroxylation thus for unknown reasons seem to be regulated partly independently [58].

In conclusion, the progress in molecular studies, although rapid and very impressive, has so far not explained all clinical and biochemical observations in the field of the congenital defects of steroidogenic enzymes. Other factors such as promoters and the influence of fusion proteins of type II *P*450 enzymes [59], adrenodoxin as an electron donor [60], cAMP-dependent expression of genes encoding the steroidogenic enzymes [61], and/or proteases [62], which are possibly also modulated by developmental factors and/or treatment of the patients with cortisol, mineralocorticoids and sex hormones, may be responsible for the discrepancies.

Table 3. Cholesterol-cleaving activity (%) of enzyme preparation of adrenal tissue of a patient with lipoid adrenal hyperplasia and of normal tissue (from [58])

Substrate	Patient	Normal ($n = 3$)
[4-^{14}C]cholesterol	<0.01	2
[7α-^{3}H]20α-hydroxycholesterol	2.6	3–5

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REFERENCES

1. Biglieri E. G., Herron M. A. and Brust N.: 17-Hydroxylase deficiency in man. *J. Clin. Invest.* 45 (1966) 1946–1954.
2. Zachmann M., Völlmin J. A., Hamilton W. and Prader A.: Steroid 17,20-desmolase deficiency: a new cause of male pseudohermaphroditism. *Clin. Endocr. (Oxf.)* 1 (1972) 369–385.
3. Goebelsmann U., Zachmann M., Davajan V., Israel R., Mestman J. H. and Mishell D. R.: Male pseudohermaphroditism consistent with 17,20-desmolase deficiency. *Gynec. Invest.* 7 (1976) 138–156.
4. Zachmann M., Werder E. A. and Prader A.: Two types of male pseudohermaphroditism due to 17,20-desmolase deficiency. *J. Clin. Endocr. Metab.* 55 (1982) 487–490.
5. Kaufman F. R., Costin G., Goebelsmann U., Stanczyk F. Z. and Zachmann M.: Male pseudohermaphroditism due to 17,20-desmolase deficiency. *J. Clin. Endocr. Metab.* 57 (1983) 32–36.
6. Zachmann M. and Prader A.: 17,20-Desmolase deficiency. In *Adrenal Diseases in Childhood* (Edited by M. I. New and L. S. Levine). Karger, NY. *Pediatr. Adolesc. Endocr.* 13 (1984) pp. 95–109.
7. Forest M. G., Lecornu M. and De Peretti E.: Familial male pseudohermaphroditism due to 17,20-desmolase deficiency. I. *In vivo* endocrine studies. *J. Clin. Endocr. Metab.* 50 (1988) 826–833.
8. De Lange W. E. and Doorenbos H.: Incomplete virilization and subclinical mineralocorticoid excess in a boy with partial 17,20-desmolase/17α-hydroxylase deficiency. *Acta Endocr. (Copenh.)* 122 (1990) 263–266.
9. Nakajin S., Shinoda M., Haniu M., Shively J. E. and Hall P. F.: C21 steroid side chain cleavage enzyme from porcine adrenal microsomes. Purification and characterization of the 17α-hydroxylase/C17,20-lyase cytochrome *P*450. *J. Biol. Chem.* 259 (1984) 3971–3976.
10. Onoda M. and Hall P. F.: Inhibition of testicular microsomal cytochrome *P*450 (17α-hydroxylase/C-17,20-lyase) by estrogens. *Endocrinology* 109 (1981) 763–767.
11. Waterman M. R. and Simpson E. R.: Regulation of steroid hydroxylase gene expression is multifactorial in nature. *Recent Prog. Horm. Res.* 45 (1989) 533–563.
12. Couch R. M., Muller J. and Winter J. S.: Regulation of the activities of 17-hydroxylase and 17,20-desmolase in the human adrenal cortex: kinetic analysis and inhibition by endogenous steroids. *J. Clin. Endocr. Metab.* 63 (1986) 613–618.
13. Yanase T., Sanders D., Shibata A., Matsui N., Simpson E. R. and Waterman M. R.: Combined 17α-hydroxylase/17,20-lyase deficiency due to a 7-basepair duplication in the N-terminal region of the cytochrome *P*450 17α (CYP17) gene. *J. Clin. Endocr. Metab.* 70 (1990) 1325–1329.
14. Biason A., Mantero F., Scaroni C., Simpson E. R. and Waterman M. R.: Deletion within the CYP17 gene together with insertion of foreign DNA is the cause of combined complete 17α-hydroxylase/17,20-lyase deficiency in an Italian patient. *Molec. Endocr.* 5 (1991) 2037–2045.
15. Ahlgren R., Yanase T., Simpson E. R., Winter J. S. and Waterman M. R.: Compound heterozygous mutations (Arg 239—stop, Pro 342—Thr) in the CYP17 (*P*450 17α) gene lead to ambiguous external genitalia in a male patient with partial combined 17α-hydroxylase/17,20-lyase deficiency. *J. Clin. Endocr. Metab.* 74 (1992) 667–672.
16. Yanase T., Waterman M. R., Zachmann M., Winter J. S., Simpson E. R. and Kagimoto M.: Molecular basis of apparent

isolated 17,20-lyase deficiency: compound heterozygous mutations in the C-terminal region (Arg(496)—Cys, Gln (461)—Stop) actually cause combined 17α-hydroxylase/17,20-lyase deficiency. *Biochim. Biophys. Acta* **1139** (1992) 275–279.
17. Winter J. S., Couch R. M., Muller J., Perry Y. S., Ferreira P., Baydala L. and Shackleton C. H.: Combined 17-hydroxylase and 17,20desmolase deficiencies: evidence for synthesis of a defective cytochrome *P*450 C17. *J. Clin. Endocr. Metab.* **68** (1989) 309–316.
18. Forest M. G., Jaccard V., Morel Y. and Pradon M.: Combined 17-hydroxylase and 17,20-desmolase deficiencies: natural history in a female and diagnosis by systematic studies in her brother. *Horm. Res.* **33** (1990) 14.
19. Zachmann M., Kempken B., Manella B. and Navarro E.: Conversion from pure 17,20-desmolase- to combined 17,20-desmolase/17α-hydroxylase deficiency with age. *Acta Endocr. (Copenh.)* **127** (1992) 97–99.
20. Nestler J. E., McClanahan M. A., Clore J. N. and Blackard W. G.: Insulin inhibits adrenal 17,20-lyase activity in man. *J. Clin. Endocr. Metab.* 74 (1992) 362–367.
21. Rösler A. and White P. C.: Mutations in human 11β-hydroxylase genes: 11β-hydroxylase deficiency in Jews of Morocco and corticosterone methyl-oxidase II deficiency in Jews of Iran. *J. Steroid Biochem. Molec. Biol.* **45** (1993) 99–106.
22. Mitsuuchi Y., Kawamoto T., Miyahara K., Ulick S., Morton D. H., Naiki Y., Kuribayashi I., Toda K., Hara T. and Orii T.: Congenitally defective aldosterone biosynthesis in humans: inactivation of the *P*450 C18 gene (CYP11B2) due to nucleotide deletion in CMO I deficient patients. *Biochem. Biophys. Res. Commun.* **190** (1993) 864–869.
23. Curnow K. M., Slutsker L., Vitek J., Cole T., Speiser P. W., New M. I., White P. C. and Pascoe L.: Mutations in the CYP11B1 gene causing congenital adrenal hyperplasia and hypertension cluster in exons 6, 7, and 8. *Proc. Natn. Acad. Sci. U.S.A.* **90** (1993) 4552–4556.
24. Naiki Y., Kawamoto T., Mitsuuchi Y., Miyahara K., Toda K., Orii T., Imura H. and Shizuta Y.: A nonsense mutation (TGG (Trp116)→TAG (Stop)) in CYP11B1 causes steroid 11β-hydroxylase deficiency. *J. Clin. Endocr. Metab.* 77 (1993) 1677–1682.
25. Yanagibashi K., Shackleton C. H. and Hall P. F.: Conversion of 11-deoxycorticosterone and corticosterone to aldosterone by cytochrome *P*450 11β/18-hydroxylase from porcine adrenal. *J. Steroid Biochem.* **29** (1988) 665–675.
26. Ho M. M. and Vinson G. P.: 11β-Hydroxylase gene expression in the rat adrenal cortex. *J. Endocr.* **139** (1993) 301–306.
27. Zhou M. Y., Gomez Sanchez C. E., Xue D. and Foecking M. F.: The hybrid rat cytochrome *P*450 containing the first 5 exons of the CYP11B1 and last 4 exons from the CYP11B2 enzyme retains 11β-hydroxylase activity, but the alternative hybrid is inactive. *Biochem. Biophys. Res. Commun.* **199** (1994) 130–135.
28. Mukai K., Imai M., Shimada H. and Ishimura Y.: Isolation and characterization of rat CYP11B genes involved in late steps of mineralo- and glucocorticoid syntheses. *J. Biol. Chem.* **268** (1993) 9130–9137.
29. Müller J. and Oertle M.: Separate induction of the two isozymes of cytochrome *P*450 (11β) in rat adrenal zona glomerulosa cells. *J. Steroid Biochem. Molec. Biol.* 47 (1993) 213–221.
30. Oertle M. and Müller J.: Two types of cytochrome *P*450 (11β) in rat adrenals: separate regulation of gene expression. *Molec. Cell. Endocr.* **91** (1993) 201–209.
31. Nomura M., Morohashi K., Kirita S., Nonaka Y., Okamoto M., Nawata H. and Omura T.: Three forms of rat CYP11B genes: 11β-hydroxylase gene, aldosterone synthase gene, and a novel gene. *J. Biochem. (Tokyo)* **113** (1993) 144–152.
32. White P. C. and Pascoe L.: Disorders of steroid 11β-hydroxylase isozymes. *Trends Endocr. Metab.* **3** (1992) 229–234.
33. Klein A., Siebenmann R., Curtius H. C. and Zachmann M.: Steroid 11β-hydroxylase activity in the microsomal fraction of human adrenals. *J. Steroid Biochem.* 7 (1976) 283–287.
34. Zachmann M., Völlmin J. A., New M. I., Curtius H. C. and Prader A.: Congenital adrenal hyperplasia due to deficiency of 11β-hydroxylation of 17α-hydroxylated steroids. *J. Clin. Endocr. Metab.* **33** (1971) 501–508.
35. Zachmann M., Tassinari D. and Prader A.: Clinical and biochemical variability of congenital adrenal hyperplasia due to 11β-hydroxylase deficiency. *J. Clin. Endocr. Metab.* **56** (1983) 222–229.
36. Pang S., Levine L. S., Stoner E., Opitz J. M., Pollack M. S. and Dupont B.: Nonsalt-losing congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency with normal glomerulosa function. *J. Clin. Endocr. Metab.* **56** (1983) 808–818.
37. Mendonca B. B., Bloise W., Arnhold I. J., Batista M. C., Toledo S. P., Drummond M. C., Nicolau W. and Mattar E.: Male pseudohermaphroditism due to nonsalt-losing 3β-hydroxysteroid dehydrogenase deficiency: gender role change and absence of gynecomastia at puberty. *J. Steroid Biochem.* **28** (1987) 669–675.
38. Zachmann M., Forest M. G. and De Peretti E.: 3β-hydroxysteroid dehydrogenase deficiency. Follow-up study in a girl with pubertal bone age. *Horm. Res.* **11** (1979) 292–302.
39. Rhéaume E., Simard J., Morel Y., Mebarki F., Zachmann M., Forest M. G., New M. and Labrie F.: Congenital adrenal hyperplasia due to point mutations in the type II 3β-hydroxysteroid dehydrogenase gene. *Nature Genet.* 1 (1992) 239–245.
40. Chang Y. T., Kappy M. S., Iwamoto K., Wang J., Yang X. and Pang S.: Mutations in the type II 3β-hydroxysteroid dehydrogenase gene in a patient with classic salt-wasting 3β-hydroxysteroid dehydrogenase deficiency congenital adrenal hyperplasia. *Pediatr. Res.* **34** (1993) 698–700.
41. Lachance Y., Luuthe V., Verreault H., Dumont M., Rhéaume E., Leblanc G. and Labrie F.: Structure of the human type-II 3β-hydroxysteroid dehydrogenase/delta5-delta4 isomerase (3β-HSD) gene—adrenal and gonadal specificity. *DNA Cell Biol.* **10** (1991) 701–711.
42. Sanchez R., Rhéaume E., Laflamme N., Rosenfield R. L., Labrie F. and Simard J.: Detection and functional characterization of the novel missense mutation Y254D in type II 3β-hydroxysteroid dehydrogenase (3β HSD) gene of a female patient with nonsalt-losing 3β HSD deficiency. *J. Clin. Endocr. Metab.* **78** (1994) 561–567.
43. Simard J., Rhéaume E., Sanchez R., Laflamme N., Delaunoit Y., Luuthe V., Vanseters A. P., Gordon R. D., Bettendorf M., Heinrich U., Moshang T., New M. I. and Labrie F.: Molecular basis of congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency. *Molec. Endocr.* 7 (1993) 716–728.
44. Simard J., Rhéaume E., Leblanc J. F., Wallis S. C., Joplin G. F., Gilbey S., Allanson J., Mettler G., Bettendorf M., Heinrich U. and Labrie F.: Congenital adrenal hyperplasia caused by a novel homozygous frameshift mutation 273 delta aa in type II 3β-hydroxysteroid dehydrogenase gene (Hsd3B2) in three male patients of Afghan/Pakistani origin. *Hum. Molec. Genet.* 3 (1994) 327–330.
45. Nishi Y. and Tezuka T.: Mild adrenal 3β-hydroxysteroid dehydrogenase deficiency in children with accelerated growth, premature pubarche and/or hirsutism. *Eur. J. Pediatr.* **151** (1992) 19–23.
46. Cathro D. M. and Golombek S. G.: Non-classical 3β-hydroxysteroid dehydrogenase deficiency in children in central Iowa. Difficulties in differentiating this entity from cases of precocious adrenarche without an adrenal enzyme defect. *J. Pediatr. Endocr.* 7 (1994) 19–32.
47. Arnaout M. A.: Late-onset congenital adrenal hyperplasia in women with hirsutism. *Eur. J. Clin. Invest.* **22** (1992) 651–658.
48. Mathieson J., Couzinet B., Wekstein Noel S., Nahoul K., Turpin G. and Schaison G.: The incidence of late-onset congenital adrenal hyperplasia due to 3β-hydroxysteroid dehydrogenase deficiency among hirsute women. *Clin. Endocr. (Oxf.)* **36** (1992) 383–388.
49. Azziz R., Bradley E. L. Jr, Potter H. D. and Boots L. R.: 3β-hydroxysteroid dehydrogenase deficiency in hyperandrogenism. *Am. J. Obstet. Gynec.* **168** (1993) 889–895.
50. Barnes R. B., Ehrmann D. A., Brigell D. F. and Rosenfield R. L.: Ovarian steroidogenic responses to gonadotropin-releasing hormone agonist testing with nafarelin in hirsute women with adrenal responses to adrenocorticotropin suggestive of 3β-hydroxy-delta5-steroid dehydrogenase deficiency. *J. Clin. Endocr. Metab.* **76** (1993) 450–455.
51. Prader A. and Gurtner H. P.: Das Syndrom des Pseudohermaphroditismus masculinus bei kongenitaler Nebennierenrinden-Hyperplasie ohne Androgenüberproduktion (adrenaler Pseudohermaphroditismus Masculinus). *Helv. Paediatr. Acta* **10** (1955) 397–412.

52. Degenhart H. J.: Prader's syndrome (congenital lipoid adrenal hyperplasia). In *Adrenal Diseases in Childhood* (Edited by M. I. New and L. S. Levine). Karger, NY. *Pediatr. Adolesc. Endocr.* **13** (1984) pp. 125–144.
53. Saenger P., Lin D., Gitelman S. E. and Miller W. L.: Congenital lipoid adrenal hyperplasia genes for *P*450scc, side chain cleavage enzyme, are normal. *J. Steroid Biochem. Molec. Biol.* 45 (1993) 87–97.
54. Hum D. W. and Miller W. L.: Transcriptional regulation of human genes for steroidogenic enzymes. *Clin. Chem.* **39** (1993) 333–340.
55. Clemens J. W., Lala D. S., Parker K. L. and Richards J. S.: Steroidogenic factor-1 binding and transcriptional activity of the cholesterol side-chain cleavage promoter in rat granulosa cells. *Endocrinology* **134** (1994) 1499–1508.
56. Rogler L. E. and Pintar J. E.: Expression of the *P*450 side-chain cleavage and adrenodoxin genes begins during early stages of adrenal cortex development. *Molec. Endocr.* 7 (1993) 453–461.
57. Tsujita M. and Ichikawa Y.: Substrate-binding region of cytochrome *P*450scc (*P*450 XIA1). Identification and primary structure of the cholesterol binding region in cytochrome *P*-450scc. *Biochim. Biophys. Acta* **1161** (1993) 124–130.
58. Degenhart H. J., Visser H. K. A., Boon H. and O'Doherty N. J.: Evidence for deficient 20α-cholesterol hydroylase activity in adrenal tissue of a patient with lipoid adrenal hyperplasia. *Acta Endocr. (Copenh.)* 71 (1972) 512–518.
59. Harikrishna J. A., Black S. M., Szklarz G. D. and Miller W. L.: Construction and function of fusion enzymes of the human cytochrome *P*450scc system. *DNA Cell Biol.* **12** (1993) 371–379.
60. Lehoux J. G. and Ducharme L.: Effects of dexamethasone on the levels of adrenal steroidogenic enzyme mRNA in rats treated with 4-aminopyrazolopyrimidine. *Molec. Cell. Endocr.* **93** (1993) 1–10.
61. Lauber M. E., Kagawa N., Waterman M. R. and Simpson E. R.: cAMP-dependent and tissue-specific expression of genes encoding steroidogenic enzymes in bovine luteal and granulosa cells in primary culture. *Molec. Cell. Endocr.* **93** (1993) 227–233.
62. Watabe S., Hara T., Kohno H., Hiroi T., Yago N. and Nakazawa T.: *In vitro* degradation of mitochondrial proteins by ATP-dependent protease in bovine adrenal cortex. *J. Biochem. (Tokyo)* **113** (1993) 672–676.