2008

SOCIETY FOR PEDIATRIC PATHOLOGY

SYMPOSIUM:
Endocrine Pathology

Moderated By:

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In accordance with ACCME guidelines, the faculty members for this Workshop were required to complete disclosure forms indicating whether or not they and/or their spouse/partner have any significant financial or other relationship with a commercial company, entity, or service (which may be discussed in this educational program) which might be perceived as influencing the content, or conclusions reached in this presentation. The speakers had nothing to disclose. The Society has also required that the speakers disclose any products that are not labeled for the use under discussion prior to their presentation.
1:30 PM – Congenital Hyperinsulinism  
Charles A. Stanley, The Children’s Hospital of Philadelphia, Philadelphia, PA

2:15 PM – Fetal and Postnatal Development of the Human Adrenal Gland  
C. Richard Parker, Jr., University of Alabama, Birmingham, AL

3:00 – 3:30 PM – Break

3:30 PM – Adrenal Cortical Neoplasia in Children  
Louis Dehner, Washington University School of Medicine, St. Louis, MO

4:15 PM – Heritable Endocrine Disorders Involving Adrenal Gland Presenting in Childhood  
J. Aidan Carney, Mayo Clinic, Rochester, MN

SYMPOSIUM OBJECTIVES:

After attending the symposium the attendees will be able to:

1. Discuss the various forms of neonatal hyperinsulinism and be able to formulate disease-specific diagnoses in clinicopathologic practice.

2. Distinguish among different types of adrenal cortical tumors of childhood and discuss their outcomes.

3. Compare and contrast patterns of adrenal enzyme expression during intrauterine life to that of adulthood and list physiologic regulators of growth and steroidogenesis.

4. Diagnose heritable syndromes of endocrine disorders that involve the adrenal glands.
Objectives
Participants will be able to:

• List the clinical features of hyperinsulinism in infants and children
• Describe the genetic mechanisms of diffuse and focal congenital hyperinsulinism.
• Discuss pathological criteria for diagnosing diffuse and focal hyperinsulinism
50 Years of Congenital Hyperinsulinism

1950s
- Idiopathic Hypoglycemia of Infancy (MacQuarrie)
- Leucine Sensitive Hypoglycemia (Cochrane)

1960s
- Insulin RIA (Yalow & Berson)
- Diazoxide Rx (Drash)

1970s
- Nesidioblastosis (Yakovac & Baker)
- Hyperinsulinism

1980s
- Somatostatin Rx

1990s
- Recessive or Dominant Inheritance
- 5 Genetic Loci Identified
- Focal HI in 50-60% of surgical cases

2000+
- Exercise-induced HI (MCT-1)
- F-DOPA PET
Types of Neonatal Hyperinsulinism (HI)

- **Non-Genetic Forms:**
  - Infant of Diabetic Mother
  - Prolonged Neonatal HI (asphyxia, SGA, toxemia)

- **Genetic Forms:**
  - \( \text{K}_{\text{ATP}} \text{-HI (SUR1 / Kir6.2)} \)
    - Recessive
    - Focal (LOH & paternal mutation)
    - Dominant
  - Dominant \( \text{GK-HI} \) (glucokinase)
  - Dominant \( \text{GDH-HI} \) (glutamate dehydrogenase)
  - Recessive \( \text{SCHAD-HI} \) (short-chain 3-OH-acyl-CoA dehydrogenase)
  - Dominant \( \text{MCT-1 HI} \) (exercise-induced HI)
Focal KATP-HI

• 40-70% of severe congenital HI cases
• Clinically identical to KATP-HI
• Diazoxide unresponsive
• Curable by surgery
• 2-Hits: clonal LOH for maternal 11p plus isodisomy for paternal KATP-channel mutation
Focal Congenital HI -- Two Hits:
(Maternal LOH & Paternal $K_{ATP}$ Defect)
Focal vs Diffuse Disease in 50 Infants with Medically-Unresponsive HI
Imaging focal HI by $[^{18}\text{F}]-\text{DOPA}$ PET scan

- F-DOPA taken up by beta-cells and stored as dopamine

- F-DOPA custom-made in cyclotron under IND ($\dagger \frac{1}{2}$ 90 min)
Pre-operative F-DOPA PET

- 50 infants with HI (24 focal)
- 75% true positive
- 0% false negative
- When positive, localization 100% correct

Hardy, et al. JCEM 2007
Congenital Hyperinsulinism Paradigm 2007

- Hypoglycemia → Fasting test → Hyperinsulinism
  - NH₃ / acyl-carn / AIR tests
  - Diazoxide trial (−) → Focal / diffuse $K_{ATP}$
    - Octreotide trial (−) → $^{18}$F-DOPA PET
    - 18F-DOPA PET → Surgery
      - Focal: 65%
        - Local resection
      - Diffuse: 35%
        - Surgeon
        - Pathologist
        - 98% Pancreatectomy
  - (+) → Geneticist
    - GDH
    - GCK
    - SCHAD
    - Dom $K_{ATP}$
    - MCT-1
    - Other?
Surgery for HI

Biopsy 3 regions of pancreas for signs of diffuse disease on frozen sections (enlarged nuclei)

Diffuse: do 97% pancreatectomy & gastrostomy

Focal: resect focal adenomatosis (if lesion in head, may need Roux-en-Y)
HISTOLOGIC FORMS OF HYPERINSULINISM

Diffuse form

Focal form
LEARNING OBJECTIVES
After attending the Symposium, the participant should be able to:
1) characterize the functional phenotype of the different cortical zones of the human fetal adrenal gland;
2) explain the structural and functional differences that exist in normal fetal adrenals versus those of the anencephalic fetus;
3) discuss the changes that occur in adrenal structure and adrenal androgen production during fetal life, infancy, and in childhood through adrenarche;
4) characterize the different steroidogenic profiles associated with various forms of Congenital Adrenal Hyperplasia, and the impact of these profiles on intruterine external genitalia in female and male fetuses.

EMBRYONIC DEVELOPMENT OF THE HUMAN FETAL ADRENAL GLAND
The early development of the human adrenal gland has been described based on studies of Carnegie staged embryos (Crowder, 1957). The primitive adrenal cortex is formed from cells of the coelomic epithelium that migrate to the suprarenal regions bilaterally during the fifth developmental week. It is believed that a single progenitor cell gives rise to cells of the inner and outer cortex whereas other cell types give rise to the adrenal stroma and the adrenal capsule. By the eighth developmental week, the fetal adrenal cortex is composed of 2 distinct cellular populations: the outer adrenal cortex, termed the neocortex, is composed of a thin band of small cells that have darkly stained nuclei and scant cytoplasm. The inner cortical zone, the fetal zone, is composed of large eosinophilic cells that have a pale nucleus. Initiating soon thereafter and continuing for several more weeks, the precursors of the adrenal medulla migrate into the primordial cortex, proliferate, and begin to differentiate from neuroblasts into pheochromocytes over the period of 8-25 weeks gestation. Details about differentiation and function of the adrenal medulla in utero and thereafter in postnatal life are beyond the scope of this presentation.

GROWTH AND CORTICAL ZONATION OF THE FETAL ADRENAL
Once the adrenal glands are formed, cortical growth appears to be achieved mainly through mitotic activity in the subcapsular region. A pattern of cell replication in this region and subsequent centripetal migration of cortical cells have been shown to occur in experimental animals and it is assumed that such may also occur in the human. The adrenals undergo extensive growth in utero and by the end of gestation, they are as large as those of adults. The fetal zone occupies an ever-increasing proportion of the entire adrenal during gestation and at term comprises up to 80% of the adrenal gland volume. The presence of a fetal zone in the adrenal occurs primarily in primates and its absence in most experimental animals has, therefore, made it difficult to explore mechanisms of its formation and function in utero. Whereas the acquisition of the capacity for catecholamine biosynthesis in the developing adrenal medulla is considered to be dependent upon corticosteroids, it is not known to what extent, if any, the
medullary/nerve elements might influence adrenal cortical growth or function in utero. Based on in vivo and in vitro studies in experimental animals and in vitro studies of the adult human adrenal, many investigators have provided evidence that several factors elaborated by adrenal nerves and cells of the medulla are likely to influence the cortex in postnatal life.

An intact hypothalamic-pituitary axis is considered to be required for normal development of the adrenal cortex, presumably due to the actions of derivatives of the pituitary polypeptide synthesized in corticotrophs, proopiomelanocortin, which is secreted in response to the hypothalamic neuropeptide, Corticotrophin Releasing Hormone (CRH). Severe CNS developmental anomalies that affect the hypothalamus also are known to markedly impair the normal development of the adrenal cortex (Detailed later). The orphan nuclear receptor steroidogenic factor 1 (SF-1, also described in early studies as Ad4-binding protein, and now officially designated NR5A1) is critical for development and function of steroidogenic organs, including the adrenal. The gene encoding SF-1 is evolutionarily conserved in vertebrates and invertebrates and even closely resembles an orphan receptor in Drosophila. The results of cell transfection studies have highlighted important roles for SF-1 in the transcriptional activation of genes that encode steroidogenic enzymes, while targeted disruption of the gene encoding SF-1 in the mouse has defined essential roles for this factor in adrenal and gonadal development. SF-1 likely plays similar roles in the human, as evidenced by adrenal insufficiency and male-to-female sex reversal in a patient with a heterozygous mutation in the human gene that encodes SF-1. Of the first 12 published mutations of the human SF-1 gene since 1999, 3 have been associated with adrenal insufficiency whereas the remainder have only displayed varying degrees of gonadal dysfunction. Another orphan nuclear receptor, DAX-1 [dosage sensitive sex reversal, adrenal hypoplasia congenital (AHC), critical region on the X chromosome gene 1], colocalizes with SF-1 during mouse development and inhibits SF-1-mediated transactivation of target genes in both steroidogenic and nonsteroidogenic tissues. In addition to the steroid hydroxylases, DAX-1 also inhibits promoters for steroidogenic acute regulatory protein (STAR), and the gonadal/adrenal form of 3beta-hydroxysteroid dehydrogenase (HSD3B2). SF-1 and DAX-1 are present in cortical cells of the adrenal at the earliest developmental periods wherein the adrenal primordium is demonstrable and they continue to be expressed throughout development. Mutations of the DAX-1 gene have been found to cause an X-linked disorder characterized by congenital adrenal hypoplasia and hypogonadotropic hypogonadism.

STEROIDGENIC PHENOTYPE OF FETAL ADRENAL CORTICAL ZONES
In addition to the morphologic differences between the cortical cells of the fetal zone (also termed the transient zone by early investigators) and those of the neocortex, there also are differences in the expression of enzymes and other regulatory factors involved in steroidogenesis. As the cortex differentiates later in gestation, the neocortex can be seen to be composed of cells arranged like those of the zona glomerulosa which surround a zone, termed the transitional zone, which in turn surrounds the fetal zone. We and others have shown that the steroidogenic pathway seen from around 15 wks gestation - term in the fetal zone is very similar to that seen in the zona reticularis of the adult adrenal and favors the production of adrenal androgens (19 Carbon containing steroids) such as DHEA and DHEA-Sulfate. These steroids are produced in the absence of 3 beta-hydroxysteroid dehydrogenase (HSD3B2), but in the presence of Cytochrome P450 C17 (CYP17), which is a dual function enzyme that can 17 hydroxylate and can also convert a 21-Carbon steroid such as 17 OH pregnenolone into
DHEA by virtue of its 17-20 lyase activity. This latter activity is promoted by an accessory protein, Cytochrome b5, which is also present in high concentrations in the fetal zone (but not in the neocortex). There have been a few observations of scattered cells within the fetal zone that contain HSD3B2; such cells have not been characterized fully but they could be a transient source of corticosteroids early in gestation. The fetal zone, like the adult zona reticularis also contains high levels of DHEA-sulfotransferase. The transitional zone, much like the zona fasciculata of the adult adrenal, expresses HSD3B2, CYP17 and other hydroxylating enzymes such as 21-hydroxylase (CYP21A2) and 11-hydroxylase (CYP11B1), which permit the formation of cortisol. Due to the absence of Cytochrome b5, the Transitional Zone is much less likely than the Fetal Zone to produce androgens. The outer zone of the neocortex is characterized by cells whose enzymatic phenotype is similar to zona glomerulosa cells of the adult. Presence of 3β HSD, CYP11B1, CYP21A2, and aldosterone synthase (CYP11B2), but the absence of CYP17, which permits the production of mineralocorticoids such as deoxycorticosterone, and aldosterone.

The fetal zone and neocortex can be crudely separated from each other by dissection of the fetal adrenal. Such tissue preparations from mid-gestation fetuses have been used extensively in culture experiments to define the regulation of steroidogenesis in the fetal adrenal. Cells of both zones secrete steroids in response to stimulation with Adrenocorticotropin (ACTH); this effect being mediated by activation of the protein kinase A pathway and c'AMP production. Direct effects of the hypothalamic/placental peptide CRH on fetal adrenal steroid production also has been demonstrated in vitro; in light of the relatively high circulating levels of CRH in fetal blood, direct effects of CRH on fetal adrenal function are of potential importance.

Many other peptides, including growth factors and cytokines have been shown to influence growth and/or steroid production by cultured human fetal adrenal cells. Steroidogenesis is augmented in the presence of serum lipoproteins, particularly LDL-Cholesterol, which is assimilated by both cell types via the LDL-receptor mediated pathway and provides cholesterol as substrate for the steroidogenic pathway. It is believed that the high rate of LDL-cholesterol uptake and utilization by the fetal zone of the adrenal cortex is one factor responsible for the relatively low levels of LDL-cholesterol in fetal blood. Conversely, failure of adrenal utilization of LDL-cholesterol as would be expected to occur in conditions of adrenal hypoplasia may explain the strikingly increased levels of cholesterol seen in umbilical cord blood of anencephalic infants.

**FETO-PLACENTAL UNIT IN ESTROGEN PRODUCTION**

Whereas the placenta is capable of producing progesterone somewhat autonomously, the placenta is an incomplete endocrine tissue with respect to estrogen production, primarily as a consequence of lacking 17-hydroxylase/17,20-desmolase activities that are essential for conversion of pregnenolone or progesterone (C-21 steroids) into androgens. The placenta contains the enzyme aromatase that converts androgens into estrogens, and also contains high concentrations of sulfatase enzyme and HSD3B2. Consequently, the placenta can make use of DHEA and DHEA Sulfate produced in the fetal and maternal adrenals as sources for estrogen formation. Estrogen production is accomplished through the first few weeks of pregnancy mainly by the maternal ovaries, but thereafter the fetal adrenals serve an increasingly important role as the source of substrate for placental estrogen synthesis. Despite adequate supplies of fetal androgen precursor production, estrogen formation in pregnancies complicated by placental sulfatase deficiency is extremely limited. Varying degrees of hypoestrogenism
also have been noted in pregnancies complicated by fetal anencephaly, intrauterine growth retardation, and others.

PARADOXICAL FETAL ADRENAL ANDROGEN RESPONSES TO INTRAUTERINE STRESS

In uncomplicated pregnancies, umbilical cord plasma levels of DHEA Sulfate and cortisol are reasonably stable during the late second and early third trimester. During the last 6 to 10 weeks of gestation, however, there are striking increases in the concentrations of DHEA Sulfate and cortisol in fetal blood. Fetal plasma cortisol is derived from fetal as well as maternal adrenals, which makes it difficult to correlate fetal adrenal activity with fetal plasma cortisol levels in various physiologic or pathophysiologic circumstances. On the other hand, fetal plasma DHEA Sulfate, which arises from fetal adrenal production, appears to be indicative of fetal responses to pregnancy conditions. For example, umbilical cord plasma levels of DHEA Sulfate have been found to be reduced in pregnancies with specific medical complications such as hypertension, Rh disease, and syphilis, as well as in pregnancies in which fetal growth is retarded. In such circumstances there is often subnormal estrogen production and morphologic evidence for reduced volume of the fetal zone of the fetal adrenal. Whereas several pregnancy complications give rise to reduced fetal DHEA Sulfate production and possible maldevelopment of the fetal zone, fetal serum or newborn urinary levels of cortisol and its metabolites seem to be normal or even increased in many such circumstances. The mechanisms for apparently impaired fetal zone androgen production in such instances to be determined; similar reductions in DHEA/DHEA Sulfate in the presence of adequate cortisol secretion also have been noted to occur in adulthood in response to severe illness.

Cortisol levels in the fetus or at delivery of the newborn, however, may not be indicative of fetal adrenal activity. This is due to the fact that the placenta is permeable to maternal serum cortisol. In normal gestation, maternal cortisol is largely converted to the inactive steroid, cortisone, in the placenta by the action of type 2 11- hydroxysteroid dehydrogenase (type 2 11-HSD), limiting availability for transport to the fetus. Due, however to the co-existence of a bi-directional steroid metabolizing enzyme in the placenta, type 1 11-HSD, that interconverts cortisol and cortisone, some maternal cortisol can reach the fetal circulation. Maternal pregnancy complications have been found to variably affect the levels of these 11-HSD's and it is likely that cortisol levels in umbilical cord blood seen in such circumstances are influenced not only by fetal adrenal secretory rates, but also variations in placental metabolism of maternal cortisol.

As mentioned earlier, the mechanisms responsible for the growth and pattern of steroidogenesis by the human fetal adrenal are not established. Although short-term correlations between fetal pituitary ACTH production and fetal adrenal activity are evident, e.g., suppressed fetal adrenal steroidogenesis after maternal glucocorticoid administration, there is little established concerning fetal pituitary ACTH production in relation to pregnancy complications. Somewhat paradoxically, fetal plasma immunoreactive ACTH levels appear to decline over the latter half of gestation, whereas fetal adrenal growth and steroid production, particularly that of the fetal zone, are markedly enhanced.

ADRENAL DEVELOPMENT IN ANENCEPHALIC FETUSES
Fetal adrenal hypoplasia has long been recognized to occur in concert with anencephaly. The most striking instances of the deficiency of adrenal development in anencephaly is seen at term when the adrenal volume may be only 10% of the normal size. The majority of the defect has been noted to be in the fetal zone of the cortex, which would normally comprise 70-80% of the gland. Although some initial studies were suggestive that fetal cortical development was not compromised until after mid gestation in anencephaly, subsequent studies, including our own, have revealed abnormalities in adrenal development as early as 14-15 weeks gestation. Despite the variably deficient development of the fetal cortical zone in relation to gestational age, the outer cortex of the adrenal appears to develop normally and the medulla has been shown to be normal or possibly even advanced in its morphologic development. The apparently normal development of the outer cortical zone (perhaps analogous to the zona glomerulosa) in anencephaly may relate to a normal renin-angiotensin system in this condition. It is presumed that the defect in adrenal development is related to mal-development of the hypothalamus and its regulation of the pituitary; particularly of the cells that produce proopiomelanocortin. Regression of the fetal zone of the primate adrenal has been noted to occur in response to intrauterine decapitation.

We and others have found that umbilical cord levels of DHEA Sulfate are extremely low at delivery of anencephalic fetuses whereas those of LDL-Cholesterol are exceptionally high (presumably due to lack of adrenal uptake and utilization for steroidogenesis). Umbilical cord levels of cortisol range from low to near normal in anencephalic newborns. In culture studies, the adrenals of anencephalic fetuses are capable of producing steroids in response to added ACTH but their basal and ACTH-stimulated steroid production is very limited compared to that of normal adrenal cultures. We find by immunohistochemical techniques that the fetal zone of anencephalic adrenals expresses CYP17, Cytochrome b5, and DHEA sulfotransferase as does the normal fetal adrenal. The failure of the adrenal of the anencephalic to maintain normal levels of circulating estrogen precursor is likely the combined result of the hypoplastic fetal zone and the deficit of normal pituitary secretagogues. By use of microarray techniques, we have recently found that the anencephalic adrenal has strikingly reduced levels (compared to age-matched adrenals of normal fetuses) of mRNA for steroidogenic enzymes (among many other gene transcripts that also are deficient) but strikingly increased expression of most genes related to the adrenal medulla. Investigation of the genes that are deficient in anencephalic adrenals may provide important insights to the factors that are required for normal adrenal growth and differentiation.

POSTNATAL ADRENOCORTICAL REORGANIZATION AND FUNCTION IN INFANCY

Soon after delivery, the adrenal cortex undergoes a dramatic involutionary process in which the fetal zone regresses and the transitional zone and outermost cortical zone become the zona fasciculata and zona glomerulosa, respectively. This process is essentially completed within the first year of postnatal life. During this time, serum and urinary levels of DHEA and DHEA Sulfate are reduced dramatically, whereas circulating levels of cortisol and aldosterone are maintained at levels that are similar to that seen in adults. Serum and/or urinary levels of other androgens also decline during early infancy, likely due to the reduction in fetal adrenal synthesis and bioconversion from circulating DHEA/DHEA Sulfate in peripheral tissues. By the second or third year of life, there are virtually no vestiges of the fetal zone remaining in the adrenal cortex. The mechanism for fetal zone involution is not completely understood but likely is achieved in part via apoptosis. It is presumed that with parturition, whatever circulating or local factors that were elaborated to permit extensive growth and steroidogenesis in the fetal zone are
thereafter unavailable, leading to the ultimate demise of this group of cortical cells. The factors that are responsible for the developmental characteristics of the fetal zone remain to be elucidated.

DISORDERS OF ADRENAL DEVELOPMENT

The most frequent disorders in adrenal development are those resulting in congenital adrenal hyperplasia (CAH). These autosomal recessive disorders arise due to mutations in the genes that encode for steroidogenic enzymes. Some of these disorders are obvious at birth, and in some cases can be diagnosed during pregnancy. The most prevalent form of CAH is due to mutations in the CYP21 gene. There are two forms of this disorder. In the classic form, which affects about 1:14,000 newborns, there is severe deficiency in 21-hydroxylase activity, leading to severe impairments in corticosteroid production in utero with an associated increase in fetal hypothalamic-pituitary activation to compensate for lack of cortisol negative feedback control. The increased production of ACTH (other peptides derived from proopiomelanocortin also are increased) by the fetal pituitary in this instance leads to chronic stimulation of the fetal adrenal both in the fetal zone and the neocortex, leading to massive increases in adrenal androgen production. In the case of an affected female fetus, there will be virilization of the genitalia and genital ambiguity at birth (pseudohermaphroditism). Male infants do not manifest any genital abnormalities at birth but may have hyperpigmentation due to excessive POMC peptide production in the pituitary. There can be salt wasting in the classic 21-hydroxylase deficiency due to impaired production of mineralocorticoids. Treatment of the developing fetus in utero with synthetic glucocorticoid such as dexamethasone can be successful in preventing virilization of the female fetus if initiated soon enough in gestation. Treatment generally should be stopped once it is determined that the affected fetus is male since there may be untoward effects of chronic intrauterine exposure to excessive levels of glucocorticoids. A less severe form of CAH, non-classic 21-hydroxylase deficiency, is the most common autosomal recessive disorder in humans with the highest frequency seen in Ashkenazi Jews. There is no evidence of salt wasting in this disorder, and usually it is manifested later in life with clinical signs of premature masculinization in males and virilization in females. Normal or near normal levels of cortisol in the circulation are achieved at the expense of substantial increases in precursor steroids. Measurement of serum 17-hydroxyprogesterone before and after exogenous ACTH administration is useful in the diagnosis of this disorder. Other, more rare, instances of CAH have been found in association with defects in HSD3B2, which also can lead to postnatal virilization due to excessive adrenal production of DHEA and DHEA-Sulfate which can be metabolized into more potent androgens in peripheral tissues by the type 1 form of this enzyme (HSD3B1), which is produced by a different gene and is unaffected. Salt wasting at birth and male genital ambiguity also can be seen in the classic form of this disorder since testicular androgen production, which is also dependent upon HSD3B2, can be affected. In 11-hydroxylase deficient CAH, there also can be prenatal and/or postnatal virilization, due to excessive adrenal androgen production in females. In this disorder, there can be salt retention due to excessive production of deoxycorticosterone, a potent mineralocorticoid. CAH due to deficiency of CYP17, which is required for both androgen and glucocorticoid production, is associated with salt retention due to excessive deoxycorticosterone and aldosterone production. This disorder also is associated with genital ambiguity in affected male infants due to impairments in androgen production in the adrenal and testes; female infants do not show any evidence of genital ambiguity. Lastly, a defect in Steroidogenic acute Regulatory Protein (STAR) also can cause a form of CAH that results in the disorder known as congenital lipoid adrenal hyperplasia.
ADRENARCHE

In the human and in some primates, a process (Adrenarche) distinct from, and independent of, gonadal maturation and puberty occurs during childhood that is associated with progressive increases in production of androgens in the adrenal cortex. Beginning around 5-6 yrs of life in boys and girls, there are increases in circulating levels of DHEA and DHEA Sulfate in the absence of increased levels of cortisol or of ACTH. Studies of the morphology of the adrenal gland have revealed that this increase in adrenal androgen production coincides with the development and progressive thickening of the zona reticularis, which lies between the zona fasciculata and the medulla. Characterization of this zone by immunohistochemical techniques has revealed that these cells express the same complement of steroidogenic factors as does the fetal zone (CYP17, Cytochrome b5, and DHEA Sulfotransferase) and that there is a similar absence of HSD3B2 as well in this zone. The cellular concentrations of these factors also appear to increase during this process, giving rise to striking capacity for DHEA/DHEA Sulfate secretion through young adulthood. Any increases in circulating levels of androstenedione and testosterone that may occur during adrenarche are likely the result of peripheral conversion from DHEA/DHEA Sulfate rather than due to increased levels of adrenal synthesis. Preliminary identification of a peptide fragment derived from proopiomelanocortin, termed the "joining peptide", as an adrenal androgen stimulating factor has subsequently been conclusively discounted by several investigators. The factors responsible for the growth (and resultant steroidogenesis) in the zona reticularis during adrenarche remain to be characterized.
Symposium: “Endocrine Pathology”
ADRENAL CORTICAL NEOPLASIA IN CHILDREN
Louis P. Dehner, MD

Objectives:
Following the completion of the presentation on adrenocortical neoplasms in children, participants will be able to:

1. Describe the variety of tumor types that can present in the adrenal other than neuroblastoma, including adrenal cortical neoplasms.

2. List common clinical features of adrenal cortical neoplasms that are typically seen in the first 4 years of life and present with virilization.

3. List two syndromes that may have adrenal cortical tumors as one of their manifestations and the genetic loci implicated in each syndrome.

4. Describe the prognostic implications of pathologic features of adrenocortical tumors in adult and pediatric patients.

The pathology of the adrenal gland in children reflects the dual progenitorship of the gland from so-called intermediate mesoderm located in the region of mesonephros and the neural crest. The neuroblasts can be identified as early as 2 weeks as they migrate into the cortex to acquire their final positioning in the medulla as pheochromocytes. During the fourth week of gestation, focal proliferation of the coelomic epithelium (mesoderm) which migrate to the cephalic end of the mesonephros. These future steroidogenic cells begin to differentiate into the fetal cortex by the eighth week and subsequent differentiation into the diminutive definitive cortex (see Mesiano and Jaffe, 1997 for additional details about cortical development).

A review of the surgical pathology files at the Lauren V. Ackerman Laboratory Surgical Pathology from 1988 through 2007 located 160 cases of adrenal resections and ectopias in children through the first two decades of life (Table 1). As anticipated, neuroblastoma accounted for 40% of cases, however, primary adrenal cortical neoplasms in the categories of adenoma, atypical adenoma and carcinomas represented 24% of all cases. The majority of these latter cases were seen in consultation as some measure of the diagnostic difficulty and dilemma posed by these neoplasms.

What is it about adrenocortical neoplasms in children which challenges the pathologist in terms of the diagnosis? For those who also do adult pathology, the distinction between an adrenal adenoma versus cortical carcinoma is straightforward in most cases though the impression may be otherwise given the number of clinicopathologic studies over the past 20 years or so which would imply otherwise. However, the reality is that the relatively rare adrenocortical
carcinoma (ACC) in the adult is a large tumor, often in excess of 500 gms, with locally aggressive behavior (invasion into surrounding organs or tissues), overtly malignant histology and regional and/or distant metastases.

Based upon our modest experience with adrenal cortical tumors (ACT) in children compared to that of the International Pediatric Adrenocortical Tumor Registry (IPATR) of 254 cases and the Armed Forces Institute of Pathology (AFIP) series of 83 cases, the observations and findings are very similar in our 39 cases to those larger series. Regardless of the specific pathology, 67% (compared to 60%) of the children were <4 years old at diagnosis and there was a striking female predilection (80% compared to 61%) (Table 2). Virilization with or without cushingoid aspects was the most common clinical presentation. One 6-month-old male in our series was known to have Beckwith-Weidemann syndrome and another infant’s mother had a history of Cushing syndrome and an adenoma resected in childhood. Another adenoma in a 2-1/2 year-old female presented as an intradural, extramedullary mass.

Any number of studies have been published on adrenocortical neoplasms in children and adults in an attempt to define a set of morphologic features which are statistically significant with clinical outcome. These features have included weight and microscopic findings to include mitotic activity, confluent necrosis and invasion into the capsule, surrounding structures and blood vessels. One of the more frequent histologic findings in ACTs in children is the presence of marked nuclear pleomorphism with nucleomegaly and hyperchromatism, but often in the absence of mitotic activity.

Weight of the adrenocortical tumor is one of the most common gross attributes in the assessment of prognosis whether in a child or adult in virtually all clinicopathologic studies. However, the problem in many of these studies is that both children and adults are included in the same analysis. An early influential study by Cagle et al (1986) indicated that 100 gm was the apparent boundary between benign and malignant adrenocortical neoplasms in children and only one example of ACC was documented in a tumor weighing less than 500 gms in a child. They reported as well that abnormal mitoses, necrosis, vascular and capsular invasion, broad fibrous bands and marked pleomorphism were found in pediatric benign tumors.

The most comprehensive studies of adrenocortical neoplasms specifically in children are those from the IPATR and AFIP (see Michalkiewicz et al 2004; Wieneke et al 2003). These tumors, both benign and malignant, presented for the most part in children less than 4 years old, have a female predilection and are completely resectable in 80% or more of cases. Adrenocortical tumors on average weigh less than 400 gm in the majority of cases. “Malignant histology” was observed in 75% or more of cases (AFIP, 89%). Event free survival was over 80% in those children whose tumors weighed less than 200 gm or measured less than 10.5 cms. In our own series of 39 cases, only 4 tumors (10%) weighed more than 200 gm (one atypical adenoma, 3 ACCs). Not to be overly sanguine nor nihilistic, the diagnosis of ACC in children should be reserved for those neoplasms in excess of 200 gms and preferably 400 gms in
older children (greater than 5 years) with several of the pathologic features associated with the ACCs in adults (capsular invasion or adjacent organ invasion, tumor necrosis, measured mitotic rate and atypical mitotic figures). A small tumor (less than 400 gms) with some of these features in a child qualified as an atypical adenoma in our series.

Recurrent chromosomal aberrations have been identified in adrenocortical tumors (ACT) in children and adults and are similar with gains/amplifications of 9q34 (close to the steroidogenic factor 1 or SF-1 at 9q33.3); this gene has a role in the control of adrenal cortical development. A germline R337H TP53 (p53) mutation when accompanied by SF-1 amplification drives cortical cell proliferation. There is also overexpression of insulin-like growth factor II in pediatric ACTs. Interestingly, Wilkin and associates have suggested that ACTs in children have phenotypic features of the fetal adrenal cortex, but with defective apoptosis. Several other genes are involved with adrenal tumorigenesis as summarized by Stratakis (2003).

One of our cases represented a bilateral adrenalectomy in a 6-year-old female with Cushing’s syndrome who proved to have micronodular adrenal disease or primary pigmented nodular adrenocortical disease (PPNAD). Most but not all cases of PPNAD are associated with the Carney complex. Multiple, small, pigmented (lipofuscin) nodules (less than 6 mm) replace an otherwise atrophic intermodular cortex. The glands are either small or normal sized. A germline mutation is found in the PRKAR1A gene (17q 22-24).

The neuroblastoma is the dysontogenic neoplasm of the adrenal medulla. Is there an equivalent or counterpart neoplasm arising from the adrenal cortex? There is a report of a case of a primitive appearing adrenal neoplasm with a “mixture of immature epithelium and mesenchyme” in a 21 month old infant that has been interpreted as an “adrenocortical blastoma” by Moberg et al (1992). More recently, Cole and Patterson (2007) reported a case in an 11-year-old female with an “undifferentiated” neoplasm in the left adrenal with caval invasion. Following chemotherapy, a resection showed a “classic adrenocortical carcinoma.” These authors suggested that a primitive adrenoblastoma had differentiated into an ACC.

**TABLE 1. ADRENALECTOMY AND ECTOPIA IN CHILDREN (1989-2007)***

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neuroblastic tumors (all types)</td>
<td>66</td>
<td>41</td>
</tr>
<tr>
<td>Incidental adrenalectomy</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Cortical adenoma</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Cortical adenoma, atypical</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Ectopia</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>Cortical carcinoma</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Cyst, with or without calcifications, hemorrhage</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Wilms tumor (direct extension)</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>Count</td>
<td>Gender</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>-------</td>
<td>--------</td>
</tr>
<tr>
<td>Pheochromocytoma</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Soft tissue neoplasms**</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Intra-adrenal Wilms tumor</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Teratoma</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Pigmented micronodular hyperplasia</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

TOTAL 160

*From the Lauren V Ackerman Laboratory of Surgical Pathology, Barnes-Jewish and St. Louis Children’s Hospitals, Washington University Medical Center, St. Louis, MO.

** One case each of malignant rhabdoid tumor, leiomyoma, desmoplastic small round cell tumor and epithelioid hemangioendothelioma.


<table>
<thead>
<tr>
<th>Type</th>
<th>Count</th>
<th>Age Range</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma</td>
<td>16 (41)</td>
<td>2 mo – 6 yr; 30, 24</td>
<td>4/12</td>
</tr>
<tr>
<td>Atypical adenoma</td>
<td>16 (41)</td>
<td>10 da – 8 yr; 30, 24</td>
<td>4/12</td>
</tr>
<tr>
<td>Cortical adenoma</td>
<td>7 (18)</td>
<td>2 yr – 12 yr; 6 yr, 3 yr</td>
<td>2/5</td>
</tr>
</tbody>
</table>

39 (100) 10M:29F

REFERENCES:
Heritable Endocrine Disorders Involving Adrenal Gland Presenting In Childhood.

J.A.Carney M.D., Ph.D.

Objectives

At the end of this presentation, the learner will be able to:

1) Describe the pathologic features of primary pigmented nodular adrenal disease (PPNAD), a heritable disorder associated with mutation of the PRKAR1A gene.

2) Describe the associations of PPNAD (Carney complex).

3) Describe the adrenal pathology caused by mutation of the PDE11A4 gene, another cause of the Cushing syndrome in children.

In 1984, Shenoy et al (1) reported 4 patients (two males and two females, aged 12, 14, 19 and 21 years) with Cushing syndrome. Results of biochemical testing suggested that the patients had an autonomously functioning adrenocortical neoplasm but radiologic examination showed no adrenal tumor. Bilateral adrenalectomy cured the Cushing syndrome in each case and none of the patients developed the Nelson syndrome postoperatively. The same similar unusual bilateral adrenal pathology found in the four patients included: 1) decreased, normal, or slightly increased total adrenal weight; 2) studding of the cut surfaces by small (<4 mm), black and brown nodules; and 3) cortical atrophy and disorganization between the nodules. The nodules were composed of enlarged cortical cells with granular eosinophilic cytoplasm that often contained lipofuscin. The term "primary pigmented nodular adrenocortical disease" (PPNAD) was suggested for the disorder. Twenty-four similar cases were found in the literature, including 6 that occurred in 2 sets of siblings. In one of the families, the patients were children, aged 12, 15 and 15 years. In 1985, Carney et al (2) reported that PPNAD was associated with spotty skin pigmentation (ephelides, lentigines and blue nevi), myxomas (heart, skin and breast), large-cell calcifying Sertoli cell tumors, growth hormone-producing pituitary adenoma and psammomatous melanotic schwannomas (Carney complex). The complex was transmitted as an autosomal dominant trait and later linked to loci on 2p16 and 17q22-24 (PRKAR1A gene) (3).

Recent studies indicate that mutation of another gene, PDE11A4, is also associated with development of PPNAD and, in addition, with emergence of a different familial adrenal pathology. One of the patients with PDE11A4
mutation had Cushing syndrome at birth and a sibling developed the condition at age 10.

In addition to the foregoing, there are a series of other childhood Cushing-associated pathologies that are not easily reconcilable morphologically with the pathology caused by the PRKARIA or PDE11A4 gene mutations.

References